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SYNTHESIS AND THE FIRST STRUCTURE–ACTIVITY RELATIONSHIP STUDY OF IMIDAZOLE ALKALOID FROM RED ASCIDIAN AS ALDOSE REDUCTASE INHIBITORS

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Abstract – We synthesized a series of 2-aroyl-4-aryl-1*H*-imidazoles (**2** and **3**) and evaluated their *in vitro* AR inhibitory activity for the first time. We observed improved activities with derivatives having multiple catechol moieties.

Diabetes is a metabolic disease characterized by chronic hyperglycemia, which eventually leads to the onset of life-threatening diabetic complications. Aldose reductase (AR) is an enzyme that relates to the onset of diabetic complications, and its inhibition is believed to be effective for preventing or treating diabetic complications. Thus, several AR inhibitors (ARIs) with diverse structures have been developed during the last three decades.[1-6] Most highly potent ARIs are classified into two structural categories: those containing a glycine unit (–NCH_2CO_2H) and those having a cyclic imide represented by a spirohydantoin or related ring systems.[7-11] These fragments, under physiologic pH conditions, dissociate to form the corresponding conjugate base forms and interact tightly with the active site of AR, which is composed of Tyr48, Lys77, His110, and Trp111, to form hydrogen bonds thus prohibiting the access of glucose to the active site. However, most of them have been omitted from preclinical and clinical trials owing to pharmacokinetic drawbacks, low *in vivo* efficacy, or adverse side effects.[12] Currently, epalrestat is the only drug approved for clinical use in Japan, China, and India,[13] but it causes side effects such as liver dysfunction.[14] Therefore, the development of new ARIs without these key structures are desired. We have been interested in using the phenolic hydroxyl group as a substitute of the glycine and spirohydantoin units, and have been focusing our interests on pyrazine and imidazole alkaloids isolated from the red ascidian *Botryllus leachi* (Figure 1).[15,16] The phenolic hydroxyl groups in these compounds are more acidic than phenol itself because electron-withdrawing pyrazine or carbonyl group attaches to the *p*-position of the hydroxyphenyl group,[17] thereby favoring proton dissociation under physiologic pH

conditions to form phenolate anions capable of interacting with the AR active site. Within this context, we previously synthesized botryllazine A, B, and their derivatives, and evaluated their AR inhibitory activity to study the relationship between their structures and activity.[5,18] However, no ARI structure–activity relationship study of imidazole **1** has been performed. In the present study, we have synthesized several 2-aroyl-4-aryl-1*H*-imidazoles (**2**–**5**) and evaluated their inhibitory activities against recombinant human AR. We herein provide the first structure–activity relationship data for this family of compounds.

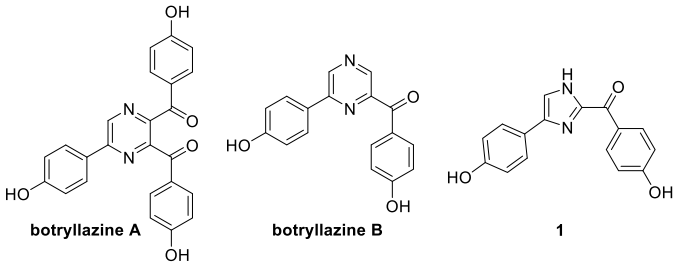
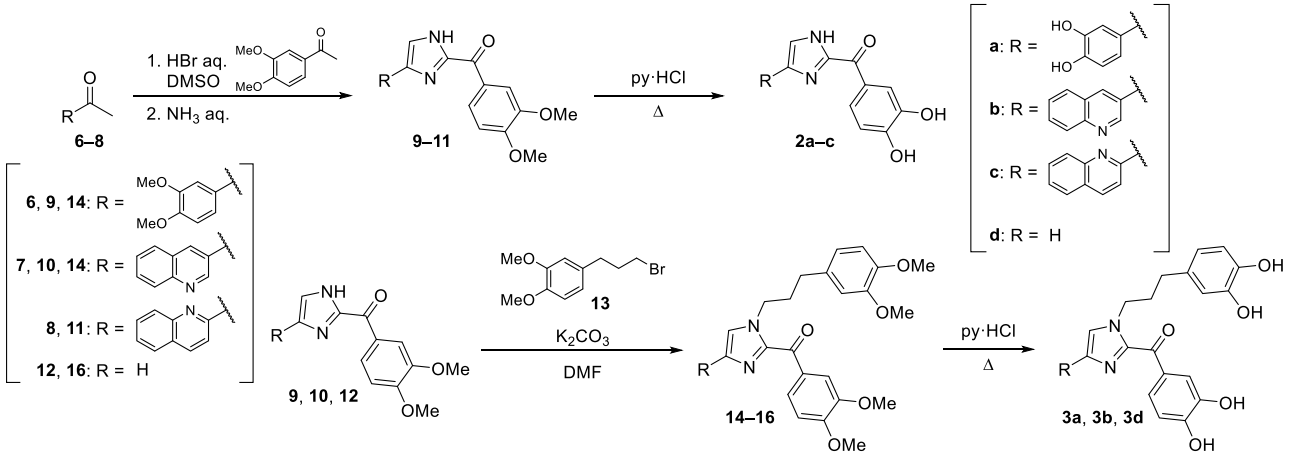
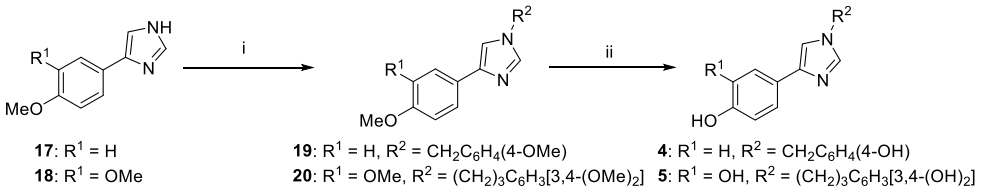


