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Original article

Syntheses of tetrahydroisoquinoline derivatives that inhibit NO production in activated BV-2 microglial cells

Jai Woong Seo [a], Ekaruth Srisook [a,1], Hyo Jin Son [b], Onyou Hwang [b,**], Young-Nam Cha [c], Dae Yoon Chi [a,*]

[a] Department of Chemistry, Inha University, 253 Yonghyundong Namgu, Inchon 402-751, Republic of Korea
[b] Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul 138-736, Republic of Korea
[c] Department of Pharmacology and Toxicology, College of Medicine, Inha University, Inchon 402-751, Republic of Korea

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Abstract

Seventeen tetrahydroisoquinoline derivatives were designed, synthesized and evaluated for inhibition of NO production in lipopolysaccharide-stimulated BV-2 microglial cells. Compounds **5a**, **9c** and **11a** potently attenuated NO production by >60%, and **5a** and **11a** inhibited BH4 production by >48% at 100 μM. In particular, *N*-ethylcarbonyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**11a**) reduced NO production by 64% and tetrahydrobiopterin (BH4) production by 49%. Introducing longer alkyl component at C1 or N2 position led to attenuation of the inhibitory effect. It is possible that **11a** inhibits NO production by blocking BH4-dependent dimerization of newly synthesized iNOS monomers.

Keywords: Tetrahydroisoquinoline; Nitric oxide; Tetrahydrobiopterin; Nitric oxide synthase

1. Introduction

Nitric oxide (NO), a radical and yet a reactive gaseous metabolite, is produced from L-arginine by the enzyme nitric oxide synthase (NOS) [1]. Since NO became known as the endothelium-derived relaxing factor [2], its biological roles in cardiovascular, immune and neuronal systems have been extensively investigated and identified [3–5].

The discovery of the subtypes of NOS, namely, the endothelial (eNOS, type-3) [6], neuronal (nNOS, type-1) [7], and inducible (iNOS, type-2) forms [8], triggered studies on their physiological functions and structures [9]. It has been found that the production of NO is controlled differently: eNOS and nNOS are constitutively expressed and activated in a Ca^{2+}-dependent manner, whereas iNOS gene expression is minimal in unstimulated state and is dramatically induced in response to stimulation [9]. Overproduction of NO by iNOS has been implicated in pathogenesis of various diseases such as stroke [10], Alzheimer's disease [11], Parkinson's disease [12], atherosclerosis [13,14], and septic shock [15].

Formation of active iNOS enzyme requires a complex intracellular process: homodimerization of newly synthesized NOS monomers and binding of FMN, FAD and NADPH to the reductase domain and heme and tetrahydrobiopterin (BH4) to the oxygenase domain [16]. All these cofactors are essential for effective electron transfer and generation of NO. Among them, BH4 plays an important role in the dimerization and catalytic activity of NOS [17,18]. Thus, inhibition of dimerization even after the number of monomers has been increased during iNOS induction would prevent NO overproduction and attenuate NO-related damages.

* Corresponding author. Tel.: +82 32 860 7686; fax: +82 32 867 5604.
** Corresponding author. Tel.: +82 2 3010 4279; fax: +82 2 3010 4248.
E-mail addresses: oyhwang@amc.seoul.kr (O. Hwang), dychi@inha.ac.kr (D.Y. Chi).
[1] Present address: Department of Chemistry, Faculty of Science, Burapha University, Chonburi 20131, Thailand.

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N-Acetyl-3-*O*-methyldopamine (NAMDA, **1**) was reported to inhibit production of NO and BH4 in BV-2 microglial cells that had been stimulated with lipopolysaccharide (LPS) but the requirement of a large dose was disadvantageous for further application [19,20]. Our initial approach aimed to find a compound that is effective at lower concentrations. In this connection, we previously synthesized NAMDA analogues by using three modification methods and identified potential lead compounds [21]. Among them, we have selected *N*-acetyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**2**) because the chemistry of tetrahydroisoquinoline is well known and C1 and/or N2 positions can easily be functionalized (Fig. 1). We have synthesized 17 derivatives of **2** and evaluated their activity against NO production in LPS-activated BV-2 cells. In addition, to elucidate whether the reduction of NO production is correlated with decreased BH4 generation, BH4 level has also been measured in some cases. Herein, we report the syntheses and structure–activity relationships (SAR) of the newly synthesized tetrahydroisoquinoline derivatives.

