Synthesis and Protein Tyrosine Phosphatase Inhibitory Activity of Dephostatin Analogs

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(Received for publication June 23, 1995)

We have synthesized derivatives of dephostatin, a protein tyrosine phosphatase (PTPase) inhibitor, to study the structure-activity relationships of this inhibitor. Inactive analogs revealed some insight into structural requirements for PTPase inhibitory activity of dephostatin. Both a nitroso group and phenolic hydroxyl groups were found to be essential for the inhibitory activity. Among the dephostatin derivatives synthesized, one of the regioisomers of dephostatin showed PTPase inhibitory activity equivalent to that of dephostatin, and also had increased stability.

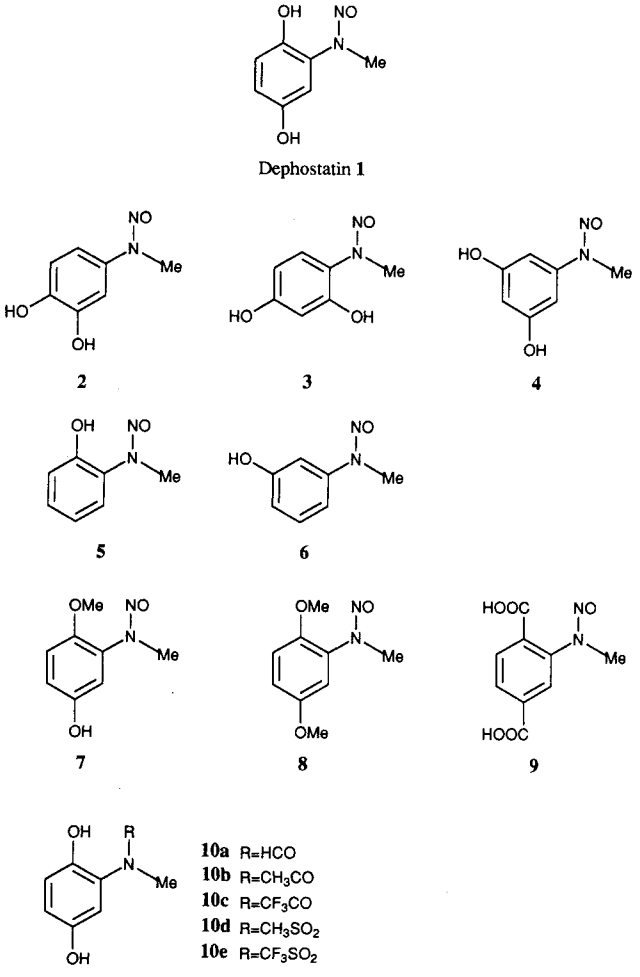
Protein tyrosine phosphatase (PTPase) has been implicated in the regulation of the cell cycle and activation of lymphocytes[1,2]. A novel protein tyrosine phosphatase inhibitor, dephostatin **1**, was isolated from the culture filtrate of *Streptomyces* sp. MJ742-NF5 in our laboratory[3,4]. In spite of its potential significance as a biochemical and therapeutic agent, sufficient amounts of dephostatin were difficult to obtain from the natural source. Therefore, we established a practical synthetic route for dephostatin[5]. However, dephostatin is not stable enough, and partial decomposition is frequently observed under storage and under conditions of biological experiments. Therefore, we synthesized several structurally related dephostatin analogs. As lability of dephostatin should arise from its hydroquinone or *N*-nitroso moiety, these structural elements were modified.

Results

Synthesis of Dephostatin Analogs

We synthesized 13 dephostatin analogs as shown in Fig. 1. Among them were some regioisomers of dephostatin (**2**, **3**, and **4**). Then, to clarify the role of the two hydroxyl groups in PTPase inhibitory activity, we synthesized two monohydroxyl analogs (**5** and **6**) and two methyl ether analogs (**7** and **8**) of the inhibitor. A carboxyl derivative (**9**), in which both of the hydroxyl groups were replaced by carboxyl groups, was also examined. Additionally, amide and sulfonamide derivatives

Fig. 1. Dephostatin and its analogs.

