Sung-Hwa YOON*,

highly reactive peroxynitrite anions (ONOO−) are produced by cells when reacting with superoxide anions to produce nitric oxide and structural proteins. Therefore, the high level of cytokines and structural proteins associated with inflammation and injury is widely known to be the hallmark of brain inflammation. Once activated, microglia release several inflammatory mediators such as reactive oxygen intermediates, nitric oxide (NO), and tumor necrosis factor α (TNF-α), as well as neurotoxic substances. These mediators are thought to be accountable for brain diseases including ischemia, AD, and neuronal death. Therefore, regulating microglial activation or suppressing these proinflammatory mediators could be a potential therapeutic approach to reducing the progression of these neurodegenerative diseases.

Although intrinsic molecules of microglia activation have not yet identified, experimental inducers of microglia, including lipopolysaccharide (LPS), amylloid-β and IFN-γ, are known. LPS, an outer-membrane component of gram-negative bacteria, is induced in inflammatory responses such as activated macrophages and microglia, producing TNF-α, IL-1β and NO, and is involved in immune responses and host defense. Among these chemicals, NO produced by inducible NO synthase (iNOS) can act as a cytotoxic agent to the cells by reacting with superoxide anions to produce highly reactive peroxynitrite anions (ONOO−) in pathophysiological state, leading oxidative damage in host tissue, modification of DNA bases, and disruption of enzyme function and structural proteins. Therefore, the high level of NO produced by iNOS has been considered an indicator of cytotoxicity in inflammation, NO production is a critical step in numerous neurodegenerative disease, and reduced NO production is a promising therapeutic strategy for the reduction of neuronal cell injury or death in various neuroinflammatory and neurodegenerative diseases.

Fluoxetine, the selective serotonin reuptake inhibitor that is widely used in clinical practice for the treatment of depression, obsessive-compulsive disorder, bulimia and panic disorder, has been shown to exert antioxidant potential via reversal of oxidative damage by enhancing the in vivo antioxidant defenses and improving the cellular antioxidant status following a stress-induced decline. Fluoxetine has been found to have beneficial effects in animal models of stroke, MS, and epilepsy, even though fluoxetine has no positive effect in clinical studies with Parkinson’s disease, AD and Huntington’s disease. Recently, we reported that fluoxetine exerts an anti-inflammatory effect by inhibiting nuclear factor-κB (NF-κB) activity in the postischemic brain and NO in LPS-treated primary microglia.

In our ongoing efforts to find a potent inhibitor of NO production, a series of fluoxetine derivatives and analogues, where the N-methylamino group in fluoxetine was replaced by various heterocyclic amines, and then the trifluoromethylphenyl ring was simplified, were investigated in this study for their inhibitory activities on NO production and iNOS expression on BV2 cells stimulated with LPS.

MATERIALS AND METHODS

**Instruments** Melting points were determined on a Fisher–Johns melting point apparatus and were left uncorrected. 1H- and 13C-NMR spectra were recorded on a Varian Gemini 400 spectrometer at 400 MHz and 100 MHz, respectively. The chemical shifts given are relative to tetramethylsilane. Infrared spectra were recorded on a Nicolet 6700 Fourier transform (FT)-IR spectrometer. Electrospray ionization (ESI)-MS spectra were obtained by Shimadzu LCMS-2010EV (Japan). Column chromatography was carried out using Merck silica gel 60 (230—400 mesh) (U.S.A.). All reactions were performed under a nitrogen atmosphere.