2. Chemistry

The synthesis of tetrahydroisoquinoline derivatives began with the well-defined Pictet–Spengler reaction between aldehyde and phenylethylamine to introduce alkyl component at C1 position [22]. Cyclization with **3** and acetaldehyde under acidic media readily afforded 1-methyl compound **4a** but this method failed to generate 1-phenyl analogue **4b** because of the solubility of benzaldehye in aqueous solvent. An alternative method using reaction under methanol to generate imine first and subsequent addition of trifluoroacetic acid (TFA) for ring formation yielded **4b** (Scheme 1). Acetylation of **4a** and **4b** with acetic anhydride produced **5a** and **5b**, respectively. Our endeavors to obtain various C1 alkyl derivatives with other aldehydes such as phenylacetaldehyde and phenylethylacetaldehyde were not fruitful because of the formation of pyridinium salt as a side reaction.

Further syntheses of tetrahydroisoquinoline derivatives were performed using the Bischler–Napieralski reaction. Compound **6**, an intermediate for branching to various alkyl amides **7a–g**, was obtained successively via protection of primary amine with

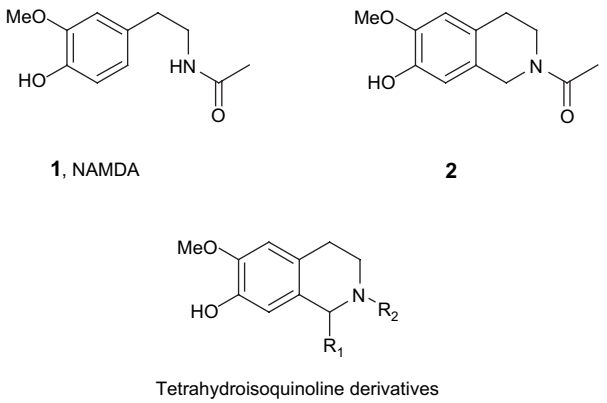
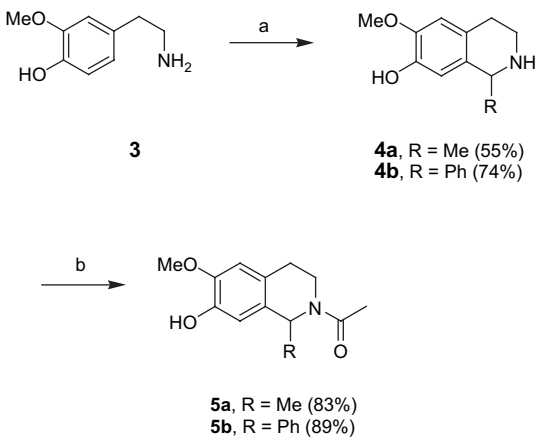


Fig. 1. NAMDA derivatives showing NO inhibitory effect.



Scheme 1. (a) Synthesis of **4a**: acetaldehyde, 1 M HCl, 100 °C, 24 h; synthesis of **4b**: (i) benzaldehyde, $MgSO_4$, TEA, MeOH, reflux, 3 h; (ii) TFA, 80 °C, 100 min; (b) Ac_2O, Et_3N, CH_2Cl_2, rt, 1 h.

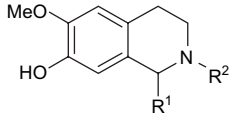
tert-butyloxycarbonyl anhydride, benzylation of phenol with benzyl bromide, and deprotection of *tert*-butyloxycarbonyl group. Acylations with various acyl chlorides, such as propionyl, butyryl, isobutyryl, α-phenylacetyl, 4-methylbutyryl, cyclopropanecarbonyl, and cyclobutanecarbonyl, afforded amide derivatives **7a–g** in high yields. These acylated compounds **7a–g** were treated with phosphorus oxychloride to obtain cyclized dihydroisoquinoline and subsequent reductions by sodium cyanoborohydride yielded 7-benzyloxy tetrahydroisoquinoline derivatives. Palladium catalyzed debenzylation gave various C1 alkyl substituted tetrahydroisoquinoline derivatives **8a–g** as hydrochloride salts. These compounds were reacted with acetic anhydride to produce target compounds **9a–g**. All C1 substituted tetrahydroisoquinoline derivatives **5a**, **b**, **9a–g** were isolated as racemic mixtures.

We also synthesized *N*2-acyl derivatives **11a–e** and their reduced forms of carbonyl groups **12a–c**. 7-Hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**10**) was prepared according to the known procedure [21]. Various *N*2-acyl tetrahydroisoquinolines **11a–e** were readily obtained in the presence of triethylamine with commercially available acyl chloride or anhydride in dichloromethane. The syntheses of *N*2-alkyl derivatives **12a–c** were accomplished from amide **11a–c** by reduction with lithium aluminium hydride.