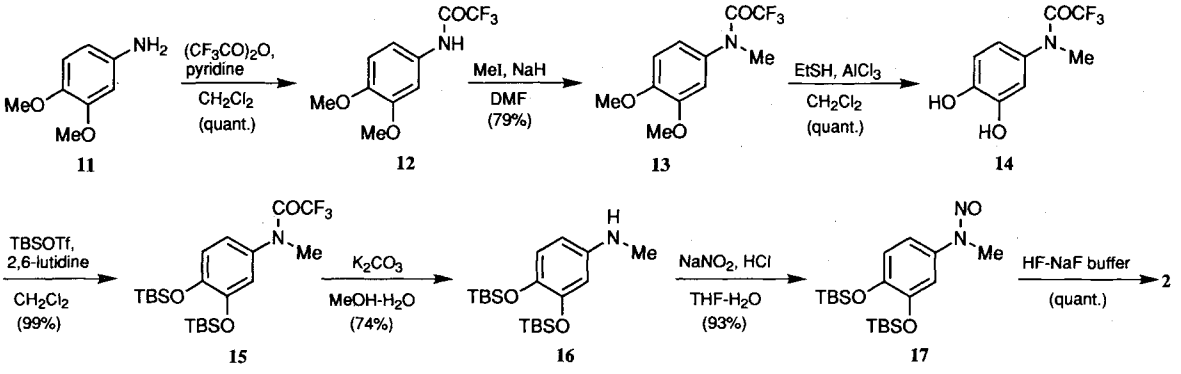


(**10a** to **10e**), in place of nitrosamine, were tested to investigate whether the labile and potentially toxic nitroso moiety could be removed from the overall structure.

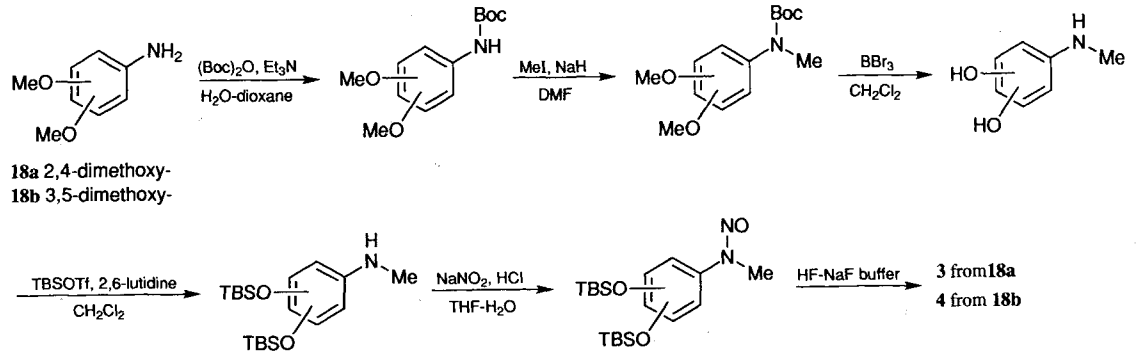
Attempts to synthesize **2**, **3** and **4** by applying our synthetic route to dephostatin[5] starting from corresponding dimethoxyanilines were not successful, therefore, modifications of reaction conditions and/or protecting groups were needed.

For the synthesis of **2** (Scheme 1), we used 3,4-dimethoxyaniline **11** as a starting material. The trifluoroacetyl group was chosen as a protecting group for the amino group because its stability under acid promoted ether cleavage (conversion from **13** to **14**). Acylation of **11** was conducted with trifluoroacetic anhydride and pyridine to give **12** in quantitative yield. Treatment of **12** with NaH in DMF followed by addition of MeI gave *N*-methylated product **13** in 79% yield. In the next step, two methyl ether groups were cleaved. This deprotection could be accomplished with BBr_3 without damaging the amide moiety, giving **14**, although the yield was not satisfactory (37%). Instead, reaction with ethanethiol (EtSH) in the presence of $AlCl_3$[6] resulted in deprotection in quantitative yield. Silylation of **14**