Figure 1. Structures of pyrazine and imidazole alkaloids isolated from *Botryllus leachi*[15,16]

Imidazoles **2** and **3**, which have catechol on C2 to mimic a carboxylate group capable of forming bidirectional hydrogen bonds, were synthesized using a method for synthesizing **1**, which involves Kornblum oxidation and subsequent treatment with aqueous ammonia.[19] As depicted in Scheme 1, ketones **6** and **8**[20] were converted into the corresponding imidazoles **9** and **11**, respectively, which were then treated with pyridinium chloride to afford **2a** and **2c**, respectively. Similarly, **2b** was synthesized from **7**[21] *via* **10** to obtain an HCl salt form because the purification of free **2b** was unsuccessful. *N*-Alkylated imidazoles **3a**, **3b**, and **3d** were prepared by alkylating **9**, **10**, and **12** with **13**[22] to give **14**–**16**, respectively, followed by deprotection of the methyl protecting groups with pyridinium chloride. Imidazoles **4** and **5** were designed to see the effect of acyl groups on C2, and synthesized by alkylating imidazole **17**[23] and **18**,[24] respectively, with the appropriate alkyl halides, followed by the cleavage of methyl protecting groups (Scheme 2).



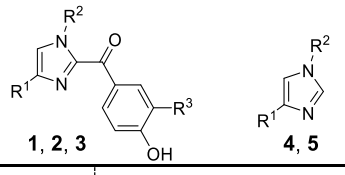
Scheme 1. Synthesis of 2-acylimidazoles **2a**–**c**, **3a**, **3b**, and **3d**

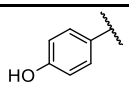
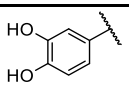
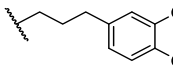
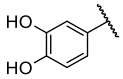
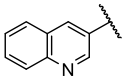
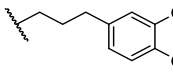
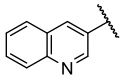
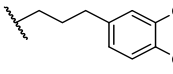
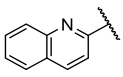
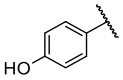
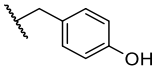
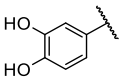
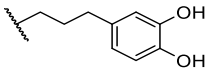


Scheme 2. Synthesis of **4** and **5**. *Reagents and conditions*: (i) For **17**: 1) NaH, THF, 40 ºC, 100 min.; 2) $ClCH_2C_6H_4$(*p*-OMe), THF, 40 ºC, 2 d. For **18**: K_2CO_3, **14**, DMF, rt, 6d. (ii) For **19**: BBr_3 CH_2Cl_2, –78 ºC to rt, 23h. For **20**: pyridinium chloride, 180 ºC, 140 min.

The AR inhibitory activities of **2–5** were evaluated *in vitro* by measuring their inhibitory effects on the reduction of D,L-glyceraldehyde with recombinant human AR in the presence of NADPH as a reductant.[5,25-27] The results are expressed as IC_{50} values which indicate the 50% inhibition concentrations of inhibitors against the reduction of D,L-glyceraldehyde by AR and are presented in Table 1, accompanied by reference IC_{50} values of epalrestat and sorbinil. The IC_{50} value of epalrestat estimated by the present study well agrees with previously-reported values,[18,28] verifying that current study experimental data represent absolute values.

Table 1. *In vitro* AR inhibitory activity of imidazoles **1**, **2a–c**, **3a**, **3b**, **3d**, **4**, and **5**



Compound	R^1	R^2	R^3	IC_{50} /μM	Compound	R^1	R^2	R^3	IC_{50} /μM
1		H	H	8.92	**3a**			OH	0.44
2a		H	OH	3.59	**3b**			OH	1.61
2b		H	OH	1.92	**3d**	H		OH	2.49
2c		H	OH	1.66	**4**			–	25% [a] (100 μM)
epalrestat				0.06	**5**			–	68.3
sorbinil				1.3 [b]					

[a] Inhibition rate at the given concentration. [b] Datum was taken from Ref 28.