**3-Chloro-1-phenylpropan-1-ol (3)** To a solution of 3-
chloro-1-propylophenone 2 (25.0 g, 148 mmol) in methanol (300 ml) was added sodium borohydride (16.8 g, 445 mmol). The mixture was stirred for 24 h at room temperature. After the reaction was complete, the solution was quenched with sodium dihydrogen phosphate (66.7 g, 556 mmol). The solvent was removed by rotary evaporator and the residue was extracted with hexane and the organic layer was washed with water, dried over Na₂SO₄ and concentrated in vacuo to give the title compound as a colorless oil (24 g, 95%). IR (KBr) cm⁻¹: 3235. ¹H-NMR (CDCl₃) δ: 1.79 (IH, s), 2.80 (1H, m), 3.03 (1H, m), 4.20 (1H, d, J = 5.6 Hz), 6.70 (1H, d, J = 8.8 Hz), 6.81 (1H, m), 6.85 (1H, s), 7.10 (1H, d, J = 6.0 Hz), 7.57 (2H, m). ¹³C-NMR (CDCl₃) δ: 23.95, 25.70, 33.50, 54.30, 57.33, 74.99, 125.18, 126.44, 127.78, 144.66.

3-Morpholino-1-phenylpropan-1-ol (5d) Morpholine (1.67 ml, 19.1 mmol) and 3-iodo-1-phenylpropan-1-ol 4 (1.0 g, 3.82 mmol) were subjected to the same reaction described for the synthesis of the compound 5a to give the title compound as a pale yellow solid (450 mg, 53%). mp: 60°C. IR (KBr) cm⁻¹: 3162. ¹H-NMR (CDCl₃) δ: 1.85 (2H, m), 2.47 (2H, s), 2.60 (4H, m), 3.72 (4H, m), 4.90 (1H, t, J = 4.8 Hz), 7.22 (1H, m), 7.32 (4H, m). ¹³C-NMR (CDCl₃) δ: 33.41, 53.52, 57.33, 66.77, 75.19, 125.22, 126.71, 129.32, 144.41. MS m/z: 222.00 ([M+H]⁺).

3-(4-Methylpiperazin-1-yl)-1-phenylpropan-1-ol (5e) 1-Methylpiperazine (2.12 ml, 19.1 mmol) and 3-iodo-1-phenylpropan-1-ol 4 (1.00 g, 3.82 mmol) were subjected to the same reaction described for the synthesis of the compound 5a to give the title compound as a white solid (450 mg, 53%). mp: 60 °C. IR (KBr) cm⁻¹: 3141, 2948, 2866. ¹H-NMR (CDCl₃) δ: 1.71 (2H, m), 2.13 (3H, s), 2.33 (4H, m), 2.48 (4H, m), 4.70 (1H, t, J = 5.6 Hz), 7.08 (1H, m), 7.20 (4H, m). ¹³C-NMR (CDCl₃) δ: 33.79, 45.27, 52.30, 54.35, 55.89, 73.91, 124.88, 126.18, 127.43, 144.40.

tert-Butyl 4-(3-Hydroxy-3-phenylpropyl)piperazine-1-carboxylate (5f) Piperazine (5.92 g, 68.7 mmol) and 3-iodo-1-phenylpropan-1-ol 1 (3.00 g, 11.4 mmol) were subjected to the same reaction described for the synthesis of the compound 5a. To a solution of crude product in THF (10 ml) was added sodium bicarbonate (3.81 g, 45.4 mmol) and di-tert-butyl dicarbonate (2.38 g, 10.9 mmol) in H₂O/THF (1/1, 60 ml). The solution was stirred for 1 h at room temperature. The mixture was extracted with ethyl acetate, washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (EA/HX, 1/1) to give the title compound as a colorless oil (1 g, 27%). IR (KBr) cm⁻¹: 3120, 1686. ¹H-NMR (CDCl₃) δ: 1.46 (9H, s), 1.80 (2H, m), 2.03 (3H, m), 2.22 (2H, m), 3.65 (4H, m). ¹³C-NMR (CDCl₃) δ: 28.47, 33.74, 53.06, 57.12, 75.19, 79.71, 125.33, 126.83, 128.09, 144.46, 154.37.