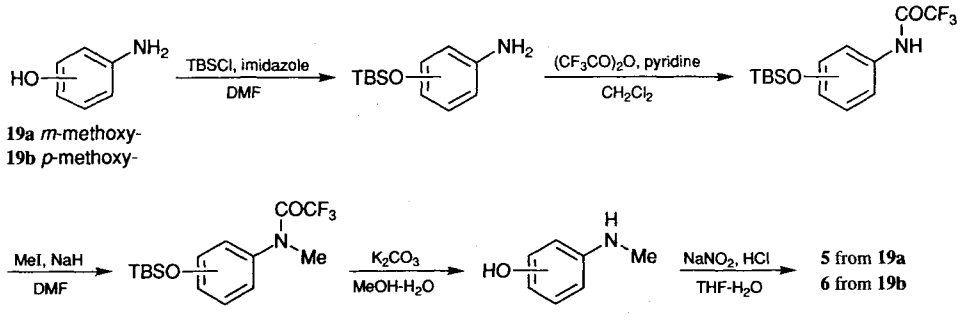
Scheme 1. Synthesis of **2**.



Scheme 2. Synthesis of **3** and **4**.

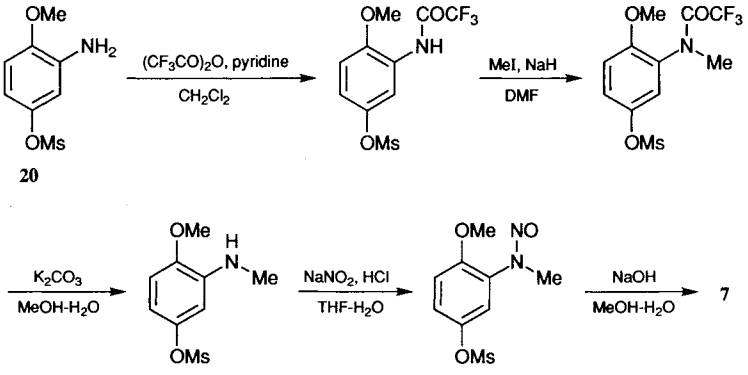


Scheme 3. Synthesis of **5** and **6**.

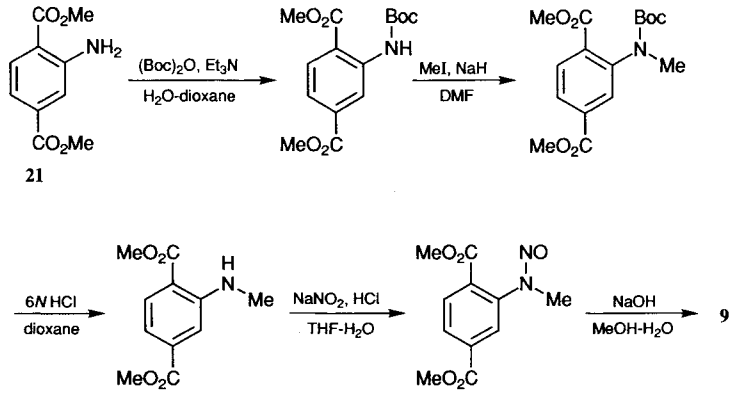


with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and 2,6-lutidine proceeded efficiently (99% yield). Subsequent treatment of **15** with aqueous K_2CO_3 yielded the deamide product **16**. Hydrolysis of silyl ether was a competing reaction in this step, and an appropriate choice of solvent was necessary to minimize this side reaction. The reaction system composed of 7% K_2CO_3 and MeOH gave a satisfactory result (74% yield). *N*-nitrosation of **16** was accomplished under the standard conditions with $NaNO_2$-HCl to give **17** in 93% yield. Final desilylation with HF-NaF buffer[7] gave the desired dephostatin analog **2** in quantitative yield. Detailed description of this synthesis is given in the experimental part.

Scheme 4. Synthesis of **7**.



Scheme 5. Synthesis of **9**.



Scheme 6. Synthesis of **10a**~**10e**.

