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Preparation and anti-inflammatory activities of diarylheptanoid and diarylheptylamine analogs

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Abstract—Seven diarylheptylamine (**12a**–**g**) and four diarylheptanoid analogs (**3**–**5**, **9**), structurally related to the natural anti-inflammatory agent oregonin (**1**), have been prepared from curcumin (**2**) for evaluation of their activity against the expression of iNOS and COX-2. Diarylheptylamine **12b** and diarylheptanoid analogs can inhibit iNOS and COX-2 responses of LPS, although less potently than **1**. These compounds, however, possess stronger potency than **1** against COX-2-derived PGE_2 formation, of which hexahydrocurcumin (**4**) is the most potent one with an IC_{50} value of 0.7 μM.

1. Introduction

Chronic inflammation leads to destruction of normal tissue integrity. Production of inflammatory mediators through up-regulation of several inducible gene products, such as inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2), contributes to inflammatory responses and tissue damage.[1–3] Studies have indicated the role of iNOS-derived NO and COX-2-derived PGE_2 in the amplification of inflammatory response. Based on these recent findings, a lot of effort has been bestowed on the development of anti-inflammatory agents with regard to interference with the transcriptional induction or inhibition of the enzymatic activities of COX-2 and iNOS.[4] On exposure to lipopolysaccharide (LPS), macrophages undergo co-induction of iNOS and COX-2 gene expression, leading to the formation of two multifunctional inflammatory mediators, nitric oxide (NO) and PGE_2.[5] Recently, oregonin (**1**), a diarylheptanoid glycoside containing 3-carbonyl and 5-xylosyloxy groups, has been reported to have anti-inflammatory activity. It can inhibit COX-2 protein expression in immortalized human mammary epithelial MCF-10A cells.[6] In addition, the presence of oregonin (**1**) could inhibit stimulation of murine macrophages by LPS, iNOS mRNA expression, and NO synthesis.[7] These studies indicate oregonin (**1**) to be a lead drug against inflammation. Recent studies in our laboratory have found that *Alnus formosana* also contains oregonin (**1**).[8] Curcumin (**2**), a naturally abundant diarylheptanoid containing two α,β-conjugated carbonyl functions, also possesses strong anti-inflammatory activity.[9–11] Various structures were derived from modification of these two conjugated systems.[12,13] Being interested in exploration of anti-inflammatory agents, we used **2** as starting material and applied the isosterism theory, i.e., converting the 5-hydroxy or 3-carbonyl function in the aglycon of **1** into 3-alkylamino groups, which will enable the preparation of organic salts and might help to improve the pharmacokinetic profile. Based on this, four diarylheptanoid analogs and a series of new diarylheptylamine analogs were prepared. Using LPS-stimulated macrophages for induction of iNOS and COX-2 as a model system, the anti-inflammatory activity of the prepared compounds was evaluated. In the following section, the outcome of these efforts is described.

2. Results and discussions

Starting from curcumin (**2**), various kinds of reductive state products **3**–**5** have been described previously in the literature.[14] Further improvement with different

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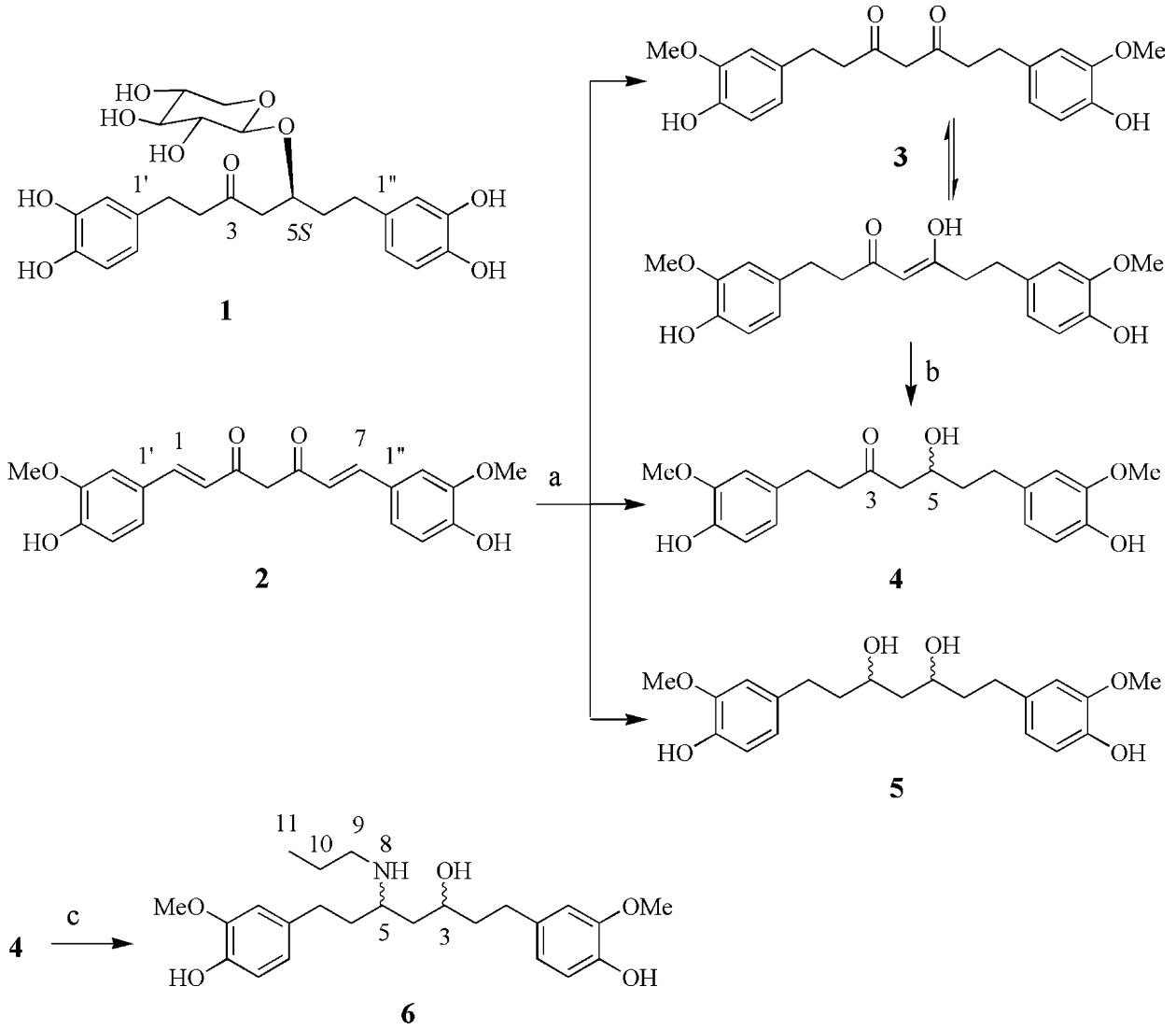
conditions of Pt/C-catalyzed hydrogenations was attempted (Scheme 1, Table 1), providing the optimum yield of hexahydrocurcumin (**4**) (30%) from **2** and octahydrocurcumin (**5**) (91.8%) under higher pressure and longer reaction time. Although **4** and **5** could also be obtained from **3** by $NaBH_4$ reduction, the yields were low. Structure **4**, possessing an asymmetrical skeleton, has more potential for further structural modification. Thus, direct reductive amination of **4** with propylamine yielded **6**, though the yields (16%) were not satisfactory. This could be due to intramolecular hydrogen bonding between 3-carbonyl and 5-hydroxy in **4**, reducing the possibility of Schiff base formation.