3. Results and discussion

We synthesized 17 tetrahydroisoquinoline derivatives that have a variable alkyl group at C1 position and/or an acyl group at N2 position. Their effects on NO production in activated microglia were evaluated. For those compounds that led to decreased NO production, we also evaluated their cytotoxicity in order to ascertain that the apparent NO decrease was not due to cell death. Generally, the compounds did not show the increase of cytotoxicity (Table 1) and these values were similar to our previously reported results [21]. To compare the NO inhibition activity of the newly synthesized derivatives with those of the previous study, we selected tetrahydroisoquinoline **2** as

Table 1
Effects on the production of NO and cell survival[a]



Comp.	R^1	R^2	Nitrite[b]	LDH[c]
2	H	Ac	46	107
5a	CH_3	Ac	40	104
9a	CH_2CH_3	Ac	50	–
9b	$CH_2CH_2CH_3$	Ac	84	–
9c	$CH(CH_3)_2$	Ac	39	–
9e	$CH_2CH(CH_3)_2$	Ac	113	–
5b	Ph	Ac	51	104
9d	CH_2Ph	Ac	96	80
9f	Cyclopropyl	Ac	60	–
9g	Cyclobutyl	Ac	69	–
11a	H	$COCH_2CH_3$	36	104
11b	H	$COCH_2CH_2CH_3$	74	91
11d	H	$COCH(CH_3)_2$	82	90
11e	H	$COCH_2CH(CH_3)_2$	90	84
11c	H	Cyclohexanecarbonyl	64	82
12a	H	$CH_2CH_2CH_3$	82	86
12b	H	$CH_2CH_2CH_2CH_3$	111	93
12c	H	Cyclohexylmethyl	63	87

[a] All values were obtained from triplicate experiments and averaged.
[b] Amount of nitrite in the presence of each drug (100 μM) in LPS-induced BV-2 cells, presented as percentage of LPS-activated BV-2.
[c] Cytotoxicity of each drug was assessed by lactate dehydrogenase (LDH) activity released into the medium, presented as percentage of LPS-activated BV-2.

a standard compound. As shown in Table 1, this compound at 100 μM inhibited NO production to 46% of that in the LPS-alone control. Among the newly synthesized compounds, **5a**, **9c** and **11a** showed a stronger inhibitory effect than **2**.

In the series of non-aromatic C1 alkyl substituents **5a**, **9a**, **b**, **c**, and **e**, the inhibitory activity was decreased with increasing carbon length at C1 position in general. In the cases of C1 phenyl compound **5b** and benzyl compound **9d**, NO production was inhibited by 49% and 4%, respectively. Although cyclopropyl compound **9f** and cyclobutyl compound **9g** have quite a large alkyl group, their inhibitory activity was more effective compared to **9b** and **9e** that have a flexible alkyl carbon. It is possible that the rigidity of the alkyl group can facilitate binding.

The compounds with acyl derivatives on N2, such as propionyl **11a**, butyryl **11b**, isobutyryl **11d**, and isopentanoyl **11e**, reduced NO production to 36%, 74%, 82%, and 90% of control, respectively. This tendency was also very similar to that observed with the C1 alkyl substituents mentioned above. The *N*2-alkyl substituents **12a**, **b**, and **c** were less effective. However, cyclohexylmethyl compound **12c** and cyclohexanecarbonyl compound **11c** were quite effective (63% and 64%, respectively), and the reason for this is unknown.

It is possible that the inhibitory effect of these compounds on NO production might be due to their effect on BH4. We therefore evaluated the degrees of BH4 production in the presence of **2**, **5a**, **11a**, and **11c**, which have exhibited the highest NO inhibitory activities. As shown in Fig. 2, the amounts of BH4 produced in the LPS-activated microglia decreased in a manner dependent on the drugs' concentration. Thus, it is possible that these drugs inhibit BH4 production and therefore lead to interruption of iNOS dimerization.

In conclusion, we have designed and synthesized 17 tetrahydroisoquinoline derivatives and found a potent compound **11a** that inhibits NO production by 64% in LPS-stimulated BV-2 cells at 100 μM. BH4 production was also reduced by 49%. It appears that a small alkyl group at C1 position or a small acyl group at N2 position yields compounds with potentially stronger inhibitory effects on BH4 and NO production. Compound **11a** might serve as a useful candidate with which inflammation-related damages can be controlled.