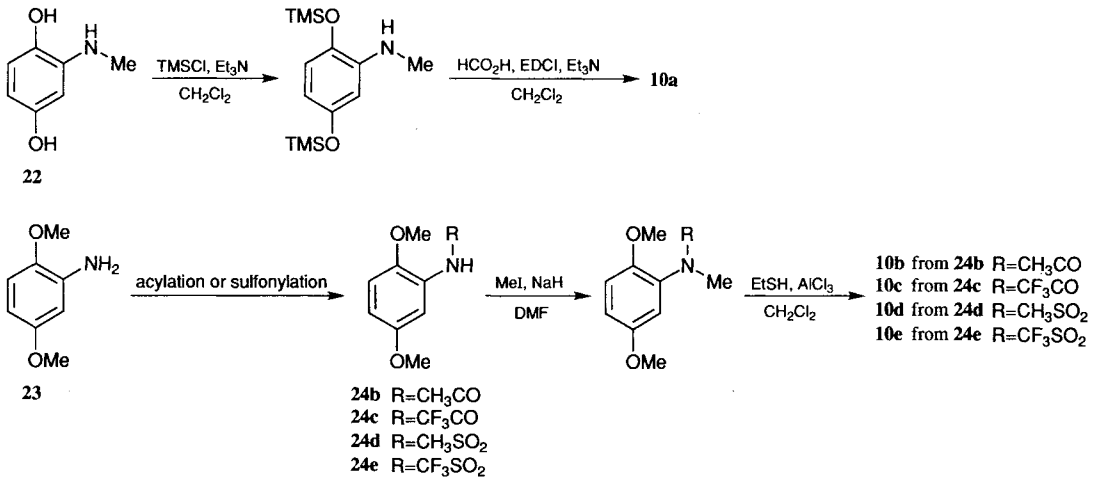


Table 1. PTPase inhibitory activities of dephostatin and its analogs.

Compound	IC_{50} (μg/ml)
1	1.8
2	3.0
3	>30
4	>30
5	>30
6	>30
7	>30
8	>30
9	>30
10a	>30
10b	>30
10c	>30
10d	>30
10e	>30

Analogs **3** and **4** were synthesized in similar manners as the case of dephostatin (Scheme 2). Other derivatives, **5** and **6**, **7**, **9** and **10a**~**10e**, were synthesized as shown in Schemes 3, 4, 5 and 6, respectively. Compound **8** was synthesized as already reported[5]. In scheme 3, the TBS group was removed without HF in the condition to eliminate the trifluoroacetyl group.

PTPase Inhibitory Activity of Dephostatin Analogs

PTPase inhibitory activities of the above analogs were examined by the method described previously[3]. The IC_{50} value of each analog is listed in Table 1. Among them, only **2** has showed inhibitory activity equivalent to that of dephostatin.

Discussion

Since all the amide and sulfonamide analogs had no effect on the PTPase, the *N*-nitroso moiety of dephostatin must play a crucial role in PTPase inhibition. An equally important structural factor is the presence of two unmasked hydoxyl groups on the benzene ring. So far only 2,5- and 3,4-substitutions (dephostatin and **2**, respectively) gave the inhibitory activity. Monohydroxyl and methoxy analogs had no inhibitory activity. Replacement of hydroxyl groups by carboxyl groups, which are similarly anionic, could not maintain the PTPase inhibitory activity.

Compound **2** was found to be more stable than dephostatin in the medium commonly used for cell culture. Dephostatin largely decomposed within 2 hours in DULBECCO's modified EAGLE's medium, whereas **2** was stable for at least 24 hours (manuscript in preparation). Compound **2** can also be stored for at least several months in a refrigerator without apparent decomposition. Since it was shown to inhibit PTPase *in situ* (unpublished result), **2** may be easier to handle than dephostatin for biological experiments. As **7** is only monomethylated at the 2-hydroxyl group and has no effect on PTPase, it should be a suitable inactive analogue for use in mechanistic studies.

Experimental

General

Melting points were determined with a Yanagimoto micro melting point apparatus and were uncorrected. IR spectra were measured with a Hitachi I-5020 FT-IR spectrometer or Hitachi Model 260-10 spectrophotometer. HRFAB-MS and FAB-MS were taken by JEOL JMS-SX 102. NMR spectra were recorded with a JEOL JNM-EX 400 spectrometer.