A synthetic scheme to improve the yield of diarylheptylamine analogs was developed as shown in Scheme 2, in which the 5-OH group was removed, followed by the protection of two phenolic groups. The reaction of curcumin (**2**) with Boc anhydride yielded **7**. Catalytic hydrogenation of **7** under neutral conditions (EtOAc) yielded **8** (92.1%), a 1,7-diarylheptan-5-ol-3-one. Since the Boc- and secondary alcoholic groups are labile under stronger acidic conditions, we attempted to develop facile reaction conditions to achieve deprotection, dehydration, and hydrogenation in sequence to give **9**. The conditions for such a purpose were found to be de-Boc- and dehydration under high concentrations of TFA for a longer time (26.5 h), followed by catalytic hydrogenation, which yielded **9** (51.5%), a key intermediate for the preparation of 1,7-diaryl-3-heptylamines. **9** was *O*,*O*-dibenzylated by $BnCl/K_2CO_3$ via a rapid microwave reaction to give dibenzylmonoketone **10** (99%). Reductive amination of **10** with six primary amines afforded the respective *N*-alkyl dibenzyldiarylheptylamines, **11a**–**f**, in yields of 43.3–67.5%, which upon catalytic hydrogenation yielded the corresponding *N*-alkyl diarylheptylamines, **12a**–**f**, in yields of 61.8–99.0%. Compound **12g**, possessing a cyclopropylamine moiety, which is labile to catalytic hydrogenation, was prepared directly from **9** through reductive amination (56.4% yield).



Scheme 1. Preparation of compounds **3**–**6** from curcumin (**2**). Reagents and conditions: (a) Pt/C, H_2, MeOH, rt*; (b) $NaBH_4$ MeOH, rt, 6 h, **4** (7%), **5** (19%); (c) H^+, propylamine, $NaBH_3CN$, MeOH, rt, 4 days, 16%. *Reaction conditions and yields see Table 1.