It was found that replacing the hydroxyphenyl groups in **1** with catechol groups improved the AR inhibitory activity (**2a**). Further improvement in the inhibitory activity was observed when the catechol on C4 in **2a** was substituted with a bicyclic heterocyclic ring system (**2b** and **2c**). AR inhibitory activities of these compounds (IC_{50} = 1.92 and 1.66 μM, respectively, for **2b** and **2c**) were comparable to that of

sorbinil, which is known to be a highly effective AR inhibitor (IC_{50} = 1.3 μM).[28] We previously reported similar chemical modification in which replacement of a phenol moiety in an imidazolone-based AR inhibitor with naphthalen-2yl group improved the AR inhibitory activity.[27] These results suggest that the present imidazole derivatives interact with AR the same way as the previous imidazolone derivatives. Compound **1** showed lower AR inhibitory activity than the corresponding botryllazine B, indicating that pyrazine moieties of botryllazine B derivatives are considered more effective in inhibiting AR than imidazole ring. It is noteworthy that the introduction of an alkyl chain having a terminal catechol group at N1 in **2a** improved the AR inhibitory activity (**3a**). Introduction of the same alkyl chain into **2b** also exhibited similar improvement in AR inhibitory activity (**3b**). These results suggest that the activity remains unimpaired by these modifications. This means additional medicinal components can be introduced at this site without impairing the AR inhibitory activity, leading to the development of multi-functional drugs. Derivatives **4** and **5** that did not possess any hydroxybenzoyl group on C2 showed decreased AR inhibitory activity, while the lack of aryl group on C4 slightly decreased the inhibitory activity of **3d**; however, it still possessed substantial activity. These results suggest that hydroxybenzoyl groups on C2 are indispensable.

In conclusion, we carried out the first structure–activity relationship study of imidazole alkaloid **1** from *B. leach* against AR, and found that introduction of quinolin-2-yl or quinolin-3-yl group into C4 position of the central imidazole as well as 3,4-dihydroxybenzoyl group at C2 position improved the AR inhibitory activity (IC_{50} = 1.92 and 1.66 μM for **2b** and **2c**, respectively). These results were comparable to that of the highly effective AR inhibitor sorbinil. Introduction of an alkyl chain-containing catechol group into the N1 position did not affect the AR inhibitory activity (**3a** and **3b**), indicating that additional medicinal components can be introduced at this site without impairing the AR inhibitory activity, leading to the development of multi-functional drugs.

EXPERIMENTAL

NMR spectra were recorded on an ECP-400 spectrometer (JEOL Ltd., Japan). Chemical shifts (δ) are reported in ppm using tetramethylsilane or an undeuterated solvent as internal standards in the deuterated solvent used. Coupling constants (J) are given in Hz. Chemical shift multiplicities are reported as s = singlet, d = doublet, t = triplet, m = multiplet. High-resolution electrospray ionization mass spectra (HRMS) were obtained using a JEOL Model JMS-T100CS mass spectrometer. All conventional chemicals used in the present study were commercially available and used as received. Column chromatography was carried out on silica gel (particle size; 46–50 μm; Fuji Silysia Chemical Ltd.).

Representative procedure for synthesizing 2-acyl-1*H*-imidazoles. A typical example: synthesis of 2-(3,4-dimethoxybenzoyl)-4-(3,4-dimethoxyphenyl)-1*H*-imidazole (9)

A mixture of **6** (3.67 g, 20.4 mmol), 48% HBr aq. (24.0 mL, 211 mmol) in DMSO (24.0 mL) was stirred for 8 h at 55 °C in a sealed reaction vessel under argon atmosphere, followed by stirring for another 20 min under argon at atmospheric pressure. After cooling down to 0 °C, 28% aqueous ammonia (24.0 mL, 355 mmol) was added to the reaction mixture, and the mixture was stirred for 45 min at 0 °C. Precipitated materials were collected, and purified by recrystallization using hot MeOH to afford **9** as orange solid (1.17 g, 31%). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 13.43 (s, 1H), 8.47 (s, 1H), 8.37 (d, J = 8.4, 1H), 7.97 (s, 1H), 7.51 (s, 1H), 7.46 (d, J = 8.4, 1H), 7.18 (d, J = 8.4, 1H), 7.01 (d, J = 8.4, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H); ^{13}C-NMR (DMSO-d_6, 0.03% TFA, 100 MHz): δ/ppm 178.6, 153.3, 149.0, 148.6, 148.2, 144.4, 140.2, 128.4, 125.6, 124.6, 120.2, 117.7, 113.3, 112.1, 111.0, 109.0, 55.8, 55.6, 55.6, 55.4. These data were in good accordance with those reported previously.[29]

2-(3,4-Dimethoxybenzoyl)-4-(quinolin-3-yl)-1*H*-imidazole (10)

This was prepared from **6** (744 mg, 4.13 mmol) and **7**[21] (704 mg, 4.11 mmol) using a method similar to that of preparing **9**: yield, 230 mg (16%) as yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 13.69 (brs, 1H), 9.50 (s, 1H), 8.78 (s, 1H), 8.54 (d, J = 8.0, 1H), 8.31 (s, 1H), 8.27 (s, 1H), 8.03 (d, J = 8.4, 2H), 7.74 (t, J = 8.4, 1H), 7.63 (t, J = 8.4, 1H), 7.22 (d, J = 8.0, 1H), 3.92 (s, 3H), 3.91 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ/ppm 178.9, 153.4, 148.5, 148.2, 146.8, 145.5, 139.7, 129.9, 129.0, 128.8, 128.3, 128.1, 127.8, 127.0, 127.0, 125.9, 119.3, 113.0, 110.9, 55.8, 55.4. HRMS Calcd for $C_{21}H_{17}N_3NaO_3$ $[M+Na]^+$: 382.11676. Found: 382.11765.