Synthesis of Dephostatin Analog **2**

3,4-Dimethoxy-*N*-trifluoroacetylaniline (**12**)

To a solution of 3,4-dimethoxyaniline **11** (10.04 g, 65.5 mmol) in dichloromethane (CH_2Cl_2) were added pyridine (5.82 ml, 72.1 mmol) and trifluoroacetic anhydride (10.0 ml, 72.1 mmol) successively under argon at 0°C. The solution was stirred at room temperature for 2 hours. After addition of CH_2Cl_2 to the reaction mixture, the organic layer was washed with 1 N HCl and brine successively, then dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by elution from a silica gel column with EtOAc-*n*-hexane (1 : 1) to give 16.30 g of **12** (65.4 mmol, quantitative yield) as a pale yellow powder: mp 101~103°C. IR (KBr) ν_{max} 3287, 1705, 1605, 1518, 1217, 1181, 841, 802 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (1H, br) 7.30 (1H, d, J=2.4 Hz), 6.99 (1H, dd, J=8.8, 2.4 Hz), 6.86 (1H, d, J=8.8 Hz), 3.90 (3H, s), 3.89 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7 (q, J=36.7 Hz), 149.3, 147.3, 128.4, 115.8 (q, J=288.7 Hz), 112.9, 111.3, 105.0, 56.1, 56.0. FAB-MS (positive): m/z 249 (M^+). *Anal.* Calcd for $C_{10}H_{10}F_3NO_3$: C, 48.20, H, 4.06, N, 5.62. Found: C, 48.12, H, 4.20, N, 5.60.

3,4-Dimethoxy-*N*-methyl-*N*-trifluoroacetylaniline (**13**)

To a suspension containing 1.45 g of 60% NaH and 10 ml of DMF was added a solution of **12** (6.00 g, 24.1 mmol) in 20 ml of DMF dropwise under argon at −23°C and the suspension was stirred at 0°C for 1 hour. Then, to the solution was added MeI (3.00 ml, 48.2 mmol) at −23°C, and the solution was stirred at room temperature for 1 hour. The solution was poured onto cold 1 N HCl and was extracted with EtOAc twice. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc-*n*-hexane (1 : 3 to 1 : 1) to give 5.00 g of **13** (19.0 mmol, 79% yield) as a white powder: mp 84~86°C. IR (KBr) ν_{max} 3445, 2843, 1700, 1518, 1213 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.86 (1H, d, J=8.8 Hz), 6.82 (1H, dd, J=8.8, 2.4 Hz), 6.73 (1H, d, J=2.4 Hz), 3.91 (3H, s), 3.88 (3H,

s), 3.34 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.1 (q, J=36.8 Hz), 149.4, 133.4, 119.7, 116.4 (q, J=288.6 Hz), 111.0, 110.6, 56.1, 56.0, 39.9. FAB-MS (positive): m/z 263 (M^+). *Anal.* Calcd for $C_{11}H_{12}F_3NO_3$: C, 50.20, H, 4.60, N, 5.32. Found: C, 50.11, H, 4.73, N, 5.24.

3,4-Dihydroxy-*N*-methyl-*N*-trifluoroacetylaniline (**14**)

To a solution of $AlCl_3$ (2.53 g, 19.0 mmol) and ethanethiol (19.0 ml) in 30 ml of CH_2Cl_2 solution was added **13** (1.00 g, 3.80 mmol) under argon at 0°C, and the solution was stirred for 5 hours[6]. Then, the solution was concentrated to dryness. The residue was chromatographed on silica gel eluted with EtOAc-*n*-hexane (1:5 to 1:1), and 893 mg of **14** (3.80 mmol, quantitative yield) was obtained as a white powder: mp 115~116°C. IR (KBr) ν_{max} 3447, 3368, 1680, 1520, 1225, 1206, 1167 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.29 (2H, br), 6.88 (1H, d, J=8.3 Hz), 6.87 (1H, d, J=2.9 Hz), 6.74 (1H, dd, J=8.3, 2.9 Hz), 3.27 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 157.4 (q, J=35.0 Hz), 147.0, 146.8, 134.2, 120.4, 118.1 (J=288.6 Hz), 116.5, 116.0, 40.4. HRFAB-MS (positive): calcd for $C_9H_8F_3NO_3$ 235.0456, found 235.0447 (M^+).

