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Enantioselective synthesis of (*R*)-(+)- and (*S*)-(−)-higenamine and their analogues with effects on platelet aggregation and experimental animal model of disseminated intravascular coagulation

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ABSTRACT

Optically active tetrahydroisoquinoline alkaloids, (*R*)-(+)-higenamine (**1*R***) and (*S*)-(−)-higenamine (**1 *S***), and their optically active 1-naphthylmethyl analogues (**2** and **3**), were synthesized by enantioselective hydrogenation of the corresponding dihydroisoquinoline intermediates **7** as a key step. The evaluation of the platelet anti-aggregation effect demonstrated clearly that the (*S*)-(−)-enantiomers, **1*S***, **2*S***, and **3*S***, had higher inhibitory potency than the corresponding (*R*)-(+)-antipodes, **1*R***, **2*R***, and **3*R***, respectively, to platelet aggregation induced by epinephrine. **1*S*** enantiomer was superior to the corresponding **1*R*** enantiomer in attenuating all of the disseminated intravascular coagulation (DIC) and multiple organ failure (MOF) parameters tested, while the ***S*** enantiomers **2*S*** and **3*S*** ameliorated some of the DIC and MOF parameters more effectively than the corresponding antipodes **2*R*** and **3*R***.

Higenamine (**1**, CAS 5843-65-2), a benzyltetrahydroisoquinoline alkaloid, was isolated as an optically inert racemic mixture from several plant materials, including Aconite root, Asiasaari radix, *Annona squamosa*, and *Gnetum parvifolium*,[1–5] and as a (*R*)-(+)-enantiomer from the embryo of *Nelumbo nucifera*.[6] Racemic higenamine was reported to possess cardiac β-adrenoceptor stimulating activity, vasodilating and platelet anti-aggregating activities through α-adrenoceptor,[7–9] and to inhibit inducible nitric oxide synthase (iNOS) expression and NO production in RAW 264.7, vascular smooth muscle and other cells.[10–12] Two of the 1-naphthylmethyl analogues of higenamine, 1-(α-naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**2**, YS-49, CAS 213179-96-5) and 1-(β-naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**3**, YS-51, CAS 213179-96-5) were also reported to possess various biological activities either equivalent or superior to higenamine.[7,13–15] In addition, these three tetrahydroisoquinolines[1–3] were reported to have therapeutic potential for disseminated intravascular coagulation (DIC) and/or accompanying multiple organ failure (MOF).[16–18] Tetrahydroisoquinolines involve one asymmetric center in their structure and exist as enantiomers. Although two optically active enantiomers have the same achiral physical properties in general, discrepancies in physiological activities are quite often observed between the two enantiomers. Therefore, the development of a single enantiomeric compound instead of racemic mixtures is strongly encouraged in new drug development these days.

Racemic higenamine (**1**) could be either partially synthesized by demethylation of racemic coclaurine (6-*O*-methylhigenamine) obtained from the natural sources[6] or by total syntheses.[19] Preparation of optically active (*R*)-(+)-higenamine (**1*R***) or (*S*)-(−)-higenamine (**1*S***) was also successful *via* resolution of the racemic norarmepavine (6,7-di-*O*-methylhigenamine) followed by demethylation.[20] However, despite the enormous potential as useful therapeutic candidates and crucial needs to secure the optically active higenamine and its derivatives, general and efficient methods to obtain sufficient amount of enantiomerically pure compounds of **1**,**2**, and **3** have not been reported yet. We report herein the first enantioselective total syntheses of both (*R*)-(+)- and (S)-(−)-enantiomers of higenamine (**1*S*** and **1*R***) and its 1-naphthylmethyl derivatives, YS-49 (**2*S*** and **2*R***) and YS-51 (**3*S*** and **3*R***) as their HBr salts in multi-gram quantities, together with their platelet anti-aggregation effects and the effects on experimental DIC and/or accompanying MOF.

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Synthesis of (*R*)-(+)-higenamine (**1*R***) is summarized in Scheme 1. A mixture of commercially available 3,4-dimethoxyphenethylamine (**4**) and 4-methoxyphenylacetic acid (**5**) was heated at 200 °C for 4 h to provide the amide **6** in 94% yield after recrystallization in ethyl acetate.[21] The amide **6** was subjected to Bischler–Napieralski cyclization ($POCl_3$, $CHCl_3$, reflux, 12 h),[22] and the crude iminium salt was subsequently converted to free imine **7** in 98% overall yield by neutralization reaction using saturated sodium bicarbonate solution at 0 °C. The imine **7** should be used immediately because it turned out to be relatively unstable at room temperature.