2-(3,4-Dimethoxybenzoyl)-4-(quinolin-2-yl)-1*H*-imidazole (11)

This was prepared from **6** (5.30 g, 29.4 mmol) and **8**[20] (5.04 g, 29.4 mmol) using a method similar to that of preparing **9**: yield, 682 mg (6.5%) as pale red solid. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 13.76 (brs, 1H), 8.55 (d, J = 7.8, 1H), 8.43 (d, J = 8.4, 1H), 8.31 (s, 1H), 8.23 (d, J = 8.4, 1H), 8.17 (s, 1H), 8.00 (d, J = 8.4, 1H), 7.96 (d, J = 8.4, 1H), 7.75 (t, J = 8.4, 1H), 7.56 (t, J = 7.8, 1H), 7.21 (d, J = 8.4, 1H), 3.91 (s, 3H), 3.90 (s, 3H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ/ppm 179.0, 153.4, 152.6, 148.3, 147.5, 145.2, 143.0, 136.9, 129.9, 128.5, 128.2, 128.0, 127.1, 126.0 (2C), 120.8, 118.0, 112.9, 111.0, 55.8, 55.5; HRMS Calcd for $C_{21}H_{17}N_3NaO_3$ $[M+Na]^+$: 382.11676. Found: 382.11839.

Synthesis of (3,4-dimethoxyphenyl)(1*H*-imidazol-2-yl)methanone (12)

Under argon atmosphere, 3,4-dimethoxybenzoyl chloride (6.02 g, 30.0 mmol) in pyridine (10.0 mL) was added to a mixture of 1*H*-imidazole (1.01 g, 14.9 mmol), triethylamine (4.1 mL, 29.4 mmol), and activated molecular sieves (4A, 500 mg) in pyridine (10.0 mL) at 0 °C. The mixture was first stirred at 0 °C for 10 min, and subsequently at room temperature for 2 h and then at 70 °C for 30 min. After that, NaOH (1.01 g, 25.3 mmol) in water (15 mL) was added to the mixture, and the mixture was refluxed for 1 h. Then, water (100 mL) was added to the reaction mixture, and organic materials were extracted with AcOEt (100 mL×3). The combined organic layer was washed with brine and dried over $MgSO_4$. Removal

of the solvent yielded crude material, which was purified by recrystallization using hot MeOH to afford **12** as a white solid (792 mg, 23%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 10.4 (brs, 1H), 8.60 (d, J = 8.6, 1H), 8.12 (s, 1H), 7.38 (s, 1H), 7.27 (s, 1H), 6.98 (d, J = 8.6, 1H), 3.99 (s, 3H), 3.98 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 180.3, 153.9, 148.9, 145.7, 131.7, 128.6, 126.9, 119.8, 112.9, 110.3, 56.2, 56.1; HRMS Calcd for $C_{12}H_{12}N_2NaO_3$ $[M+Na]^+$: 255.07456. Found: 255.07623.

Representative procedure for alkylating imidazole derivatives with compound 13. A typical example: Synthesis of 2-(3,4-dimethoxybenzoyl)-4-(3,4-dimethoxyphenyl)-1-[3-(3,4-dimethoxyphenyl)-propyl)-1*H*-imidazole (14)

A mixture of **9** (1.00 g, 2.72 mmol), **13**[22] (765 mg, 2.95 mmol), and K_2CO_3 (1.88 g, 13.6 mmol) in DMF (30.0 mL) was stirred at room temperature under argon for 16 h. The white solid formed during the reaction was removed by filtration and the filtrate was concentrated to afford deep orange oil. The resulting mixture was purified by column chromatography on silica gel (90 g, eluted with $CHCl_3$/AcOEt = 39/1) followed by recrystallization using $CHCl_3$/MeOH to afford **14** as a yellowish solid (772 mg, 52%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 8.28 (dd, J = 8.4 and 1.2, 1H), 8.14 (d, J = 1.2, 1H), 7.41 (s, 1H), 7.35 (d, J = 8.4, 1H), 7.32 (s, 1H), 6.97 (d, J = 8.4, 1H), 6.90 (d, J = 8.4, 1H), 6.78 (d, J = 8.0, 1H), 6.73 (d, J = 8.0, 1H), 6.72 (s, 1H), 4.47 (t, J = 7.2, 2H), 3.99 (s, 3H), 3.98 (s, 3H), 3.95 (s, 3H), 3.91 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 2.69 (t, J = 7.2, 2H), 2.27−2.20 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 181.9, 153.3, 149.3, 149.0, 148.8, 148.5, 147.5, 142.7, 141.4, 133.4, 130.3, 126.7, 126.6, 120. 8, 120.3, 117.6, 113.6, 111.7, 111.5, 111.4, 110.1, 108.5, 56.2, 56.1, 56.0, 56.0, 56.0, 56.0, 48.7, 32.8, 32.5; HRMS Calcd for $C_{31}H_{34}N_2NaO_7$ $[M+Na]^+$: 569.22637. Found: 569.22343.