1-(3-Phenyl-1-(4-(trifluoromethyl)phenoxy)propyl)-pyrrolidine (6a) To a solution of NaH (250 mg, 10.0 mmol) in N,N-dimethylethlyanide (DMA) (10 ml) was added 1-phenyl-3-(pyrrolidin-1-yl)propan-1-ol 5a (410 mg, 2.00 mmol). The mixture was stirred at 70 °C for 30 min. p-Chlorobenzotrifluoride (1.34 ml, 10.0 mmol) was added and the mixture was heated to 100 °C for 2 h. After being cooled to room temperature, the mixture was quenched with 1 N NaOH solution (10 ml) and extracted with tolune. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (EA/HX, 1/1) to give the title compound as a pale yellow oil (200 mg, 29%). ¹H-NMR (CDCl₃) δ: 1.43 (2H, m), 1.60 (4H, m), 2.05 (1H, m), 2.22 (2H, m), 2.43 (4H, s), 2.68 (1H, q, J = 4.8 Hz), 6.88 (2H, d, J = 8.8 Hz), 7.22 (1H, m), 7.19 (4H, m), 7.36 (2H, d, J = 8.8 Hz), 13C-NMR (CDCl₃) δ: 23.52, 34.32, 52.43, 35.66, 54.40, 110.60, 126.64 (δC₆H₅ = 31.9 Hz), 125.55, 126.65, 126.68, 128.27, 128.87, 138.60, 159.36. MS m/z: 350.15 ([M+H]⁺).

1-(3-Phenyl-3-(4-(trifluoromethyl)phenoxy)propyl)-1H-imidazole (6b) Sodium hydride (178 mg, 7.42 mmol) and 3-
The crude oil was dissolved in 1,4-dioxane (5 ml) and 4 N HCl in 1,4-dioxane (30 ml) added. This solution was stirred for 3 h at room temperature. After the solution was evaporated in vacuo, the residue was dissolved in ethyl acetate and then basified with 1 N NaOH solution. The organic layer was washed with brine, dried over Na2SO4 and concentrated in vacuo to give the title compound as a yellow oil (500 mg, 44%). 1H-NMR (CDCl3) δ: 1.84 (1H, s), 2.00 (1H, m), 2.20 (1H, m), 2.45 (6H, m), 2.90 (4H, m), 5.27 (1H, q, J = 5.2 Hz), 6.89 (2H, d, J = 8.8 Hz), 7.23 (1H, m), 7.32 (4H, m), 7.41 (2H, d, J = 8.4 Hz). 13C-NMR (CDCl3) δ: 34.55, 46.05, 54.46, 54.98, 74.88, 115.49, 122.18 (J C-6 = 31.8 Hz), 122.48, 126.42, 126.46, 127.53, 128.45, 140.77, 160.30. MS m/z: 364.90 ([M+H]+).

3-Iodo-1-phenylprop-1-one (9a) To a solution of 3-chloro-1-phenylpropan-2-one (5.00 g, 29.7 mmol) in acetonitrile (100 ml) was added sodium iodide (7.56 g, 50.4 mmol). The mixture was heated to reflux temperature for 4 h. After the solution was cooled to room temperature, the solvent was evaporated in vacuo. The residue was extracted with diethyl ether, the organic layer was washed with brine, dried over Na2SO4 and evaporated in vacuo to provide the title compound as a yellow solid (4.14 g, 54%). mp: 56 °C. IR (KBr) cm⁻¹: 1675. 1H-NMR (CDCl3) δ: 3.32 (2H, t, J = 6.4 Hz), 3.48 (2H, t, J = 7.6 Hz), 7.34 (2H, m), 7.44 (1H, m), 7.79 (2H, m). 13C-NMR (CDCl3) δ: 3.58, 42.31, 127.62, 128.36, 133.13, 135.63, 196.90.