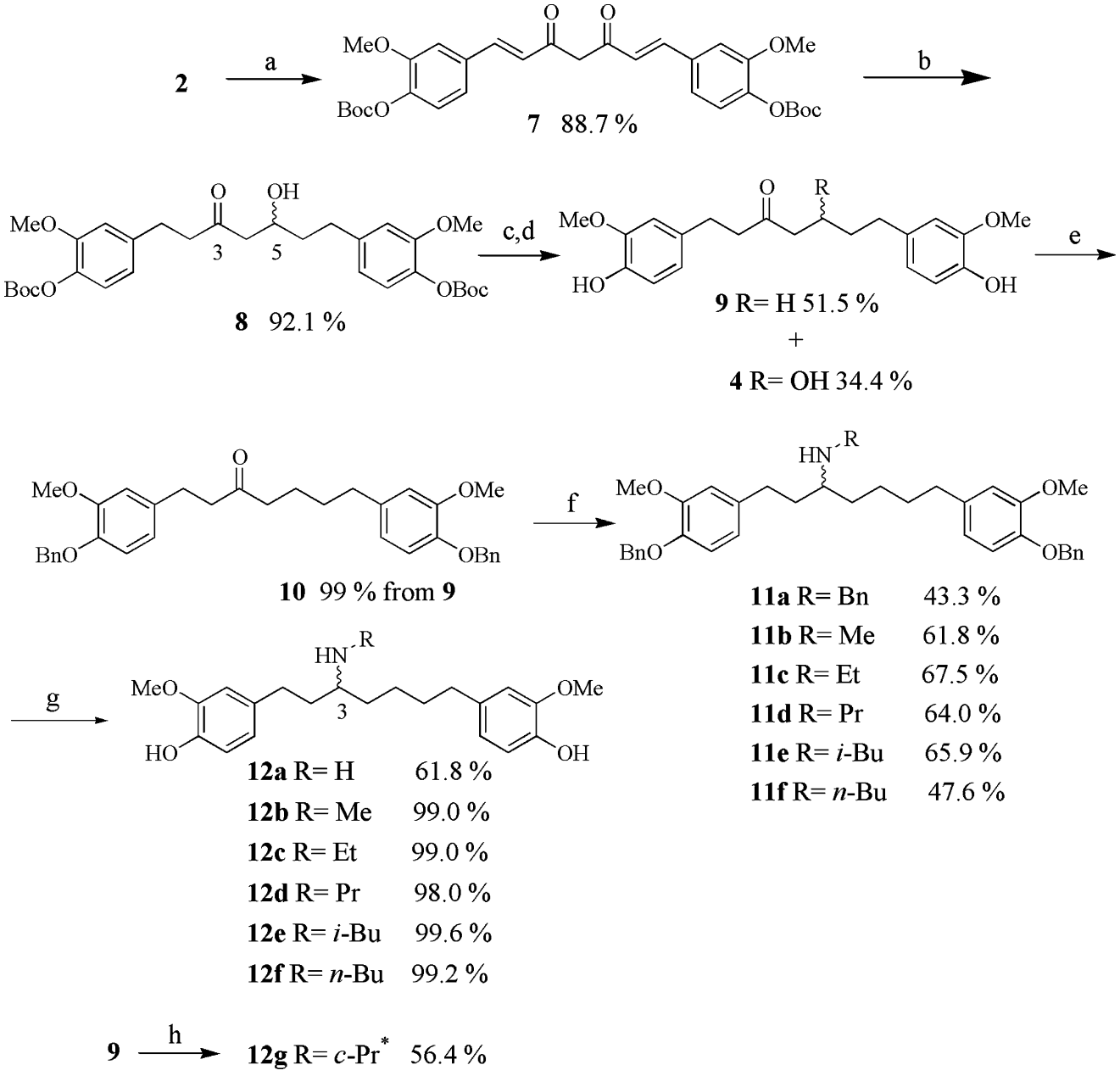
Table 1. Hydrogenation conditions for curcumin (**2**) and yields of **3**–**5**

Reaction condition	Product (yields)
H_2 (1 atm), Pt/C, MeOH, rt, 1 h	**3** (71.6%)
H_2 (200 psi), Pt/C, MeOH, rt, 16 h	**4** (30.2%), **5** (66.8%)
H_2 (200 psi), Pt/C, MeOH, rt, 27 h	**5** (91.8%)

3. Bioactivity

Compounds **1**, **3**–**5**, **9**, and **12a**–**g** were tested in LPS (1 μg/mL)-stimulated RAW264.7 macrophages pertaining to: (1) inhibition of iNOS expression; (2) inhibition of NO production; (3) inhibition of biosynthesis of COX-2 downstream product PGE_2; and (4) cytotoxicity.

MTT assay as an index of cell viability indicated that at concentrations up to 100 μM, oregonin (**1**) and compounds **3**–**5**, **9**, and **12b** had no significant effect on cell viability. However, a significant reduction of MTT values, cell toxicity, was observed for the other compounds at 100 μM. The cytotoxic IC_{50} values are given in Table 2. In the measurement of NO production by LPS, we found that, compared to oregonin (**1**), all these compounds showed less potency (Table 2). As NO reduction of 100 μM compounds **12a**, **12c**, **12d**, **12e**, **12f**, and **12g**



Scheme 2. Preparation of diarylheptylamine **12a**–**g** from curcumin (**2**). Reagents and conditions: (a) $(Boc)_2O$, NaOH, MeOH, rt, 3 h; (b) Pd/C, H_2 200 psi, EtOAc, rt, 60 h; (c) TFA-CH_2Cl_2 (2–3), rt, 26.5 h; (d) Pd/C, H_2, MeOH, rt, 1 h; (e) BnCl, K_2CO_3, DMF, microwave, 20 min; (f) H^+/NH_2-R, $NaBH_3CN$, MeOH–CH_2Cl_2, rt; (g) Pd/C, H_2, MeOH, rt, 1 h; (h) (i) H^+, NH_2–$CH(CH_2)_2$, toluene, △, 2 h; (ii) $NaBH_3CN$, toluene, 40 °C, 18 h *c-Pr, cyclopropyl.

Table 2. IC_{50} values (μM) of oregonin (**1**) and diarylheptanoid compounds on cytotoxicity and inhibition of NO and PGE_2 production

Compound	Inhibition of cell viability	Inhibition of LPS (1 μg/ml)-induced NO production	Inhibition of LPS (1 μg/ml)-induced PGE_2 production
Oregonin (**1**)	>100	18	6.1
3	>100	77	4.5
4	>100	100	0.7
5	>100	42	2.3
9	>100	72	1.4
12a	95	61	ND
12b	>100	100	1.0
12c	73	68	7.4
12d	73	62	16
12e	78	61	6.6
12f	69	67	ND
12g	86	58	ND

ND, not determined.

was accompanied by displaying cell toxicity, NO response had conceivably resulted from a toxic event. In Figures 1 and 2, reduction of LPS-stimulated iNOS promoter activity and protein expression by nontoxic compounds, respectively, at 30 and 100 μM are given, which implies that iNOS inhibition takes place primarily occurs at the transcriptional level. In contrast to moderate inhibition of NO, these compounds showed significant activities inhibiting the biosynthesis of PGE_2 (Table 2). It should be pointed out that compounds **4** and **5** existed as mixtures of enantiomeric and diastereomeric isomers, respectively, which still await further resolution, and all comparisons were made to a presumed active isomer in the mixture.

From the scant information of these bioassays, it is still too early for us to deduce meaningful SAR of this series of compounds. However, most of them exhibit marked inhibition of COX-2-derived PGE_2 formation and have the potential to develop as anti-inflammatory agents.