2-(3,4-Dimethoxybenzoyl-1-[3-(3,4-dimethoxyphenyl)propyl]-4-(quinolin-3-yl)-1*H*-imidazole (15)

This was prepared from **10** (900 mg, 2.50 mmol) with **13** (715 mg, 2.76 mmol) using a method similar to that of preparing **14**: yield, 999 mg (74%) as a pale red solid. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 9.36 (d, J = 2.0, 1H), 8.57 (d, J = 2.0, 1H), 8.34 (dd, J = 8.4 and 2.0, 1H), 8.11 (d, J = 8.4, 1H), 8.09 (d, J = 2.0, 1H), 7.86 (d, J = 8.4, 1H), 7.69 (td, J = 6.8 and 2.0, 1H), 7.57 (s, 1H), 7.56 (t, J = 6.8, 1H), 7.01 (d, J = 8.0, 1H), 6.81−6.74 (m, 3H), 4.52 (t, J = 8.0, 2H), 4.04 (s, 3H), 4.01 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 2.73 (t, J = 7.2, 2H), 2.35−2.23 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 182.0, 153.7, 149.1, 148.8, 148.5, 147.6, 147.6, 143.4, 138.5, 133.2, 130.9, 130.0, 129.5, 129.3, 128.3, 128.0, 127.2, 126.9, 126.7, 122.0, 120.3, 113.3, 111.7, 111.4, 110.2, 56.3, 56.1, 56.1, 56.0, 49.0, 32.8, 32.6; HRMS Calcd for $C_{32}H_{32}N_3NaO_5$ $[M+Na]^+$: 560.21614. Found: 560.21657.

2-(3,4-Dimethoxybenzoyl)-1-[3-(3,4-dimethoxyphenyl)propyl]-1*H*-imidazole (16)

Compound **12** (702 mg, 3.02 mmol) was reacted with **13** (878 mg, 3.39 mmol) using a method similar to that of preparing **14**, a 1.44 g of a yellow liquid was obtained after removing precipitates and the solvent from the reaction mixture. This material was used directly in the next step without further

purification (yield, quant.). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 8.14 (dd, J = 8.4 and 2.0, 1H), 7.83 (d, J = 2.0, 1H), 7.22 (d, J = 1.2, 1H), 7.12 (s, 1H), 6.94 (d, J = 8.4, 1H), 6.79 (d, J = 8.4, 1H), 6.72 (dd, J = 8.4 and 1.2, 1H), 4.44 (t, J = 7.2, 2H), 6.71 (s, 1H), 3.96 (s, 3H), 3.96 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 2.65 (t, J = 7.2, 2H), 2.23–2.16 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 182.7, 153.3, 149.1, 148.7, 147.5, 143.1, 133.4, 130.3, 129.2, 126.7, 125.4, 120.3, 112.8, 111.7, 111.4, 110.1, 56.2, 56.1, 56.0, 56.0, 48.4, 32.9, 32.5; HRMS Calcd for $C_{23}H_{26}N_2NaO_5$ $[M+Na]^+$: 433.17394. Found: 433.17308.

Synthesis of 4-(3,4-dimethoxyphenyl)-1-[3-(3,4-dimethoxyphenyl)propyl]-1*H*-imidazole (20)

This was prepared from **18**[24] (1.15 g, 5.65 mmol) with **13** (1.61 g, 6.23 mmol) using a method similar to that of preparing **14**. After purification of the crude product by column chromatography, a pink liquid (1.87 g) was obtained. This was found to be a mixture of **20** and DMF (**20**/DMF = 1/0.043) by ^{1}H-NMR measurement. Yield and amount of **20** was calculated based on this ratio (4.84 mmol, 86%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.47 (d, J = 1.6, 1H), 7.40 (d, J = 1.6, 1H), 7.26 (dd, J = 8.4 and 1.6, 1H), 7.13 (d, J = 1.6, 1H), 6.88 (d, J = 8.4, 1H), 6.81 (d, J = 8.0, 1H), 6.71 (d, J = 8.0, 1H), 6.67 (d, J = 1.6, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 2.60 (t, J = 7.2, 2H), 2.19−2.09 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 149.3, 149.1, 148.1, 147.6, 142.3, 137.2, 132.8, 127.5, 120.3, 117.0, 113.9, 111.8, 111.5 (2C), 108.3, 56.0, 56.0, 56.0, 56.0 50.7, 46.4, 32.4, 32.1; HRMS Calcd for $C_{22}H_{27}N_2O_4$ $[M+H]^+$: 383.19708. Found: 383.20100.