2-Bromo-1-phenylethanone (9b) To a solution of triphenylphosphine (2.89 g, 11.1 mmol) and carbon tetrabromide (3.69 g, 11.1 mmol) in acetonitrile (10 ml) and dichloromethane (15 ml) at 0 °C was added 2-hydroxyacetophenone (750 mg, 3.67 mmol) in acetonitrile (2 ml). After being stirred for 15 min, the mixture was filtered off and the residue was concentrated in vacuo. The crude product was purified by silica column chromatography (EA/HX, 1/3) to give the title compound as a yellow oil (200 mg, 73%). IR (KBr) cm⁻¹: 1680. 1H-NMR (CDCl3) δ: 4.37 (2H, s), 3.79 (2H, t, J = 7.6 Hz), 7.51 (1H, t, J = 7.6 Hz), 7.87 (2H, m). 13C-NMR (CDCl3) δ: 31.06, 128.68, 128.72, 133.76, 190.93.

4-Iodo-1-phenylbutan-1-one (9c) To a solution of 4-chloro-1-phenylbutan-1-one (1.00 g, 5.47 mmol) in acetone (30 ml) was added sodium iodide (1.22 g, 8.21 mmol). The mixture was heated to reflux temperature for 18 h. After the solution was cooled to room temperature, the solvent was evaporated in vacuo. The residue was extracted with diethyl ether, the organic layer was washed with brine, dried over Na2SO4 and evaporated in vacuo to provide the title compound as a yellow solid (800 mg, 80%). IR (KBr) cm⁻¹: 1693. 1H-NMR (CDCl3) δ: 2.19 (2H, q, J = 6.4 Hz), 3.06 (2H, t, J = 6.8 Hz), 3.25 (2H, t, J = 6.8 Hz), 7.38 (2H, m), 7.48 (1H, m), 7.88 (2H, m). 13C-NMR (CDCl3) δ: 6.97, 27.61, 38.99, 127.87, 128.51, 133.08, 136.53, 198.29.

3-Morpholin-1-phenylpropan-1-one (10a) Morpholine (8.40 ml, 96.0 mmol) and 3-iodo-1-phenylpropan-1-one (9a) (500 mg, 1.92 mmol) were subjected to the same reaction described for the synthesis of the compound 5a to give the title compound as a yellow oil (350 mg, 83%). The title compound was previously synthesized from dimethylaminopropionophenone as a hydrochloride salt (mp²) = 175—177 °C. IR (KBr) cm⁻¹: 1685. 1H-NMR (CDCl3) δ: 2.43 (4H, t, J = 4.4 Hz), 2.75 (2H, t, J = 7.2 Hz), 3.10 (2H, t, J = 7.6 Hz), 3.63 (4H, t, J = 4.8 Hz), 3.73 (2H, t, J = 8.0 Hz), 7.47 (1H, t, J = 7.2 Hz), 7.86 (2H, m). 13C-NMR (CDCl3) δ: 35.98, 53.51, 53.66, 66.84, 127.81, 128.44, 132.95, 136.63, 198.57. MS
m/z: 220.00 ([M + H]^+) *.  

2-Morpholino-1-phenylethanone (10b) Morpholine (439 µl, 5.00 mmol) and 2-bromo-1-phenylethanone 9b (200 mg, 1.00 mmol) were subjected to the same reaction described for the synthesis of the compound 5a to give the title compound as a colorless oil (500 mg, 25%). IR (KBr) cm⁻¹: 1680. ¹H-NMR (CDCl₃) δ: 2.62 (4H, m), 3.78 (4H, m), 3.83 (2H, s), 7.45 (2H, m), 7.56 (1H, m), 7.97 (2H, m). ¹³C-NMR (CDCl₃) δ: 53.88, 64.64, 66.78, 127.90, 128.44, 133.19. MS m/z: 206.00 ([M + H]^+).  

4-Morpholino-1-phenylbutan-1-one (10e) Morpholine (1.12 ml, 12.8 mmol) and 4-iodo-1-phenylbutan-1-one 9c (0.70 g, 2.55 mmol) were subjected to the same reaction described for the synthesis of the compound 5a to give the title compound as a white powder (30 mg, 10%). mp: 143 °C.  