Catalytic asymmetric reduction of **7**, a key step in this route, was carried out in DMF–HCO_2H–TEA at rt for 12 h, using Noyori's RuCl[(*S,S*)-*TsDPEN](p*-cymene)] (**8*S***) (1 mol%) as a catalyst.[23] The reaction proceeded smoothly to give a product **9** along with a significant amount of greenish by-product, which interfered the purification of the desired secondary amine **9** in large scale reaction. Fortunately, purification of the free amine **9** was successfully carried out by a combination of acid–base reaction during the work-up procedure, precipitation as its hydrobromide salt, and neutralization reaction in 62% yield with >99% ee by HPLC analysis.[24] The purified amine **9** turned out to be rather unstable at room temperature unless it was very pure and therefore converted into its hydrobromide salt **10** immediately by treatment with 48% HBr–AcOH solution at rt in 84% yield.[24] The salt **10** was then subjected to BBr_3 in methylene chloride at 0 °C for 3 h to afford the optically active (*R*)-(+)-higenamine (**1*R***) (78%) in excellent purity (>98%) and enantioselectivity (>99% ee).[25]

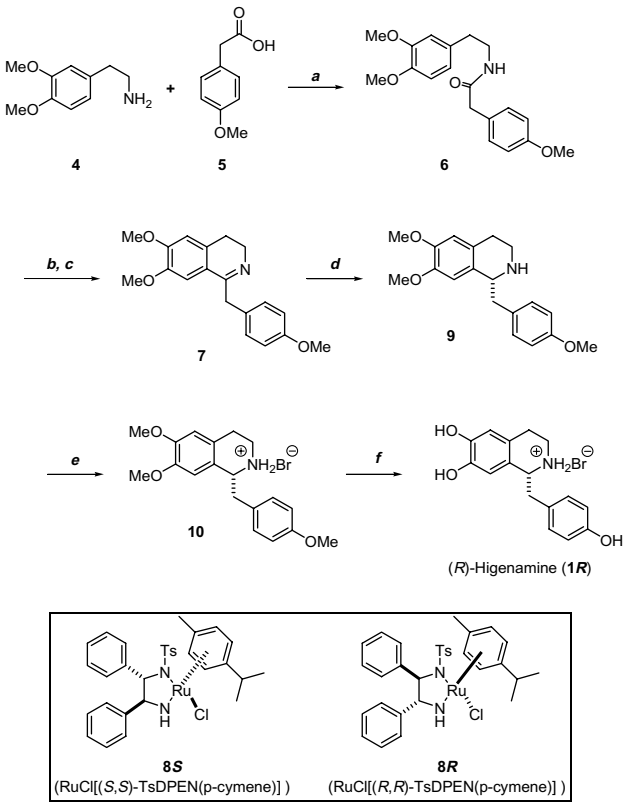
(*S*)-(−)-Higenamine (**1*S***), the antipode of **1*R***, was prepared following the same protocol as the synthesis of **1*R***, except that (*R,R*)-Noyori catalyst (**8*R***) was employed in place of (*S,S*)-catalyst (**8*S***) in reaction (d) of Scheme 1 (Scheme 2).

Other optically active higenamine derivatives such as (*R*)-(+)-YS-49 (**2*R***), (*S*)-(−)-YS-49 (**2*S***), (*R*)-(+)-YS-51 (**3*R***) and (*S*)-(−)-YS-51 (**3*S***) were also synthesized in a similar manner with >98% ee in all cases (Scheme 3), starting from the commercially available 1-naphthylacetic acid (**11**) and 2-naphthylacetic acids (**12**), respectively.[26,27]