Synthesis of 1-(4-methoxybenzyl)-4-(4-methoxyphenyl)-1*H*-imidazole (19)

To a slurry of sodium hydride (398 mg, 9.11 mmol) in THF (70 mL), **17**[23] (1.05 g, 6.01 mmol) was added and the mixture was stirred at 40 °C for 2 h. Then, 4-methoxybenzyl chloride (1.08 mL, 7.93 mmol) was slowly added to the reaction mixture at −78 °C, and the mixture was stirred at 40 °C for 2 days. Water (100 mL) was added to the reaction mixture at 0 °C, and organic materials were extracted with $CHCl_3$ (100 mL×4). The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The resulting crude product was purified by column chromatography on silica gel (75 g) with $CHCl_3$/AcOEt = 7/1 as eluents followed by recrystallization from $CHCl_3$/hexane to afford **19** as a yellowish solid (440 mg, 25%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.67 (d, J = 8.4, 2H). 7.53 (s, 1H), 7.14 (d, J = 8.4, 2H), 7.05 (s, 1H), 6.89 (d, J = 8.4, 2H), 6.88 (d, J = 8.4, 2H), 5.03 (s, 2H), 3.81 (s, 3H), 3.80 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 159.7, 158.7, 142.5, 137.3, 129.0, 128.2, 127.2, 126.1, 114.5, 114.1, 114.0, 55.5, 55.4, 50.6; HRMS Calcd for $C_{18}H_{18}N_2O_2$ $[M+Na]^+$: 317.12660. Found: 317.12891.

Representative procedure for cleaving methyl protecting groups with pyridinium chloride. A typical example: synthesis of 2-(3,4-dihydroxybenzoyl)-4-(3,4-dihydroxyphenyl)-1*H*-imidazole (2a)

Under argon atmosphere, a mixture of **9** (467 mg, 1.27 mmol) and pyridinium chloride (9.39 g, 81.3 mmol) was stirred at 200 °C for 1 h. After cooling to room temperature, water (20 mL) was added to the

reaction mixture, and the solution was neutralized to around pH 6 with aqueous NaOH. The resulting precipitates were collected, and purified by column chromatography (eluted with $CHCl_3$/MeOH = 9/1) on silica gel (60 g) to afford **2a** as a yellow solid (100 mg, 320 μmol, 25%). ^{1}H-NMR (DMSO-d_6, 0.03% TFA, 400 MHz): δ/ppm 8.08 (d, J = 8.4, 1H), 7.98 (s, 1H), 7.70 (s, 1H), 7.32 (s, 1H), 7.21 (d, J = 8.4, 1H), 6.90 (d, J = 8.4, 1H), 6.79 (d, J = 8.4, 1H); ^{13}C-NMR (DMSO-d_6, 0.03% TFA, 100 MHz): δ/ppm 178.8, 151.2, 145.5, 145.4, 145.0, 144.4, 140.5, 127.5, 124.5, 123.4, 119.0, 117.8, 116.9, 115.9, 115.1, 113.0. *Anal*. Calcd for $C_{16}H_{12}N_2O_5$•0.5CH_3OH: C, 60.36; H, 4.30; N, 8.53. Found: C, 60.33; H, 4.39; N, 8.58.

2-(3,4-Dihydroxybenzoyl)-4-(quinolin-3-yl)-1*H*-imidazole (2b)

Compound **10** (320 mg, 891 μmol) was treated with pyridinium chloride (9.04 g, 78.3 mmol) using a method similar to that of preparing **2a**. After completion of the reaction, water (20 mL) was added to the reaction mixture, and the solution was neutralized to around pH 7 with aqueous NaOH. The resulting precipitates were collected and dissolved in AcOEt. The AcOEt solution was then mixed with HCl in 1,4-dioxane (4 mol/L). The resulting precipitates were collected and purified by recrystallization using hot MeOH to afford **2b** in a hydrochloride salt form as a yellow solid (59.0 mg, 18%). ^{1}H-NMR (DMSO-d_6, 0.03% TFA, 400 MHz): δ/ppm 9.77 (s, 1H), 9.49 (s, 1H), 8.40 (s, 1H), 8.34 (d, J = 8.4, 1H), 8.31 (d, J = 8.4, 1H), 8.24–8.18 (m, 2H), 8.01 (td, J = 8.0 and 1.2, 1H), 7.88 (t, J = 8.0 Hz, 1H), 6.95 (d, J = 8.4, 1H); ^{13}C-NMR (DMSO-d_6, 0.03% TFA, 100 MHz): δ/ppm 179.0, 151.5, 146.0, 145.1, 143.9, 138.6, 137.2, 136.9, 132.8, 129.4, 129.0, 128.6, 127.9, 127.0, 124.5, 122.4, 120.9, 118.1, 115.3; *Anal*. Calcd for $C_{19}H_{14}ClN_3O_3$•1.1H_2O: C, 58.87; H, 4.21; N, 10.84. Found: C, 58.82; H, 4.51; N, 10.60.