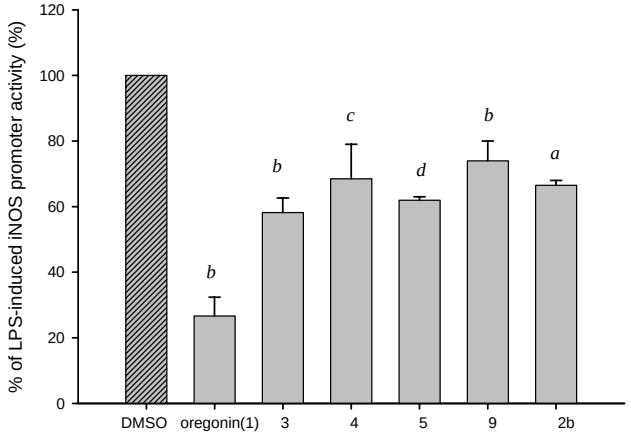


Figure 1. Inhibitory effect of diarylheptanoid compounds (30 μm) on LPS-induced iNOS promoter activity. *P* value: $a < 0.005$, $b < 0.05$, $c < 0.01$, and $d < 0.001$.

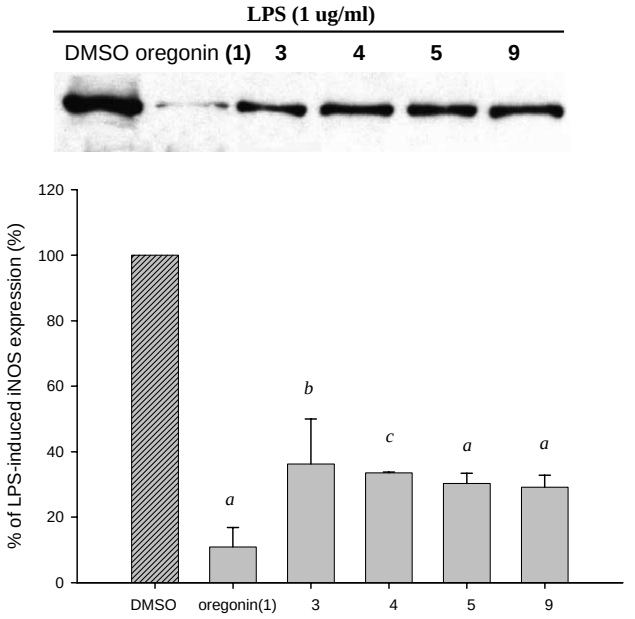


Figure 2. Inhibitory effects of diarylheptanoid compounds (100 μM) on LPS (1 μg/ml)-stimulated iNOS protein expression. Immunoblotting of iNOS was performed from cell lysates (A) and immunoreactivity quantified by densitometer is presented as percentages of control LPS response without treatment with diarylheptanoid compounds. *P* value: $a < 0.005$, $b < 0.05$, and $c < 0.00005$.

4. Experimental

4.1. General experimental conditions

The physical data of the prepared compounds were obtained from the following instruments: Melting points: Fisher–Johns melting apparatus (uncorrected); IR (KBr): JASCO FT/IR-410 spectrometer; UV (MeOH): Hitachi U-2000 spectrophotometer; NMR: Brucker DPX-200 and Avance-400 in deuterated solvent, using the residual solvent peak as internal standard ($CDCl_3$: δ_H 7.24 and δ_C 77.0; methanol-d_4: δ_H 3.30, δ_C 49.0); Mass: Finnigan Mat TSQ-7000 Mass spectrometer (ESIMS), VG 70-250S GC/MS (HFABMS). Chromatographic system: 230–400 mesh silica gel for column.

4.2. 1,7-Bis(4-hydroxy-3-methoxyphenyl)-heptane-3,5-dione (tetrahydrocurcumin, 3)

To a solution of curcumin (**2**) (1.00 g, 2.7 mmol) in MeOH (100 mL) was added 10% Pt/C (100 mg). After degassing, the mixture was hydrogenated (H_2, 1 atm) at rt for 1 h, filtered though a Celite pad, and concentrated. The residue (1.05 g) was purified via a silica gel column (40 g, $CHCl_3$) to give **3** (724.3 mg, 71.6%) as a white powder.

Compound ***3***: mp 95–97 °C; R_f 0.53 (1% MeOH–$CHCl_3$); key ^1H NMR ($CDCl_3$, 400 MHz) δ 2.85 (4H, t, $J = 7.4$, 8.2 Hz, H-1, 7), 2.76 (2H, m, H-4), 2.53 (4H, t, $J = 7.4$, 8.2 Hz, H-2, 6); key ^{13}C NMR ($CDCl_3$) δ 203.4 (s, C-3, 5).

4.3. 1,7-Bis(4-hydroxy-3-methoxyphenyl)-5-hydroxy-3-heptanone (Hexahydrocurcumin, 4) and 1,7-Bis(4-hydroxy-3-methoxyphenyl)-3,5-heptanediol (octahydrocurcumin, 5)

The mixture of **2** (1.64 g, 4.4 mmol), MeOH (175 mL), and Pt/C (10%, 163 mg) in a stainless steel autoclave was hydrogenated (H_2, 200 psi) at rt for 16 h. The residue obtained (1.65 g) after a similar workup procedure as above was chomatographed over a silica gel column (50 g, $CHCl_3$) to give **5** (1.12 g, 66.8 %) and **4** (502 mg, 30.2%). While prolonging the reaction time to 27 h yielded **5** exclusively (91.8%).

Compound ***4***. Greenish crystal, mp 80–82 °C; R_f 0.42 (3% MeOH–$CHCl_3$); key ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.02 (1H, *m*, H-5), 3.84 and 3.83 (6H, s, 3′,3″-OMe), 2.84–2.50 (8H, m, H-1, 2, 4, 7), 1.78–1.58 (2H, m, H-6).