Preparative Thin-Layer Chromatography (dichloromethane/methanol, 20/1) to give the crude products of corresponding amine-propanols 6a—f. 

- 4-Morpholino-1-phenylpropan-1-one oxime (11a) 3-Morpholino-1-phenylpropan-1-one 10a (500 mg, 1.60 mmol) in ethanol (10 ml) was added hydroxylamine hydrochloride (0.5% sulfanilamide and 0.05% N-1-naphthylethenediamine, and incubated for 10 min at room temperature. The absorbance of the mixture at 550 nm was measured using a microplate reader. ²⁴  

- Cell Viability Cell respiration, an indicator of cell viability, was determined by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoium (MTT; Sigma-Aldrich Corp., U.S.A.) to formazan. MTT solution (50 µl, 0.5 mg/ml) was added to BV2 cell cultures. The conversion of MTT to formazan by metabolically visible cells was measured with a microplate reader at 595 nm. ²¹  

- Reverse Transcription-Polymerase Chain Reaction Total RNA was prepared with Trizol reagent (Gibco BRL, U.S.A.), and 1-µg RNA samples were used for cDNA synthesis using RT-PCR kit (Roche, Germany). The primer sequences of rat inducible NO synthase (iNOS) was described previously. ²³  

- Immunoblotting BV2 cells were washed twice with cold phosphate buffered saline (PBS) and lysed in RIPA buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40, 0.25% sodium-deoxycholate, 150 mM NaCl, 1 mM Na₂VO₃, 5% FBS (Hyclone, U.S.A.), and 1-mg/ml Penicillin–streptomycin (Gibco, U.S.A.). ²²  

RESULUTS AND DISCUSSION  

- Chemistry of Fluoxetine Analogues The syntheses of the heterocyclic analogues of fluoxetine started from commercially available 3-chloropropiophenone 2, as depicted in Chart 1. Since racemic fluoxetine is used therapeutically, all compounds were synthesized as a racemic mixture. Chloropronone 2 was first reduced to chloropropanol 3 using 1 eq of NaBH₄ in methanol at room temperature for 24 h in excellent yield (95%). The chloropropanol 3 was converted to isopropanol 4 using NaI with acetone via a Finkelstein reaction in 97% yield and the completion of the reaction was judged by the ¹H-NMR spectrum. ²⁵ Reactions of isopropanol 4 with various heterocyclic amines produced the crude products of corresponding amine-propanols 5a—f containing excess amines, which were purified by chromatography on silica gel with dichloromethane/methanol (v/v, 15/1) to give pure amine-propanols (5a—f). Finally, the heterocyclic analogues 6a—f were prepared by nucleophilic aromatic substitution of p-chlorobenzotri fluoride with the corresponding alkoxide anions of compounds (5a—f), which were generated by reacting with sodium hydride in DMA. The simplified analogues of fluoxetine, where the trifluo-
romethyl phenyl ring was removed, were synthesized as depicted in Chart 2. Chloropropanone 2 was converted to iodopropanone 9a using NaI in acetone, which was then reacted with excess morpholine at reflux temperature for 4 h to give the crude residue containing product as well as excess morpholine. The desired morpholino-propanone 10a was obtained by chromatography on silica gel with dichloromethane/methanol (v/v, 15/1). The various oxime analogues (11a—c) were prepared by condensation reaction of 10a with corresponding hydroxylamine hydrochlorides.

To investigate the influence of the alkyl chain length in 10a on the activity, two different chain-length analogues (10b, c) were synthesized as depicted in Chart 2, respectively. For the synthesis of 10b, the alcohol 7 was first converted into the bromide 9b using triphenylphosphine (PPh₃) and tetrabromide (CBr₄) via an Appel reaction, and then reacted with excess morpholine to produce the crude product 10b, which was purified by silica gel column chromatography to give pure 10b. Similarly, the crude product 10c was prepared from the conversion of 4-chlorobutanone 8 to its corresponding iodide 9c using NaI in acetone followed by the reaction of 9c with excess morpholine. Pure product 10c was obtained by column chromatography on silica gel.