3,4-Bis[(*tert*-butyldimethylsilyl)oxy]-*N*-methyl-*N*-trifluoroacetylaniline (**15**)

To a solution of **14** (507 mg, 2.15 mmol) in 15 ml of CH_2Cl_2 were successively added 2,6-lutidine (1.90 ml, 16.3 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.78 ml, 7.74 mmol) under argon at 0°C, and the solution was stirred at room temperature for 12 hours. The solution was poured into water and extracted with EtOAc twice. The organic layer was washed with saturated $NaHCO_3$ and brine successively, then, dried over Na_2SO_4 and concentrated to dryness. The resulting dark brown oil was purified by silica gel column chromatography with EtOAc-*n*-hexane (1:20 to 1:10) as eluent to give 887 mg of colorless needles **15** (2.13 mmol, 99%): mp 88~89°C. IR (KBr) ν_{max} 3438, 2861, 1713, 1510, 1327, 1254, 909, 843 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.82 (1H, d, J=9.3 Hz), 6.67~6.71 (2H, m), 3.31 (3H, s), 0.99 (9H, s), 0.98 (9H, s), 0.22 (6H, s), 0.22 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.1 (q, J=36.8 Hz), 147.7, 147.3, 133.7, 121.0, 120.4, 120.3, 116.4 (q, J=286.7 Hz), 39.8, 25.9, 25.8, 18.5, 18.4, −4.1, −4.3. HRFAB-MS (positive): calcd for $C_{21}H_{37}F_3NO_3Si_2$ 464.2264, found 464.2235 $(M+H)^+$.

3,4-Bis[(*tert*-butyldimethylsilyl)oxy]-*N*-methylaniline (**16**)

Compound **15** was dissolved in 10 ml of aqueous methanol solution of K_2CO_3 (7% K_2CO_3-MeOH=2:5). The solution was stirred at room temperature for 3 hours. Then, the reaction mixture was extracted with CH_2Cl_2 twice. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by elution from a silica gel column with EtOAc-*n*-hexane (1:15 to 1:10) to give 142 mg of **16** (0.387 mmol, 74% yield) as colorless needles: mp 34~35°C. IR (KBr) ν_{max} 3428, 2857, 1617, 1514, 1275, 1251, 840 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.67 (1H, d, J=8.8 Hz), 6.17 (1H, d, J=2.9 Hz), 6.12 (1H, dd, J=8.8, 2.9 Hz), 2.76 (3H, s), 0.98 (9H, s), 0.97 (9H, s), 0.19 (6H, s), 0.15 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.3, 144.2, 138.6, 121.5, 106.0, 105.5, 31.4, 26.0, 26.0, 18.5, 18.4, −4.1, −4.2. HRFAB-MS (positive): calcd for $C_{19}H_{37}NO_3Si_2$ 367.2363, found 367.2365 (M^+).

3,4-Bis[(*tert*-butyldimethylsilyl)oxy]-*N*-methyl-*N*-nitrosoaniline (**17**)

To a solution of **16** (130 mg, 0.353 mmol) in 15 ml of THF were added 3 ml of 1 N HCl and $NaNO_2$ (26.7 mg, 0.387 mmol) successively at 0°C. The solution was stirred at 0°C for 3 hours. Then, the reaction mixture was extracted with CH_2Cl_2 twice. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with EtOAc-*n*-hexane (1:15 to 1:10) to give 131 mg of **17** (0.329 mmol, 93% yield) as a bright yellow powder: mp 37~38°C. IR (KBr) ν_{max} 3461, 2861, 1603, 1518, 1445, 1345, 905, 841 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.08 (1H, d, J=1.5 Hz), 6.91 (2H, d, J=1.5 Hz), 3.41 (3H, s), 1.01 (9H, s), 1.00 (9H, s), 0.23 (12H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.5, 146.6, 136.1, 121.1, 113.0, 112.3, 32.0, 25.9, 18.5, −4.1. HRFAB-MS (positive): calcd for $C_{19}H_{37}N_2O_3Si_2$ 397.2343, found 397.2338 $(M+H)^+$.

3,4-Dihydroxy-*N*-methyl-*N*-nitrosoaniline (**2**)

To a solution of **17** (58.3 mg, 0.147 mmol) in 6 ml of THF was added 1.5 ml of NaF-HF buffer (pH 4.98, prepared as reported[7]) under argon at 0°C. The solution was stirred at room temperature for 24 hours. The reaction mixture was extracted with $CHCl_3$ three times. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified on preparative TLC developed with MeOH-$CHCl_3$ (1:5) to give 24.5 mg of **2** (0.147 mmol, quantitative yield) as a brown powder: mp 120~122°C. IR (KBr) ν_{max} 3405, 1607, 1537, 1466, 1368, 1256, 1192, 870, 801 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.32 (2H, br), 7.12 (1H, d, J=2.4 Hz), 6.88~6.95 (2H, m), 3.35 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 147.0, 146.0, 136.8, 116.7, 112.7, 109.1, 32.6. HRFAB-MS (positive): calcd for $C_7H_9N_2O_3$ 169.0613, found 169.0608 $(M+H)^+$.