Compound ***5***. Yellowish viscous liquid, R_f 0.36 (5% MeOH–$CHCl_3$); key ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.84 (2H, m, H-3, 5), 3.79 (6H, s, 3′-,3″-OMe); key ^{13}C NMR ($CDCl_3$) δ 72.4 (d, C-3, 5), 55.9 (q, 3′, 3″-OMe), 42.9 (t, C-4), 40.0 (t, C-2, 6), 31.4 (t, C-1,7).

4.4. 1,7-Bis(4-hydroxy-3-methoxyphenyl) 5-(propylamino)-3-heptanol (6)

The mixture of **4** (100 mg, 0.3 mmol), MeOH (15 mL), 12 N HCl (5 drops), and propylamine (115 mg, 1.9 mmol) was stirred at rt for 1 h. Then, $NaBH_3CN$ (82 mg, 1.3 mmol) and molecular sieve (4 Å) were added and the reaction mixture was stirred for 4 days. After the removal of molecular sieve by filtration, the filtrate was concentrated and the residue suspended in 1 N HCl (50 mL) was extracted with CH_2Cl_2 (50 mL) to remove unreacted **4**. The aqueous layer after neutralization with ammonia water was partitioned with CH_2Cl_2 (50 mL × 3). The combined CH_2Cl_2 layers were dried over Na_2SO_4 and concentrated to give a crude product (42 mg), which was purified via a silica gel column (5 g, 3–10% MeOH in $CHCl_3$) to give **6** (18 mg, 16%) as a yellowish solid.

Compound ***6***. R_f 0.10 (3% MeOH–$CHCl_3$); UV λ_{max} (log ε) 227.2 (3.92), 280.2 (3.59) nm; key ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.83 (1H, m, H-3), 2.85–2.45 (7H, m, H-5, 1, 7, $NCH_2C_2H_5$), 1.73–1.23 (8H, m, H-2, 4, 6, $NCH_2CH_2CH_3$), 0.89 (3H, t, J = 7.2 Hz, $NC_2H_4CH_3$); key ^{13}C NMR ($CDCl_3$) δ 72.0 (d, C-3), 58.5 (d, C-5), 46.8 (t, $NCH_2C_2H_5$), 40.3 (t, C-4), 38.5 (t, C-2), 35.1 (t, C-6), 31.7 (t, C-7), 31.4 (t, C-1), 22.4 (t, $NCH_2CH_2CH_3$), 11.5 (q, $NC_2H_4CH_3$); ESIMS: *m*/*z* $[M+H]^+$ 418.2.

4.5. *O*,*O*-Di-^{t}butyloxycarbonylcurcumin (7)

The mixture of **2** (5.00 g, 13.6 mmol), MeOH (200 mL), NaOH (1.60 g, 40.7 mmol), and $(Boc)_2O$ (11.8 g, 54.3 mmol) was stirred under N_2 at rt for 3 h and then filtered. The residue was washed with water and dried to give **7** (6.84 g, 88.7%) as a yellow powder.

Compound ***7***. mp 139.5–142 °C; R_f 0.4 (50% hexane–$CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.59 (2H, d, J = 15.8 Hz, H-1, 7), 7.12 (4H, br s, H-5′,5″,6′,6″), 7.10 (2H, br s, H-2′,2″), 6.53 (2H, d, J = 15.8, H-2,6), 5.84 (1H, s, H-4), 3.88 (6H, s, 3′,3″-OCH_3), 1.54 (18H, s, Boc-CH_3); ESIMS *m*/*z* (rel. int. %) $[M+Na]^+$ 591.2 (100), $[M+H]^+$ 569.1 (37).

4.6. *O*,*O*-Di-^{t}butyloxycarbonyl-hexahydrocurcumin (8)

To a stainless steel autoclave were added **7** (3.20 g, 5.6 mmol), EtOAc (150 mL), and 10% Pd/C (600 mg) in sequence. After degassing, the mixture was hydrogenated (H_2, 200 psi) at rt for 60 h. The usual workup procedure gave a crude product (3.39 g), which was purified via a silica gel column (100 g, $CHCl_3$) to yield **8** as a colorless solid (2.98 g, 92.1%).

Compound ***8***. R_f 0.34 (1% MeOH–$CHCl_3$); key ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.02 (1H, m, H-5), 1.52 (18H, s, Boc-CH_3); key ^{13}C NMR ($CDCl_3$) δ 209.6 (s, C-3), 83.1 and 83.0 (s, Boc-O*C*Me_3), 66.5 (d, C-5); ESIMS *m*/*z* $[M+Na]^+$ 597.3.

4.7. 1,7-Bis(4-hydroxy-3-methoxyphenyl)-3-heptanone (9)

The mixture of **8** (2.39 g, 4.1 mmol), CH_2Cl_2 (15 mL), and TFA (10 mL) was stirred under N_2 at rt for 14 h and then concentrated to give an oily residue, which was partitioned between water (100 mL) and CH_2Cl_2 (100 mL × 3). The combined CH_2Cl_2 layers were concentrated to give a viscous residue (1.65 g), which without further purification was catalytically hydrogenated (MeOH, 40 mL; 10% Pd/C, 146 mg; H_2, 1 atm; 1 h). After the usual workup procedure, the crude residue (1.39 g) was separated over a silica gel column (40 g, 25% hexane–$CHCl_3$) to give **4** (532 mg, 34.4%) and **9** (761 mg, two steps 51.5%).