Biological Effect of Fluoxetine Analogues Since LPS considerably induced NO production in BV2 cells and the level of NO production was detected by nitrite, in this study, fluoxetine and its analogues (6a—f) were first evaluated for inhibitory effects on the production of NO in LPS-induced BV2 cells. After BV2 cells were co-treated with fluoxetine or its analogues at a concentration of 10 μM and LPS (100 ng/ml) for 24 h, the nitrite concentration was determined in the supernatant by the Griess assay, and their results are presented in Table 1. Among the fluoxetine analogues possessing various heterocyclic amine rings (6a—f), 6d, 6e, and 6f showed inhibitory activities (6.00±0.9%, 64.3±6.7% and 79.6±2.3%, respectively) comparable to that of fluoxetine (20.8±0.9%), while the other heterocyclic analogues exhibited no activity at a concentration of 10 μM in NO production.

To determine whether the inhibitory effects of fluoxetine and its analogues on NO production were related to their toxicity, the cytotoxicity of fluoxetine analogues was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay.27) As shown in Fig. 1, the relative cell viability of the BV2 cells treated with fluoxetine at the concentrations of 10 μM, 20 μM and 40 μM was 52.3±3.4%, 73.9±2.2% and 20.1±0.6%, respectively, which indicates that fluoxetine did not suppress the cell viability of the BV2 cell at the concentrations of 10 μM or 20 μM, but did exhibit cytotoxicity at the concentration of 40 μM. This result was consistent with a previous report.22) The relative cell viability

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Table 1. Effects of Fluoxetine Heterocyclic Analogues on LPS-Induced NO Production

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substituent (R₁)</th>
<th>Inhibition of NO release (%) at 10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NHCH₃</td>
<td>20.8±0.9</td>
</tr>
<tr>
<td>6a</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>6c</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>6d</td>
<td></td>
<td>6.00±0.9</td>
</tr>
<tr>
<td>6e</td>
<td></td>
<td>Cytotoxic (64.3±6.7)</td>
</tr>
<tr>
<td>6f</td>
<td></td>
<td>Cytotoxic (79.6±2.3)</td>
</tr>
</tbody>
</table>

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Reagents and conditions: (a) NaI, acetone, reflux, 48 h; (b) CBr₄, PPh₃, ACN/CH₂Cl₂ (1/1), 0 °C, 15 min; (c) NaI, acetone, reflux, 18 h; (d) morpholine, THF, reflux, 4 h; (e) R₁, hydroxylamine hydrochloride, EtOH, pyridine, reflux, 1 h.
of the BV2 cells treated with fluoxetine analogues (6e, f) was also decreased to 32.1 ± 0.7% and 20.2 ± 0.5%, respectively (Fig. 1), which clearly indicated that the inhibitory effect on NO production of the fluoxetine analogues (6e, f) was due to their cytotoxicity rather than their intrinsic activities. In contrast, 6d containing the morpholine moiety did not show any cytotoxicity even at the concentration of 40 μM (data not shown), despite of its weak inhibitory activity in NO production. Due to the absence of any cytotoxic effect, 6d was selected as a lead compound for further modification study.

The structure of 6d was simplified by removing the trifluoromethyl phenyl ring. Various functional moieties such as hydroxyl, ketone and oxime groups were introduced for replacing the ether linkage in fluoxetine while the morpholine moiety was fixed. The simplified compounds were tested for their inhibitory effects in NO production on BV2 cells. As shown in Table 2, none of the tested compounds (5d, 11a—c) exhibited any inhibitory effect in NO production on BV2 cells, except 10a containing a ketone group. 10a revealed almost the same inhibitory activity as that of fluoxetine at the concentration of 10 μM. When we tested the inhibitory activities at different concentrations, 10a reduced the LPS-induced nitrite production in a dose-dependent manner. Accordingly, the percentage of inhibited NO production in 10a was 22.1 ± 4.8, 35.2 ± 3.4 and 83.7 ± 1.4% at the concentrations of 10, 20 and 40 μM, respectively (Table 2).