Physico-chemical Data of Other Dephostatin Analogs

2,4-Dihydroxy-*N*-methyl-*N*-nitrosoaniline (**3**)

Brown powder: mp 116~118°C. IR (KBr) ν_{max} 3347, 1609, 1518, 1503, 1424, 1372, 1244, 1169, 835, 791 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.91 (1H, s), 8.65 (1H, s), 7.14 (1H, d, J=8.3 Hz), 6.59 (1H, d, J=2.4 Hz), 6.47 (1H, dd, J=8.3, 2.4 Hz), 3.33 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 160.3, 153.6, 128.9, 124.1, 108.2, 104.8, 35.4. HRFAB-MS (positive): calcd for $C_7H_9N_2O_3$

169.0613, found 169.0603 $(M+H)^+$.

3,5-Dihydroxy-*N*-methyl-*N*-nitrosoaniline (**4**)

Brown powder: mp 164~166°C. IR (KBr) ν_{max} 3094, 1611, 1526, 1422, 1364, 1275, 1175, 851, 789 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.64 (2H, s), 6.61 (2H, d, J=2.0 Hz), 6.37 (1H, t, J=2.0 Hz), 3.37 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 160.4, 145.6, 102.5, 99.3, 31.7. HRFAB-MS (positive): calcd for $C_7H_9N_2O_3$ 169.0613, found 169.0604 $(M+H)^+$.

2-Hydroxy-*N*-methyl-*N*-nitrosoaniline (**5**)

Brown powder: mp 106~107°C. IR (KBr) ν_{max} 3115, 1603, 1516, 1472, 1418, 1375, 1233, 752 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 7.99 (1H, s), 6.97~7.36 (4H, m), 3.56 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 152.4, 131.8, 131.1, 127.9, 121.3, 118.2, 35.0. HRFAB-MS (positive): calcd for $C_7H_9N_2O_2$ 153.0664, found 153.0676 $(M+H)^+$.

3-Hydroxy-*N*-methyl-*N*-nitrosoaniline (**6**)

Brown powder: mp 125~127°C. IR (KBr) ν_{max} 3206, 1611, 1599, 1472, 1431, 1372, 1246, 868 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.32~7.37 (2H, m), 7.00 (1H, br), 6.90~6.98 (2H, m).*3.50 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 145.1, 131.6, 115.4, 111.5, 107.6, 31.8. HRFAB-MS (positive): calcd for $C_7H_9N_2O_2$ 153.0664, found 153.0666 $(M+H)^+$.

5-Hydroxy-2-methoxy-*N*-methyl-*N*-nitrosoaniline (**7**)

Brown powder: mp 114~116°C. IR (KBr) ν_{max} 3187, 2840, 1520, 1470, 1424, 1364, 1225, 1088, 862, 815 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.94 (3H, br), 5.67 (1H, br), 3.93 (3H, s), 3.40 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.4, 146.2, 131.1, 117.1, 113.9, 113.5, 56.3, 35.7. HRFAB-MS (positive): calcd for $C_8H_{11}N_2O_3$ 183.0770, found 183.0777 $(M+H)^+$.

2,5-Dimethoxy-*N*-methyl-*N*-nitrosoaniline (**8**)

Colorless needles, mp 83~84°C. IR (KBr) ν_{max} 2840, 1515, 1485, 1445, 1390, 1280, 1080, 1045, 1015, 865, 815, 735 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 7.19 (1H, d, J=9.3 Hz), 7.07 (1H, dd, J=9.3, 3.3 Hz), 3.79 (3H, s), 3.76 (3H, s), 3.27 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.6, 146.9, 131.7, 115.2, 113.2, 112.4, 56.3, 55.9, 35.0. FAB-MS (positive): m/z 197 $(M+H)^+$. *Anal.* Calcd for $C_9H_{12}N_2O_3$: C, 55.09, H, 6.16, N, 14.28. Found: C, 55.31, H, 6.16, N, 14.31.