2-(3,4-Dihydroxybenzoyl)-4-(quinolin-2-yl)-1*H*-imidazole (2c)

This was prepared from **11** (328 mg, 912 μmol) with pyridinium chloride (4.61 g, 39.9 mmol) using a method similar to that of preparing **2a**: yield, 113 mg (37%) as a yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 13.6 (brs, 1H), 9.89 (brs, 1H), 9.58 (brs, 1H), 8.44 (d, J = 8.8, 1H), 8.31 (d, J = 8.8, 1H), 8.23 (d, J = 8.4, 1H), 8.21 (s, 1H), 8.14 (s, 1H), 8.00 (d, J = 8.4, 1H), 7.97 (d, J = 8.4, 1H), 7.75 (t, J = 7.2, 1H), 7.56 (t, J = 7.2, 1H), 6.94 (d, J = 8.4 Hz, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ/ppm 179.1, 152.7, 151.3, 147.5, 145.4, 144.9, 142.9, 136.8, 129.8, 128.5, 128.0, 127.2, 127.1, 125.9, 124.5, 120.4, 118.3, 117.9, 115.2; *Anal.* Calcd for $C_{19}H_{13}N_3O_3$•0.1H_2O: C, 68.50; H, 3.99; N, 12.62. Found: C, 68.40; H, 4.12; N, 12.54.

2-(3,4-Dihydroxybenzoyl)-4-(3,4-dihydroxyphenyl)-1-[3-(3,4-dihydroxyphenyl)propyl]-1*H*-imidazole (3a)

This was prepared from **14** (598 mg, 1.09 mmol) with pyridinium chloride (10.84 g, 93.8 mmol) using a method similar to that of preparing **2a**: 80.8 mg (16%) as a yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 9.88 (s, 1H), 9.36 (s, 1H), 8.95 (s, 1H), 8.93 (s, 1H), 8.71 (s, 1H), 8.62 (s, 1H), 7.83 (s, 1H), 7.82 (dd, J = 8.0 and 2.0, 1H), 7.78 (d, J = 2.0, 1H), 7.27 (d, J = 2.0, 1H), 7.11 (dd, J = 8.0 and 2.0, 1H), 6.86

(d, J = 8.0, 1H), 6.75 (d, J = 8.0, 1H), 6.60 (d, J = 8.0, 1H), 6.56 (d, J = 2.0, 1H), 6.41 (dd, J = 8.0 and 2.0, 1H), 4.33 (t, J = 7.2, 2H), 2.42 (t, J = 7.2, 2H), 2.04−1.97 (m, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ/ppm 181.5, 150.7, 145.4, 145.1, 144.8, 144.6, 143.3, 142.1, 140.6, 131.7, 128.7, 125.2, 124.5, 121.0, 118.8, 118.0, 116.2, 115.8, 115.5, 115.5, 114.8, 112.4, 47.7, 32.7, 31.6; *Anal*. Calcd for $C_{25}H_{22}N_2O_7$•H_2O: C, 62.49; H, 5.04; N, 5.83. Found: C, 62.58; H, 5.15; N, 5.89.

2-(3,4-Dihydroxybenzoyl)-1-[3-(3,4-dihydroxyphenyl)propyl]-4-(quinolin-3-yl)-1*H*-imidazole (3b)

This was prepared from **15** (799 mg, 1.49 mmol) with pyridinium chloride (8.95 g, 77.4 mmol) using a method similar to that of preparing **2a**: yield, 110 mg (15%) as a yellowish solid. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 9.89 (brs, 1H), 9.43 (d, J = 2.0, 1H), 8.72 (s, 1H), 8.71 (s, 1H), 8.35 (s, 1H), 8.04 (s, 1H), 8.02 (s, 1H), 7.90 (d, J = 2.0, 1H), 7.84 (dd, J = 8.0 and 2.0, 1H), 7.73 (t, J = 8.0, 1H), 7.62 (t, J = 8.0, 1H), 6.90 (d, J = 8.0, 1H), 6.61 (d, J = 8.0, 1H), 6.58 (d, J = 1.6, 1H), 6.44 (dd, J = 8.0 and 1.6, 1H), 4.38 (t, J = 7.2, 2H), 2.47 (t, J = 8.0, 2H), 2.11−2.03 (m, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ/ppm 181.6, 151.1, 148.4, 146.7, 145.1, 144.8, 143.4, 143.1, 137.1, 131.7, 129.8, 129.0, 128.8, 128.3, 128.2, 127.8, 127.1, 126.7, 124.6, 123.5, 118.8, 118.0, 115.6, 115.5, 115.1, 48.0, 32.6, 31.6; *Anal*. Calcd for $C_{28}H_{23}N_3O_5$•0.9H_2O: C, 67.57; H, 5.02; N, 8.44. Found: C, 67.43; H, 4.94; N, 8.41.