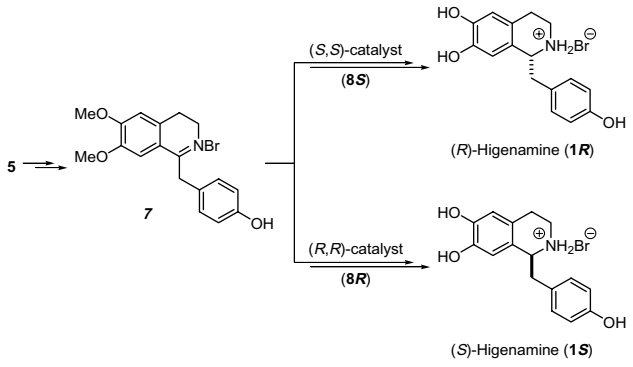
The yields in each step of the synthesis of (*R*)-higenamine (**1*R***), (*R*)-YS-49 (**2*R***), and (*R*)-YS-51 (**3*R***) are summarized in Table 1. In general, amide condensation, Bischler–Napieralski cyclization, and neutralization reaction proceeded smoothly in all cases (at least >85% yield in entries 1–3). And chiral reduction, salt formation, and final demethylation reaction also occurred in moderate yields of 61–89% (entries 4–6).

Platelet anti-aggregating activities of six enantiomers were then measured as follows. Blood, collected from the rat heart after surgery using syringe containing 0.1 volume of 2.2% sodium citrate, was centrifuged at 200*g* for 10 min to obtain platelet rich plasma (PRP). The supernatant PRP was diluted with saline to adjust the number of platelets (400–450 × 10^6 platelets/ml) with the aid of platelet counter. Platelet poor plasma (PPP) was prepared from the residue by centrifugation at 900*g* for 30 more minutes. After 3-min pre-incubation of the adjusted PRP, sample or vehicle was added and an aggregation inducing agent [ADP (2–5 μM) or collagen (2–5 μg/ml)] was added at 1 min after the sample addition on platelet aggregometer.[28] Epinephrine (1–4 μM), arachidonic acid (AA, 10–40 μM), and U46619 (1–5 μM) induced rat platelet aggregation were measured in the presence of the threshold concentration of collagen (0.5–1.0 μg/ml), since rat platelets were observed not to aggregate in response to epinephrine, AA, or U46619 (PGH_2/TXA_2 receptor agonist) alone in the concentration dependent manner.[29] The concentrations of the compounds causing 50% inhibitory effects (IC_{50}) were determined from the Regression Wizard from the SigmaPlot equation library.

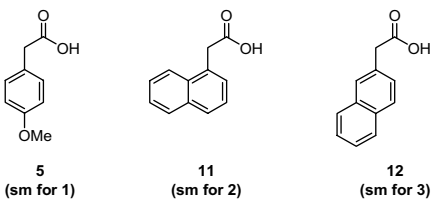
Effects of these enantiomers on LPS-induced DIC model in the rat were evaluated as follows. Six enantiomers suspended in dis-



Scheme 1. Synthesis of (*R*)-(+)-higenamine (**1*R***). Reagents and conditions: (a) 200 °C, neat, 4 h, 94%; (b) $POCl_3$, $CHCl_3$, reflux, 12 h; (c) saturated $NaHCO_3$, 0 °C, 98% overall yield; (d) (i) RuCl[(*S,S*)-*TsDPEN*(*p*-cymene)] (**8*S***) (1 mol%), HCO_2H/TEA = 5:2, DMF, rt, 12 h; (ii) purification, 62%; (e) 48% HBr, AcOH, rt, 84%; (f) CH_2Cl_2, BBr_3, −78 to 0 °C, 3 h, 78%.



Scheme 2. Concepts in the synthesis of **1*R***, and **1*S***.



Scheme 3. Starting materials for the synthesis of **1**, **2** and **3**.

Table 1
Yields (%) in each step for the route to (*R*)-higenamine (**1*R***), (*R*)-YS-49 (**2*R***), and (*R*)-YS-51 (**3*R***)

Entry	Reaction types	Higenamine (**1**)	YS-49 (**2**)	YS-51 (**3**)
1	Amide condensation (a)	97	92	92
2	Bischler–Napieralski cyclization (b)	98	94	99
3	Neutralization (c)	85	96	88
4	Chiral reduction[a] (d)	65	70	71
5	Salt formation (e)	70	70	70
6	Deprotection[a] (f)	75	61	89

[a] The purities of the isolated compounds were >98% pure and >99% ee in all cases as determined by chiral HPLC analysis.