Compound ***9***. Colorless liquid, R_f 0.60 (2% MeOH–$CHCl_3$); UV λ_{max} (log ε) 228.4 (4.11), 281.2 (3.82) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.80 (2H, d, J = 7.9 Hz, H-5′,5″), 6.66–6.61 (4H, m, H-2′,2″,6′,6″), 5.47 (1H, s) and 5.45 (1H, s) (O*H*), 3.85 and 3.83 (6H, s, 3′,3″-OMe), 2.80 (2H, t, J = 7.4 Hz, H-1), 2.66 (2H, t, J = 7.6 Hz, H-2), 2.51 (2H, t, J = 7.3 Hz, H-7), 2.37 (2H, t, J = 6.7 Hz, H-4), 1.58–1.53 (4H, m, H-6, 5); ^{13}C NMR ($CDCl_3$) δ 210.4 (s, C-3), 146.3 (s, C-3′,3″), 143.8 and 143.5 (s, C-4′,4″), 133.0 and 134.1 (s, C-1′,1″), 120.7 and 120.8 (d, C-6′, 6″), 110.9, 111.0 and 114.1, 114.3 (each d) (C-2′,2″,5′,5″), 55.8 (q, 3′,3″-OMe), 44.6 (t, C-2), 42.8 (t, C-4), 35.3 (t, C-7), 31.2 (t, C-6), 29.5 (t, C-1), 23.3 (t, C-5); HRFABMS *m*/*z* 358.1779 (Calcd for $C_{21}H_{26}O_5$, 358.1780).

4.8. 1,7-Bis(4-benzyloxy-3-methoxyphenyl)-3-heptanone (10)

The mixture of **9** (1.07 g, 3.0 mmol), DMF (15 mL), BnCl (3.16 g, 24.9 mmol), and K_2CO_3 (1.22 g, 8.9 mmol) was reacted for 15 min in a microwave digester (Prolabo Maxidigest MX350) with 60% power. After cooling, the reaction mixture was concentrated and the residue

dissolved in CH_2Cl_2 (80 mL) was washed with water (80 mL × 3). The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated to give a solid residue (1.68 g), which upon treatment with acetone gave pure **10** (1.59 g, 99 %) as a white powder: R_f 0.60 (0.5% MeOH–$CHCl_3$); key ^{1}H NMR ($CDCl_3$) δ 7.43–7.24 (10H, m, $OCH_2C_6H_5$), 5.09 (4H, s, $OCH_2C_6H_5$).

4.9. *N*-Alkyl-1,7-bis(4-benzyloxy-3-methoxyphenyl)-3-heptylamines (11a: *N*-Bn; 11b: *N*-Me; 11c: *N*-Et; 11d: *N*-^{n}Pr; 11e: *N*-^{i}Bu; 11f: *N*-^{n}Bu)

The mixture of **10** (436 mg, 0.8 mmol), MeOH–CH_2Cl_2 (5:4, 18 mL), benzylamine (893 mg, 8.3 mmol, 10.3 equiv), and 12 N HCl (3 drops) was stirred at rt under N_2 for 30 min and then $NaBH_3CN$ (524 mg, 8.3 mmol, 10.3 equiv) was added. The reaction took place for 89 h. The reaction mixture was concentrated and the residue was partitioned between CH_2Cl_2 (200 mL) and water (50 mL × 3). The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated to give a residue (1.10 g), which was purified via two silica gel columns (20 g, 50–100% $CHCl_3$–hexane, satd with NH_4OH; 10 g 0–3% MeOH–$CHCl_3$) to give compound **11a** as colorless oil (211 mg, 43.3%).

Under similar reaction conditions and workup procedures for the preparation of **11a**, compounds **11b** (253 mg, 61.8%), **11c** (288 mg, 67.5%), **11d** (327 mg, 64.1%), **11e** (233 mg, 65.9%), and **11f** (197 mg, 62.9%) were produced from five batches of **10** (398 mg, 0.7 mmol for **11b**; 406 mg, 0.8 mmol for **11c**; 473 mg, 0.9 mmol for **11d**; 320 mg, 0.6 mmol for **11e**; 283 mg, 0.5 mmol for **11f**) by reacting with methylamine solution (25%, 922 mg, 7.4 mmol, 10 equiv for **11b**), ethylamine (491 mg, 7.6 mmol, 10 equiv for **11c**), propylamine (591 mg, 11.0 mmol, 12 equiv for **11d**), isobutylamine (382 mg, 5.2 mmol, 9 equiv for **11e**), and butylamine (412 mg, 5.6 mmol, 11 equiv for **11f**), respectively.

*Compound **11a***. R_f 0.33 ($CHCl_3$, satd with $NH_{3(aq)}$); key ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44–7.29 (15H, m, 2× $OCH_2C_6H_5$, $NCH_2C_6H_5$), 3.74 (2H, s, $NCH_2C_6H_5$); key ^{13}C NMR ($CDCl_3$) δ 56.2 (d, C-3), 53.0 (t, $NCH_2C_6H_5$).

*Compound **11b***. R_f 0.51 (3% MeOH–$CHCl_3$, satd with $NH_{3(aq)}$); key ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.54 (4H, t, J = 7.7 Hz, H-1 and H-7), 2.43 (1H, m, H-3), 2.37 (3H, s, NMe); key ^{13}C NMR ($CDCl_3$) δ 58.6 (d, C-3), 33.5 (q, *N*-Me).

*Compound **11c***. R_f 0.40 (1% MeOH–$CHCl_3$, satd with $NH_{3(aq)}$); key ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.62–2.52 (7H, m, H-1, 3, 7, NCH_2CH_3), 1.07 (3H, t, J = 7.1 Hz, NCH_2CH_3); key ^{13}C NMR ($CDCl_3$) δ 57.0 (d, C-3), 41.2 (t, NCH_2CH_3), 15.6 (q, NCH_2CH_3).