To confirm that the suppressed effect of 10a on NO production was not caused by cytotoxicity, the cell viability was measured. LPS dramatically increased the concentration of nitrite from the cells (47.1 ± 2.3 μM) whereas a small amount of NO was produced (5.47 ± 1.22 μM) in unstimulated BV2 cells. The relative cell viability of the BV2 cells treated with 10a at the concentrations of 10 μM, 20 μM and 40 μM was 60.2 ± 1.2%, 61.1 ± 1.9% and 68.8 ± 1.6%, respectively (Fig. 1), which indicated that 10a did not exhibit any cytotoxicity even at the concentration of 40 μM, while fluoxetine at the same concentration did. 10a also exhibited NO inhibitory activity in a dose-dependent manner (Fig. 2).

To investigate the influence of the alkyl chain length between the ketone group and the morpholine moiety in 10a on the activity, two analogues (10b, c) were also evaluated for the NO production activities in BV2 cells. Interestingly, these two analogues did not exhibit any effect at a concentration of 10 μM, which implied that the chain length between the oxo group and the heteroamine moiety plays an important role in maintaining the activity.

NO is biosynthesized by NOS, including, for example, endothelial NOS (eNOS), neuronal NOS (nNOS) and iNOS. The iNOS-derived NO is generated from various cells such as macrophages, monocytes and microglial cells and the overproduction of NO is caused by inflammation. Therefore, the reduced NO production is due to the inhibited iNOS expression. To examine whether the NO production was caused by a decrease in the mRNA level of iNOS, LPS-stimulated BV2 cells were treated with 10a and then the amount of iNOS was measured by reverse transcription polymerase chain reaction (RT-PCR). The expression of iNOS mRNA in LPS-treated BV2 cells was significantly increased in comparison with the non-treated control cells. When the LPS-treated BV2 cells were treated with 10a at different concentrations...
human blood platelets. The expression levels of iNOS mRNA in fluoxetine (A) and 10a (B) were determined by RT-PCR. The production of iNOS protein level in fluoxetine and 10a (C) was determined by immunoblotting with antibody iNOS.

(10, 20, 40 μM), the cells were decreased to the density of iNOS mRNA in a dose-dependent manner (Figs. 3A, B). To confirm the effects of 10a on iNOS protein expression, the amount of iNOS protein expression was measured by the immunoblotting method. As shown in Fig. 3C, no iNOS protein was detected in unstimulated BV2 cells, whereas iNOS protein was induced in LPS-stimulated BV2 cells. When the LPS-treated BV2 cells were treated with 10a at different concentrations (10, 20, 40 μM), the densities of iNOS protein in the cells were decreased in a dose-dependent manner. These results indicated that the inhibitory action of 10a on NO production related to a modulation of iNOS induction.

10a was shown to have weak activities against the EMT6 tumor in vitro and against ADP-induced aggregation of human blood platelets in vitro,25,26 while there was no effect in inhibition of edema in the test for anti-inflammatory activity in CF1 mice at 8 mg/kg as a series of β-alkylamino-ketones.30 However, there is no previous report regarding the suppression of NO production by 10a in LPS-induced BV2 cells.

In conclusion, by modifying the structure of fluoxetine, we discovered an analogue of fluoxetine 10a, which inhibited not only NO production but also iNOS expression in LPS-induced BV2 cells without much cytotoxicity. The trifluromethyl phenyl moiety was not essential for the suppression in NO production. The simplified structure of 10a can be used for the further development of potent inhibitors of NO production.

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REFERENCES