Table 2
IC_{50} (μM) values for platelet anti-aggregating activities of each enantiomer of higenamine (**1**), YS-49 (**2**) and YS-51 (**3**) in rat platelets

Sample	ADP[a]	Collagen[b]	Epinephrine[c,f]	NaAA[d,f]	U46619[e,f]
ASA[g]	>1000	420	53	66	340
1*R*	>1000	300	14	11	15
1*S*	>1000	450	1.6	9.1	25
2*R*	540	140	2.4	11	20
2*S*	270	69	0.38	5.6	16
3*R*	540	150	15	7.9	49
3*S*	460	150	0.9	6.2	61

[a] ADP $2–5 \times 10^{-6}$ M.
[b] Collagen $2–5 \times 10^{-6}$ g/ml.
[c] Epinephrine $1–4 \times 10^{-6}$ M.
[d] NaAA $1–4 \times 10^{-5}$ M.
[e] U46619 $1–5 \times 10^{-6}$ M.
[f] With the threshold.

tilled water were administered orally to rats (Sprague–Dawley, male, 230–270 g) once a day for two consecutive days at the dose of 10 mg or 25 mg/10 ml/day after fasting 2 h. At 1 h after the second oral administration of vehicle (for normal and LPS groups) or test sample, rats were anesthetized with ketamine (250 mg/kg, im). After 30 min, physiological saline (10 ml/kg for normal group) or LPS (20 mg/10 ml of saline/kg) was continuously infused into the tail vein for 3 h through the scalp vein set (needle size; 25 G × 19 mm) with the aid of a syringe pump (KASP 005/150MT, Keun-A Mechatronics, Korea). Blood from the abdominal aorta was collected either in a glass tube containing soy bean trypsin inhibitor and Bothrops atrox venom for the measurement of fibrinogen/fibrin degradation product (FDP, Murex Diagnostics, England) or in a plastic tube containing 2.2% sodium citrate (1/10, v/v). After centrifugation of the coagulated blood at 1200g for 5 min twice, the supernatant serum was separated and stored in the freezer for at least 12 h before FDP testing. The citrated blood was centrifuged at 2000g for 30 min and the supernatant plasma was used for the measurement of prothrombin time (PT), activated partial thrombin time (aPTT), and fibrinogen level (Sigma Chem. Co., USA). A part (0.4 ml) of the plasma was centrifuged with 100 mM $CaCl_2$ (0.1 ml) to obtain serum for the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen (BUN), and creatinine levels. Platelet count was measured from the citrated whole blood using an automatic platelet counter (PLT-4, Texas International Lab). The PT and aPTT time were measured on a Beckton–Dickinson BBL fibrosystem (Coctkeysville, USA) according to the specifications set by the manufacturer. Thrombo-wellcotest kit was used for FDP assay. The assay was performed semi-quantitatively, with the up and down dilution method according to the procedure in the kit. AST, ALT, BUN, and creatinine levels were determined according to the specifications set by the manufacturer with the use of an Autobiochemical analyzer (HITACH 747, Japan) in the Green Cross Reference Laboratory. The experimental data were expressed as means ± standard error of the mean (SEM). The results obtained from the experiments were analyzed with Turkey's honesty significant difference test for multiple comparisons to make the paired comparisons between groups after the analysis of one-way ANOVA test. P values less than 0.05 were considered as significant.

The anti-platelet activity of each enantiomer (**1*R***, **1*S***, **2*R***, **2*S***, **3*R***, and **3*S***) was summarized in Table 2. All the compounds showed dose-dependent inhibitory activities to ADP, collagen, epinephrine, AA, and U46619 induced platelet aggregation. Both R-(+)- and S-(−)-enantiomers of **1**, **2**, and **3** showed the same order of inhibitory effects on ADP, collagen, AA, and U46619 induced aggregation. However, **1*S*** and **2*S*** isomers were approximately 10-fold stronger inhibitors than the corresponding **1*R*** and **2*R*** isomers, respectively, and **3*S*** isomer was about 20 times more active than the **3*R*** antipode on epinephrine-induced aggregation, suggesting that these enantiomers, at least partly, differentially interfere with the adrenergic α-receptors.