*Compound **11d***. R_f 0.36 (3% MeOH–$CHCl_3$); key ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.56–2.47 (7H, m, H-1, 3, 7, $NCH_2C_2H_5$), 1.51–1.36 (6H, m, H-4, 5, $NCH_2CH_2CH_3$), 0.89 (3H, t, J = 7.3 Hz, $NC_2H_4CH_3$); key ^{13}C NMR ($CDCl_3$) δ 57.0 (d, C-3), 48.9 (t, $NCH_2C_2H_5$), 23.5 (t, $NCH_2CH_2CH_3$), 11.9 (q, $NC_2H_4CH_3$).

*Compound **11e***. R_f 0.31 ($CHCl_3$, satd with $NH_{3(aq)}$); key ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.53 (6H, m, H-1, 3, 7 and N*H*), 2.37 (2H, d, J = 6.7 Hz, $NCH_2{}^i$Pr), 0.87 (6H, d, J = 6.6 Hz, $NCH_2CH(CH_3)_2$); key ^{13}C NMR ($CDCl_3$) δ 57.1 (d, C-3), 54.4 (t, $NCH_2{}^i$Pr), 28.1 (d, $NCH_2CH(CH_3)_2$), 20.7 (q, $NCH_2CH(CH_3)_2$).

*Compound **11f***. R_f 0.33 ($CHCl_3$, satd with $NH_{3(aq)}$, develop twice); key ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.65 (4H, m, H-1 and H-7), 2.59 (1H, m, H-3), 2.53 (2H, t, J = 7.7 Hz, $NCH_2C_3H_7$), 1.59 (6H, m, H-2, 6, $NCH_2CH_2C_2H_5$), 0.87 (3H, t, J = 7.4 Hz, $NC_3H_6CH_3$); key ^{13}C NMR ($CDCl_3$) δ 57.2 (d, C-3), 45.8 (t, $NCH_2C_3H_7$), 20.4 (t, $NC_2H_4CH_2CH_3$), 13.7 (q, $NC_3H_6CH_3$).

4.10. 1,7-Bis(4-hydroxy-3-methoxyphenyl)-3-heptylamine (12a)

To a stainless steel autoclave were added **11a** (129 mg 0.2 mmol), MeOH (10 mL), and 10% Pd/C (29 mg) in sequence. After degassing, the mixture was hydrogenated (H_2, 200 psi) for 17 h. The usual workup procedure gave the product **12a** (48 mg, 61.8%) as a greenish liquid.

*Compound **12a***. R_f 0.22 (3% MeOH–$CHCl_3$, satd with $NH_{3(aq)}$); IR ν_{max}: 3445, 2929, 1519 cm^{-1}; UV λ_{max} (log ε) 226.6 (3.87), 280.6 (3.53) nm; ^{1}H NMR (CD_3OD, 200 MHz) δ 6.47 (2H, m, H-5′,5″), 6.70 (2H, m, H-2′,2″), 6.62 (2H, m, H-6′,6″), 3.80 (6H, s, 3′,3″-OCH_3), 2.58–2.54 (5H, m, H-1, 3, 7), 1.64–1.61, 1.35–1.13 (8H, m, H-2, 4, 5, 6); HRFABMS *m*/*z* $[M+H]^+$ 360.2173 (Calcd for $C_{21}H_{30}O_4N$, 360.2175).

4.11. *N*-Alkyl-1,7-bis-(4-hydroxy-3-methoxyphenyl)-3-heptylamines (12b: *N*-Me; 12c: *N*-Et; 12d: *N*-^{n}Pr; 12e: *N*-^{i}Bu; 12f: *N*-^{n}Bu)

To a solution of **11b** (137 mg 0.3 mmol) in MeOH (10 mL) was added 10% Pd/C (12 mg). After degassing, the mixture was hydrogenated (H_2, 1 atm) for 1 h. After the usual workup procedure, the product **12b** (91 mg, 99%) was obtained as a brownish liquid. Compound **12b** tartrate was prepared by evaporating the methanolic solution of **12b** and L-(+)-tartaric acid (1 equiv).

Under similar reaction conditions and workup procedures for the preparation of **12b**, compounds **12c** (140 mg, 99%), **12d** (139 mg, 98%), **12e** (144 mg, 99%), and **12f** (103 mg, 99%) were produced from **11c** (209 mg, 0.4 mmol), **11d** (201 mg, 0.4 mmol), **11e** (207 mg, 0.4 mmol), and **11f** (150 mg, 0.3 mmol), respectively.

*Common physical data for **12b–f***. UV λ_{max} (log ε) 228 (ca. 3.9), 281 (ca. 3.5) nm; ^{1}H NMR δ ($CDCl_3$) 6.79 (2H, d, J = 7.7 Hz, H-5′,5″), 6.64 (4H, d, J = 8.1 Hz, H-2′,2″,6′,6″), 3.84 and 3.83 (6H, s, 3′,3″-OMe); ^{13}C NMR δ ($CDCl_3$) 146.5 and 146.4 (s, C-3′,3″), 143.7

and 143.6 (s, C-4′,4″), 134.5 and 134.3 (s, C-1′,1″), 120.8 and 120.7 (d, C-6′,6″), 114.3 and 111.0 (d, C-2′,2″,5′,5″), 55.8 (q, 3′,3″-OMe).

Compound ***12b*** *tartrate*. R_f 0.38 (7% MeOH–$CHCl_3$, satd with $NH_{3(aq)}$); IR ν_{max} 3123, 1519, 1683 cm^{-1}; key ^1H NMR (CD_3OD, 400 MHz) δ 2.64 (3H, s, NCH_3); key ^{13}C NMR (CD_3OD) δ 60.0 (d, C-3), 30.8 (q, NCH_3); HRFABMS *m/z* $[M+H]^+$ 374.2334 (Calcd for $C_{22}H_{32}O_4N$, 374.2331).