4. Experimental protocols

4.1. Chemistry

4.1.1. General information

Reagents were purchased from Aldrich Company and anhydrous solvents were purchased form Aldrich or Burdick & Jackson Company. Reaction progress was followed by thin layer chromatography using silica gel glass plates containing F-254 indicator (Merck). Visualization of organic compounds on TLC was monitored by UV light or phosphomolybdic acid indicator. HPLC solvents were purchased from Burdick & Jackson Company Analysis of mass trace with standard compounds was performed with Gilson HPLC system. ^1H and ^{13}C NMR spectra were btained on a Varian Gemini-200 or -400 and chemical shifts are reported in δ unit (ppm) relative to tetramethylsilane. Coupling constants are reported in hertz. Melting points were checked using OptiMelt apparatus (Stanford research systems) and are uncorrected. Low-resolution electron impact (EI, 70 eV) spectra were obtained on a Varian 1200L single quadruple GC–MS system with CP-3800GC

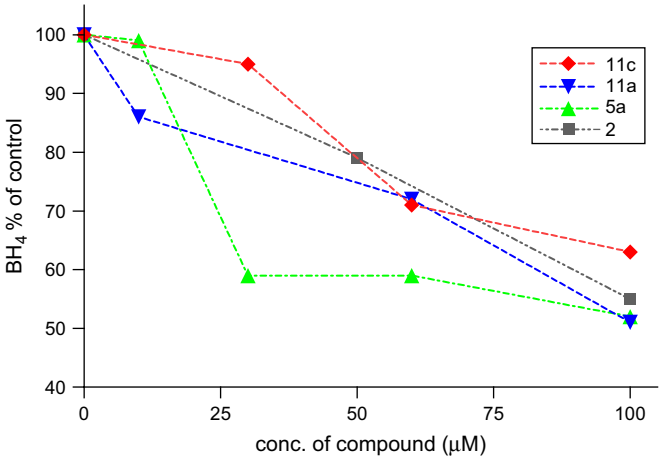


Fig. 2. Amount of BH4 produced in LPS-activated BV-2 cells in the presence of various concentrations of drugs. Data points: **2** (50 μM – 79%, 100 μM – 55%), **5a** (10 μM – 99%, 30 μM – 59%, 60 μM – 59%, 100 μM – 52%), **11a** (10 μM – 86%, 60 μM – 72%, 100 μM – 51%), **11c** (30 μM – 95%, 60 μM – 71%, 100 μM – 63%). The data are presented as percentage of LPS-alone control.

and ESI low-resolution mass spectra were obtained on a Varian 1200L quadruple LC/MS system. Elemental analyses were performed on Flash EA1112 (Thermo Electron Corporation) by the Center for Collaborative Instruments at Inha University.

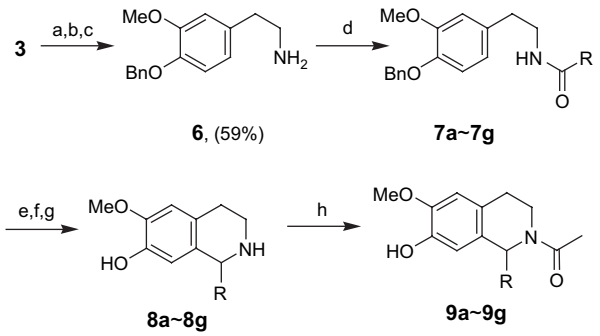
*4.1.2. Procedures for the preparation of **5a** and **5b** (Scheme 1)*