Lipopolysaccharide (LPS, endotoxin), an integral component of outer membrane of Gram-negative bacteria, has been considered one of the major pathogenic factors contributing to symptoms of septicemia and sepsis-related DIC and MOF. The iv infusion of LPS to animals induces human DIC-like symptoms and has been established as an experimental animal DIC model.[30–32] The activation of blood coagulation system results in lowering of blood platelet count and fibrinogen level, enhancing FDP concentration and the prolongation of coagulation time. In the LPS-treated group of rats (LPS control), most of parameters related to DIC deviated significantly from those of the normal group of rats as shown in Figure 1. The oral administration of each enantiomer (**1*R***, **1*S***, **2*R***, **2*S***, **3*R***, and **3*S***) significantly ameliorated the increase of FDP and BUN level, and prolongation of PT and aPTT time induced by the iv infusion of LPS with showing more favorable effects with ***S*** enantiomer than with corresponding ***R*** enantiomer. The decrease of platelet counts was also suppressed with **1*S***, **2*R***, **3*R***, and **3*S***. The prevention of LPS-induced decrease of fibrinogen level was observed only with **1*S*** and **3*S***. The LPS-induced AST increase was suppressed by **1*R***, **1*S***, **3*R***, and **3*S***. No significant differences were observed in ALT and creatinine levels among normal, LPS control, and all of the compounds treated groups of rats. The overall effects showed that **1*S*** enantiomer was superior to the corresponding **1*R*** enantiomer while the enantiomers of **2** and **3** did not show significant differences, although the ***S*** enantiomers **2*S*** and **3*S*** attenuated some DIC and MOF parameters more effectively than the corresponding antipodes **2*R*** and **3*R*** (Fig. 2).

In summary, enantioselective synthesis of optically active **1*R***, **1*S***, **2*R***, **2*S***, **3*R***, and **3*S*** were completed in multi-gram quantities using Noyori's asymmetric hydrogenation protocol as a key step in 27–37% overall yields, respectively. The (*S*)-(−)-enantiomers, **1*S***, **2*S*** and **3*S***, showed selectively higher inhibitory potency than the (*R*)-(+)-enantiomers, **1*R***, **2*R***, and **3*R*** in platelet anti-aggregating activities induced by epinephrine. **1*S*** enantiomer was superior to

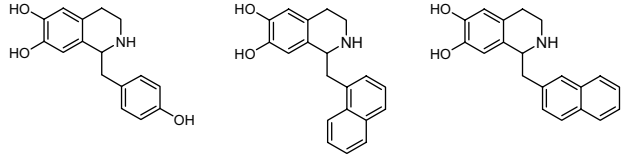


Figure 1. Higenamine (**1**) and its derivatives (**2**, **3**).