2-(3,4-Dihydroxybenzoyl)-1-[3-(3,4-dihydroxyphenyl)propyl]-1*H*-imidazole (3d)

This was prepared from **16** (712 mg) with pyridinium chloride (11.2 g, 97.2 mmol) using a method similar to that of preparing **2a**: yield, 268 mg (51% from **12**) as yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 9.48 (brs, 2H), 8.78 (brs, 2H), 7.75 (s, 1H), 7.72 (d, J = 8.0, 1H), 7.56 (s, 1H), 7.16 (s, 1H), 6.83 (d, J = 8.0, 1H), 6.59 (d, J = 8.0, 1H), 6.54 (s, 1H), 6.39 (d, J = 8.0, 1H), 4.33 (t, J = 7.2, 2H), 2.38 (t, J = 7.8, 2H), 1.99–1.92 (m, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ/ppm 181.7, 150.8, 145.1, 144.6, 143.3, 142.5, 131.7, 128.5, 128.4, 126.0, 124.4, 118.7, 117.9, 115.5, 115.5, 114.8, 47.5, 32.7, 31.5; *Anal*. Calcd for $C_{19}H_{18}N_2O_5$•0.2CH_3OH: C, 63.92; H, 5.25; N, 7.77. Found: C, 63.85; H, 5.22; N, 7.82.

4-{3-[4-(3,4-Dihydroxyphenyl)-1*H*-imidazol-1-yl]propyl}benzene-1,2-diol (5)

This was prepared from **20** (1.32 g, 3.46 mmol) with pyridinium chloride (20.0 g, 173 mmol) using a method similar to that of preparing **2a**: yield, 327 mg (29%) as a yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 8.75 (brs, 4H), 7.57 (s, 1H), 7.40 (s, 1H), 7.17 (d, J = 2.0, 1H), 6.99 (dd, J = 8.0 and 2.0, 1H), 6.69 (d, J = 8.0, 1H), 6.64 (d, J = 8.0, 1H), 6.56 (d, J = 2.0, 1H), 6.43 (dd, J = 8.0 and 2.0, 1H), 3.91 (t, J = 6.9, 2H), 2.37 (t, J = 7.5, 2H), 2.04−1.89 (m, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ/ppm 145.1, 145.1, 143.9, 143.3, 141.1, 137.2, 131.7, 126.5, 118.8, 115.7, 115.6, 115.5, 115.5, 113.8, 112.1, 45.7, 32.3, 31.4; *Anal*. Calcd for $C_{18}H_{18}N_2O_4$•0.2CH_3OH: C, 65.69; H, 5.70; N, 8.42. Found: C, 65.63; H, 5.73; N, 8.45.

Synthesis of 4-[1-(4-hydroxybenzyl)-1*H*-imidazol-4-yl]phenol (4)

Under argon atmosphere, a solution of BBr_3 in CH_2Cl_2 (1 mol/L; 45.0 mL, 45.0 mmol) was slowly added to a stirred solution of **19** (304 mg, 1.03 mmol) in CH_2Cl_2 (10 mL) at −78 °C. The mixture was stirred at room temperature for 23 h. Then, MeOH (20 mL) and water (24 mL) were added to the reaction mixture, and the solution was neutralized to pH 7 with NaOH aq. (1 mol/L). Organic materials were extracted with AcOEt (100 mL×3), and the organic phase was dried over $MgSO_4$. After removing the solvent, the resulting solid was washed with MeOH and purified by recrystallization using hot MeOH to afford **4** as a white solid (83.2 mg, 312 μmol, 30%). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ/ppm 9.45 (brs, 1H), 9.29 (brs, 1H), 7.69 (s, 1H), 7.51 (d, J = 8.6, 2H), 7.40 (s, 1H), 7.14 (d, J = 8.4, 2H), 6.74 (d, J = 8.6, 2H), 6.71 (d, J = 8.4, 2H), 5.03 (s, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ/ppm 157.0, 155.9, 141.2, 137.1, 129.1, 127.9, 125.8, 125.4, 115.3, 115.2, 113.8, 49.3; *Anal*. Calcd for $C_{16}H_{14}N_2O_2$•0.1CH_3OH: C, 71.75; H, 5.39; N, 10.40. Found: C, 71.48; H, 5.42; N, 10.33.

***In vitro* AR inhibitory assay**[5]

Enzyme activity was measured by monitoring the decay in absorbance at 340 nm, which accompanies the oxidation of NADPH catalyzed by recombinant human AR. The reaction mixture contained 0.2 M phosphate buffer (pH 6.2), 0.15 mM NADPH, 100 mM D,L-glyceraldehyde, and 3.6 mU/mL AR, in a total volume of 1.0 mL. All the above reagents, except D,L-glyceraldehyde, were incubated at 25 °C for 1 min. The reaction was initiated by adding D,L-glyceraldehyde, and it was monitored spectrophotometrically for 3 min at the same temperature. Inhibitory activity was tested in the above assay conditions by including the inhibitors dissolved in DMSO or 0.2 M phosphate buffer (pH 6.2) at desired concentrations in the reaction mixture. The final concentration of DMSO in the reaction mixture was kept at a constant concentration of 0.3%. Reference blank assays, lacking either the substrate or enzyme, were routinely included; the rates were subtracted from the reaction rates to correct the nonenzymatic oxidation of NADPH. IC_{50} values, which express the inhibitor concentrations that produce 50% inhibition on the oxidation of NADPH catalyzed by AR, were calculated using a log linear regression analysis of the log dose-inhibition plots. Each plot was provided using at least three concentrations of inhibitor with three replicates at each concentration.

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