Compound ***12c***. R_f 0.18 (3% MeOH–$CHCl_3$, satd with $NH_{3(aq)}$); IR ν_{max} 3318, 1558, 1134 cm^{-1}; key ^1H NMR ($CDCl_3$) δ 2.61 (2H, q, J = 7.1 Hz, NCH_2CH_3), 1.07 (3H, t, J = 7.1 Hz, NCH_2CH_3); key ^{13}C NMR ($CDCl_3$) δ 57.0 (d, C-3), 41.2 (t, NCH_2CH_3), 15.5 (q, NCH_2CH_3); HRFABMS *m/z* $[M+H]^+$ 388.2486 (Calcd for $C_{23}H_{34}O_4N$, 388.2488).

Compound ***12d***. R_f 0.27 (3% MeOH/$CHCl_3$, satd with $NH_{3(aq)}$); IR ν_{max} 2934, 1698, 1508 cm^{-1}; key ^1H NMR ($CDCl_3$, 400 MHz) δ 2.56–2.49 (7H, m, H-1, 3, 7, $NCH_2C_2H_5$), 1.49–1.43 (4H, m, H-4, 10), 0.89 (3H, t, J = 7.4 Hz, $NC_2H_4CH_3$); key ^{13}C NMR ($CDCl_3$) δ 57.1 (d, C-3), 49.0 (t, $NCH_2C_2H_5$), 23.5 (t, $NCH_2CH_2CH_3$), 11.9 (q, $NC_2H_4CH_3$); HRFABMS *m/z* $[M+H]^+$ 402.2645 (Calcd for $C_{24}H_{36}O_4N$, 402.2644).

Compound ***12e***. R_f 0.42 (3% MeOH–$CHCl_3$, satd with $NH_{3(aq)}$); IR ν_{max} 3324, 1518, 1732, 1698, 1263 cm^{-1}; key ^1H NMR (CD_3OD, 200 MHz) δ 2.54 (5H, m, H-1, 3, 7), 2.38 (2H, d, J = 6.8 Hz, $NCH_2{}^iPr$), 0.887 (3H, d, J = 6.6 Hz) and 0.880 (3H, d, J = 6.6 Hz) ($NCH_2CH(CH_3)_2$); key $^{13}CNMR$ (CD_3OD) δ 58.4 (d, C-3), 55.5 (t, $NCH_2{}^iPr$), 28.8 (d, $NCH_2CH(CH_3)_2$), 21.0 (q, $NCH_2CH(CH_3)_2$); HRFABMS *m/z* $[M+H]^+$ 416.2803 (Calcd for $C_{25}H_{38}O_4N$, 416.2801).

Compound ***12f***. R_f 0.13 (3% MeOH–$CHCl_3$, satd with $NH_{3(aq)}$); IR ν_{max} 3225, 1716, 1698, 1507 cm^{-1}; key ^1H NMR (CD_3OD, 400 MHz) δ 2.52 (7H, m, H-1, 3, 7, $NCH_2C_3H_7$), 1.67 (2H, m), 1.59 (2H, m), 1.47 (2H, m), 1.39 (2H, m), 1.30 (4H, m), 0.90 (3H, t, J = 7.2 Hz, $NC_3H_6CH_3$); key ^{13}C NMR (CD_3OD) δ 58.1 (d, C-3), 47.6 (t, C-9), 21.6 (t, $NC_2H_4CH_2CH_3$), 14.4 (q, $NC_3H_6CH_3$); HRFABMS *m/z* $[M+H]^+$ 416.2799 (Calcd for $C_{25}H_{38}O_4N$, 416.2801).

4.12. *N*-Cyclopropyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-3-heptylamine (12g)

The mixture of **9** (203.5 mg, 0.57 mmol), toluene (7 mL), cyclopropylamine (269 mg, 4.7 mmol) and 12 N HCl (4 drops) was stirred at 100 °C under N_2 for 2 h and then $NaBH_3CN$ (199 mg, 3.2 mmol) was added and stirred at 40 °C for additional 13 h. After a similar workup as that for the preparation of **11a**, the crude product obtained (199 mg) was purified via a flash column (silica gel, 10 g, 0–1% MeOH–$CHCl_3$, saturated with NH_4OH) to give **12g** as a bluish liquid (129 mg, 56%).

Compound ***12g***. R_f 0.42 (7% MeOH–$CHCl_3$); IR ν_{max} 3322, 2938, 1601, 1517, 1269 cm^{-1}; key ^1H NMR (CD_3OD, 400 MHz) δ 2.60 (1H, m, H-3), 2.04 (1H, m, $NCH(CH_2)_2$), 0.43 (2H, m) and 0.31 (2H, m) ($NCH(CH_2)_2$); key ^{13}C NMR (CD_3OD) δ 58.7 (d, C-3), 29.5 (d, $NCH(CH_2)_2$), 6.5 (t, $NCH(CH_2)_2$); HRFABMS *m/z* $[M+H]^+$ 400.2488 (Calcd for $C_{24}H_{34}O_4N$, 400.2488).

4.13. Biological assay

The samples tested were dissolved in DMSO and the final concentration of DMSO for each assay was 0.1%. Measurements of nitrite production, as an assay of NO release, and PGE_2 production were carried out, as we have previously described.[15] Immunoblotting was performed to assess the protein level of iNOS. Promoter activity of the iNOS gene as reflected by the reporter gene assay was performed, as we have previously described.[15] Cells were co-transfected with iNOS promoter–luciferase reporter and β-galactosidase expression vector. After compounds' treatment, luciferase activity was determined and normalized to transfection efficiency, as indicated by the expressed β-galactosidase activity.

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