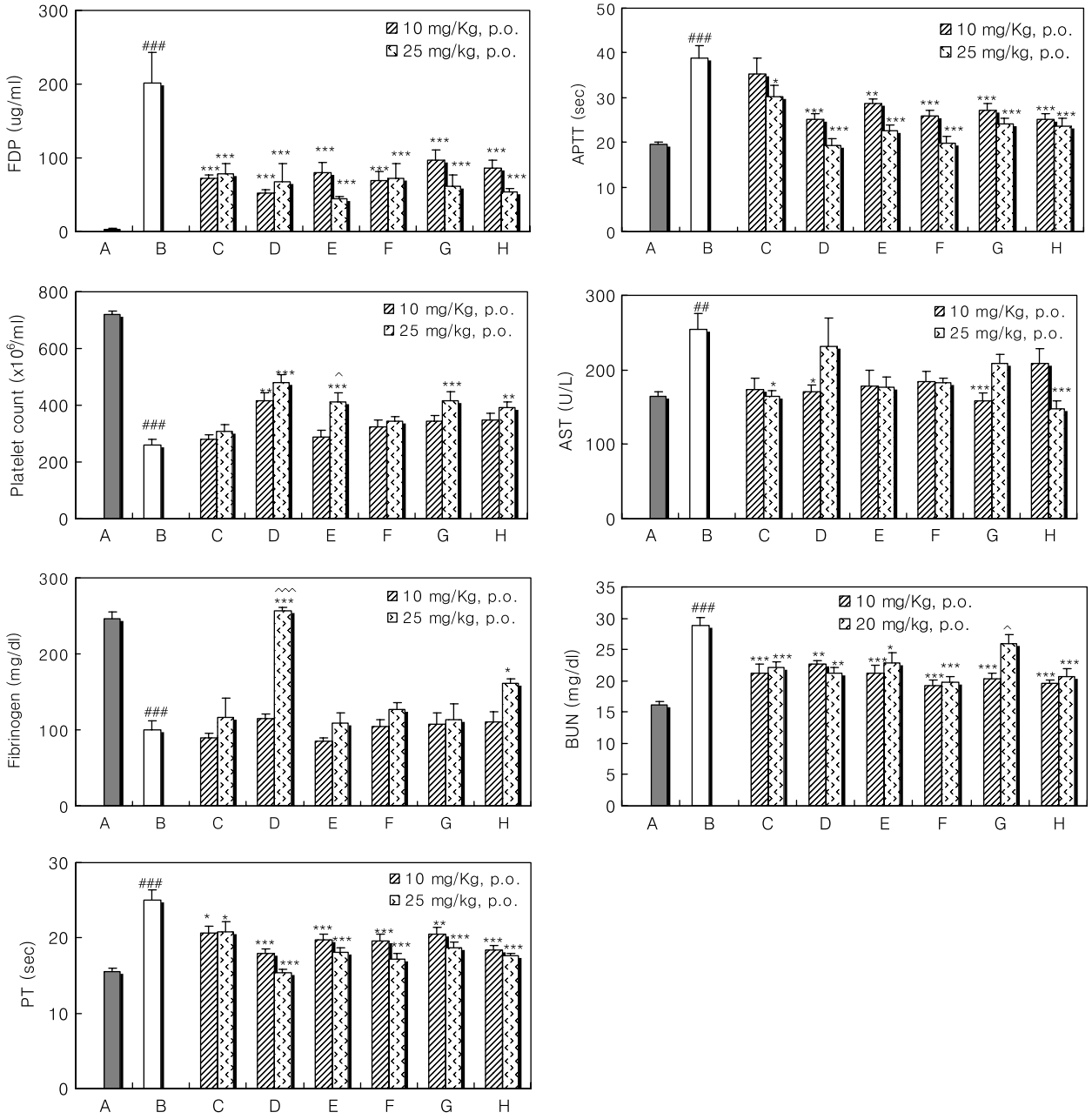


Figure 2. Effects of each enantiomer of tetrahydroisoquinoline alkaloids (po) on LPS (20 mg/kg, 3 h iv infusion) induced DIC model in the rat. Each column represents the relative mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significantly different from the control group (LPS group), $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, significantly different from the normal group, $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$, significantly different from the same sample, different dose treated group from Tukey's HSD test after the one-way ANOVA test. (A) Normal ($n = 8$), (B) LPS control ($n = 13$), (C) **1*R*** 10 mg/kg ($n = 6$), 25 mg/kg ($n = 9$), (D) **1*S*** 10 mg/kg ($n = 8$), 25 mg/kg ($n = 7$), (E) **2*R*** 10 mg/kg ($n = 9$), 25 mg/kg ($n = 7$), (F) **2*S*** 10 mg/kg ($n = 11$), 25 mg/kg ($n = 9$), (G) **3*R*** 10 mg/kg ($n = 10$), 25 mg/kg ($n = 11$), H: **3*S*** 10 mg/kg ($n = 11$), 25 mg/kg ($n = 9$).

the corresponding **1*R*** enantiomer in attenuating DIC and MOF parameters, while the ***S*** enantiomers **2*S*** and **3*S*** ameliorated some of the DIC and MOF parameters more effectively than the corresponding antipodes **2*R*** and **3*R***.