2-(*N*-Methyl-*N*-nitrosoamino)-1,4-benzenedicarboxylic acid (**9**)

Yellow powder: mp 210~212°C. IR (KBr) ν_{max} 3065, 2813, 2618, 1728, 1701, 1503, 1439, 1422, 1258 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.16 (1H, dd, J=8.3, 1.5 Hz), 8.05~8.08 (2H, m), 3.42 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 167.7, 167.1, 142.8, 136.1, 133.1, 132.5, 130.7, 128.0, 35.8. HRFAB-MS (negative): calcd for $C_9H_7N_2O_5$ 223.0355, found 223.0346 $(M-2H)^-$.

2,5-Dihydroxy-*N*-methyl-*N*-formylaniline (**10a**)

Brown amorphous. IR (KBr) ν_{max} 3300, 1659, 1609, 1514, 1460, 1366, 1196, 1090, 1007, 816, 779 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.13 (3H, br), 6.85 (1H, d, J=8.8 Hz), 6.69 (1H, dd, J=8.8, 2.9 Hz), 6.65 (1H, d, J=2.9 Hz), 3.12 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 163.8, 151.6, 146.3, 130.5, 118.2, 116.1, 115.1, 32.4. HRFAB-MS (positive): calcd for $C_8H_{10}NO_3$ 168.0661, found 168.0669 $(M+H)^+$.

2,5-Dihydroxy-*N*-methyl-*N*-acetylaniline (**10b**)

White powder: mp 201~204°C. IR (KBr) ν_{max} 3355, 3264, 1644, 1524, 1456, 1252, 816, 787 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.15 (2H, br), 6.86 (1H, d, J=8.8 Hz), 6.73 (1H, dd, J=8.8, 2.9 Hz), 6.69 (1H, d, J=2.9 Hz), 3.09 (3H, s), 1.77 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 170.5, 151.7, 146.7, 132.7, 118.1, 116.7, 115.9, 35.6, 21.8. HRFAB-MS (positive): calcd for $C_9H_{12}NO_3$ 182.0817, found 182.0828 $(M+H)^+$.

2,5-Dihydroxy-*N*-methyl-*N*-trifluoroacetylaniline (**10c**)

Brown powder: mp 158~160°C. IR (KBr) ν_{max} 3401, 1688, 1518, 1458, 1341, 1244, 1215, 1198, 1161, 823, 783 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.23 (2H, br), 6.76~6.87 (3H, m), 3.09 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 158.1 (q, J=34.9 Hz), 151.7, 147.6, 129.2, 118.4, 118.3, 117.9 (q, J=288.7 Hz), 116.9, 38.5. HRFAB-MS (positive): calcd for $C_9H_9F_3NO_3$ 236.0535, found 236.0531 $(M+H)^+$.

2,5-Dihydroxy-*N*-methyl-*N*-methanesulfonylaniline (**10d**)

White powder: mp 116~118°C. IR (KBr) ν_{max} 3476, 1510, 1316, 1201, 1145, 820, 787 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.01 (2H, br), 6.81 (1H, d, J=8.3 Hz), 6.81 (1H, d, J=2.9 Hz), 6.71 (1H, dd, J=8.3, 2.9 Hz), 3.23, (3H, s), 2.96 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 151.4, 148.0, 129.3, 118.0, 117.8, 117.1, 38.2, 37.3. FABHR-MS (positive): calcd for $C_8H_{12}NO_4S$ 218.0487, found 218.0497 $(M+H)^+$.

2,5-Dihydroxy-*N*-methyl-*N*-trifluoromethanesulfonylaniline (**10e**)

White powder: mp 131~133°C. IR (KBr) ν_{max} 3476, 3320, 1518, 1379, 1325, 1227, 1194, 970, 820 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ 8.10~8.70 (2H, br), 6.76~6.88 (3H, m), 3.41, (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ 151.6, 148.5, 127.3, 121.8 (q, J=324.5 Hz), 118.8, 118.5, 118.0, 40.2. HRFAB-MS (positive): calcd for $C_8H_8F_3NO_4S$ 271.0126, found 271.0123 $(M+H)^+$.

Acknowledgments

We thank Dr. H. NAGANAWA and Dr. R. SAWA, Institute of Microbial Chemistry, for valuable suggestions on structure determination.

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