Acknowledgments

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24. *Spectral data for* **9**. TLC R_f 0.32 (EtOH/hexane = 3:1); $[\alpha]_D^{28} = +25.0$ (*c* 0.05, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (d, 2H, *J* = 8.5 Hz), 6.87 (d, 2H, *J* = 8.5 Hz), 6.64 (s, 1H), 6.59 (s, 1H), 4.10 (m, 1H), 3.86 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.10–3.26 (m, 2H), 2.81–2.97 (m, 2H), 2.62–2.81 (m, 2H); MS (*m*/*z*) calcd for $C_{19}H_{23}NO_2$ (M^+) 313.39, found 313.55; t_R (Daicel Chiralcel, 4.6 mm × 25 cm, 40:10:0.05 hexane/2-propanol/diethylamine, flow rate: 0.5 ml/min) 25.4 min for (*R*)-isomer (**9*R***), 20.6 min for (*S*)-isomer (**9*S***).
25. *Spectral data for* (*R*)-*higenamine* (**1*R***)-*HBr*. TLC R_f 0.50 (benzene/acetone/MeOH = 5:4:2, then three drops of 28% ammonium hydroxide solution); mp 249 °C; $[\alpha]_D^{28} = +25.0$ (*c* 0.05, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.35 (s, 1H), 9.06 (s, 1H), 8.84 (s, 1H), 7.10 (d, 2H, *J* = 8.5 Hz), 6.70 (d, 2H, *J* = 8.5 Hz), 6.56 (s, 1H), 6.52 (s, 1H), 4.5 (br s, 1H), 3.32 (m, 1H), 3.15 (m, 2H), 2.97 (m, 1H), 2.86 (m, 1H), 2.77 (m, 1H); IR (KBr pellet) 3231, 2798, 1627, 1520, 1454 cm^{-1}; Anal. Calcd for $C_{16}H_{18}BrNO_3$: C, 54.56; H, 5.15; N, 3.98. Found: C, 54.55; H, 5.12; N, 4.02; t_R (CHIREX 3020G-EO column by Phenomenex Co. 4.6 mm × 25 cm, 53:35:12 hexane/dichloromethane/(trifluoroacetic acid/EtOH = 1:20), flow rate 0.9 ml/min) 19.6 min for (*R*)-higenamine (**1*R***)-HBr, 23.8 min for (*S*)-higenamine (**1*S***)-HBr.
26. *Spectral data for* (*R*)-*YS-49* (**2*R***)-*HBr*. TLC R_f 0.50 (benzene/acetone/MeOH = 5:4:2, then three drops of 28% ammonium hydroxide solution); mp 255 °C; $[\alpha]_D^{28} = -68.7$ (*c* 0.048, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.80 (s, 1H), 8.20 (d, 1H, *J* = 8.5 Hz), 8.02 (d, 1H, *J* = 8.0 Hz), 7.92 (d, 1H, *J* = 8.0 Hz), 7.57–7.65 (m, 2H), 7.49 (t, 1H, *J* = 8.0 Hz), 7.42 (d, 1H, *J* = 8.5 Hz), 6.61 (s, 1H), 6.39 (s, 1H), 4.68 (t, 1H, *J* = 6.5 Hz), 3.79 (m, 1H), 3.52 (m, 2H), 3.20 (m, 1H), 2.98 (m, 1H), 2.83 (m, 1H); IR (KBr pellet) 3419, 2935, 2788, 2559, 1632, 1535 cm^{-1}; Anal. Calcd for $C_{20}H_{20}BrNO_2$: C, 62.19; H, 5.22; N, 3.63. Found: C, 62.21; H, 5.26; N, 3.66; t_R (CHIREX 3020G-EO column by Phenomenex Co. 4.6 mm × 25 cm, 53:35:12 hexane/dichloromethane/(trifluoroacetic acid/EtOH = 1:20), flow rate 0.9 ml/min) 9.9 min for (*R*)-YS-49 (**2*R***)-HBr, 11.9 min for (*S*)-YS-49 (**2*S***)-HBr.
27. *Spectral data for* (*R*)-*YS-51* (**3*R***)-*HBr*. TLC R_f 0.50 (benzene/acetone/MeOH = 5:4:2, then three drops of 28% ammonium hydroxide solution); mp 244 °C; $[\alpha]_D^{28} = +10.4$ (*c* 0.051, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.84 (s, 1H), 7.89–7.96 (m, 2H), 7.82–7.89 (m, 2H), 7.46–7.56 (m, 3H), 6.59 (s,1H), 6.57 (s, 1H), 4.72 (t, 1H, *J* = 6.5 Hz), 3.47 (m, 1H), 3.35 (m, 1H), 3.17 (m, 2H), 2.88 (m, 1H); IR (KBr pellet) 3162, 2966, 2800, 1628, 1541, 1441 cm^{-1}; Anal. Calcd for $C_{20}H_{20}BrNO_2$: C, 62.19; H, 5.22; N, 3.63. Found: C, 62.19; H, 5.22; N, 3.67; t_R (CHIREX 3020G-EO column by Phenomenex Co. 4.6 mm × 25 cm, 53:35:12 hexane/dichloromethane/(trifluoroacetic acid/EtOH = 1:20), flow rate 0.9 ml/min) 9.7 min for (*R*)-higenamine (**3*R***)-HBr, 10.9 min for (*S*)-higenamine (**3*S***)-HBr.
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