*4.1.2.1. 2-Acetyl-7-hydroxy-6-methoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (**5a**).* To a solution of **3** (1.96 mmol, 400 mg) in 1 M HCl solution (10 mL) under pressure tube, acetaldehyde (15.7 mmol, 692 mg) was added. After the tube was surely capped, the reaction was heated at 100 °C for 24 h. The solution was neutralized by saturated $NaHCO_3$ solution and evaporated under vacuum. The powder was dissolved with methanol and precipitates were removed. After the solution was concentrated, flash column chromatography (1% MeOH/EtOAc) was performed. Compound **4a** was obtained as a hydrochloride salt from MeOH and 1.0 M hydrogen chloride in diethyl ether (off-white powder, 250 mg, 55% yield): m.p. 175–177 °C dec. (lit. 174–175 °C [23]); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (br s, 1H), 9.40 (br s, 1H), 9.06 (s, 1H), 6.72 (s, 1H), 6.67 (s, 1H), 4.32–4.30 (m, 1H), 3.47 (s, 3H), 3.28–3.36 (m, 1H), 3.21–3.22 (m, 1H), 2.29–2.00 (m, 1H), 1.52 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.1, 145.4, 126.0, 122.1, 112.7, 111.9, 55.6, 49.7, 38.6, 24.6, 19.1; MS (CI) 194 ($M^+ + 1$, 100), 178, 164. Registry: 98321-33-6. Compound **4a** (0.44 mmol, 100 mg) was poured in CH_2Cl_2 (5 mL) and then Et_3N (1.0 mmol) and acetic anhydride (0.44 mmol, 45 mg) were slowly added at room temperature. After 1 h, solvent was evaporated under vacuum. The residual solution was neutralized by saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and solvent was evaporated under reduced pressure. Compound **5a** (91 mg, 83%) was obtained as a white solid by crystallization in CH_2Cl_2 and hexane: m.p. 175–177 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, one conformer of C5–H), 6.66 (s, one conformer of C5–H), 6.59 (s, one conformer of C8–H), 6.57 (s, one conformer of C8–H), 5.83 (s, one conformer of O–H), 5.78 (s, one conformer of O–H), 5.53 (q, $J = 6.6$ Hz, one conformer of C1–H), 4.83 (q, $J = 6.6$ Hz, one conformer of C1–H), 4.70–4.65 (m, one conformer of C3–H), 3.82–3.76 (m, one conformer of C3–H), 3.52–3.44 (m, one conformer of C3–H), 3.02–2.95 (m, one conformer of C3–H), 2.90–2.79 (m, 1H), 2.75–2.70 (m, one conformer of C4–H), 2.69–2.60 (m, one conformer of C4–H), 2.18 (s, one conformer of C1–CH_3), 2.15 (s, one conformer of C1–CH_3), 1.49 (d, $J = 6.4$ Hz, one conformer of $COCH_3$), 1.40 (d, $J = 6.8$ Hz, one conformer of $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 168.7, 145.5, 145.3, 144.3, 144.1, 131.3, 130.0, 125.6, 124.4, 112.8, 112.3, 110.8, 110.4, 55.9, 52.3, 47.9, 40.4, 34.8, 29.0, 28.2, 22.5, 21.9, 21.5, 21.4; MS (EI) 235 (M^+, 100), 220. HRMS (EI) *m/z* calcd for $C_{13}H_{17}NO_3$ $[M]^+$ 235.1208; found 235.1209.

*4.1.2.2. 2-Acetyl-7-hydroxy-6-methoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline (**5b**).* A mixture of **3** (1.0 mmol, 204 mg), $MgSO_4$ (2.49 mmol, 300 mg), benzaldehyde (1.0 mmol, 106 mg), and Et_3N (2.0 mmol, 202 mg) in anhydrous MeOH was refluxed for 3 h. The mixture was filtered through celite and the solution was evaporated under reduced pressure. The residual solid was refluxed in trifluoroacetic acid (TFA) for 1 h and then TFA was evaporated. After neutralization by $NaHCO_3$ solution, organics were extracted by CH_2Cl_2. The organic layer was dried over Na_2SO_4 and flash column chromatography (5:95, MeOH:CH_2Cl_2) gave **4b** (188 mg, 74%) as a white foam: m.p. 176–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (br s, 1H),7.22–7.32 (m, 5H), 6.63 (s, 1H), 6.04 (s, 1H), 3.72 (s, 3H), 3.01–3.10 (m, 1H), 2.76–2.89 (m, 2H), 2.55–2.65 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.0, 145.6, 144.1, 130.7, 128.9, 128.0, 126.8, 125.7, 114.4, 112.2, 60.8, 55.5, 42.0, 28.8; MS (CI) 284, 256 ($M^+ + 1$, 100), 178. Registry: 72105-97-6. Compound **4b** (57 mg, 0.223 mmol) dissolved in chloroform (5 mL) was reacted with acetic anhydride (23 mg, 0.223 mmol) at room temperature for 1 h. After neutralization by $NaHCO_3$ solution, organics were extracted by CH_2Cl_2. Compound **5b** (59 mg, 89%) was recrystallized from CH_2Cl_2 and hexane as a white solid: m.p. 168–169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.18 (m, 5H), 6.86–6.58 (m, several conformer peaks of C5–H and C8–H, 3H), 5.84–5.30 (m, several conformer peaks of O–H, 1H), 4.33–4.27 (m, one conformer of C1–H), 3.89 (s, 3H) 3.72–3.66 (m, one conformer of C1–H), 3.46–3.38 (m, one conformer of C3–H), 3.27–3.21 (m, one conformer of C3–H), 2.96–2.82 (m, one conformer of C4–H), 2.76–2.60 (m, one conformer of C4–H), 2.74 (s, one conformer of $COCH_3$), 2.15 (s, one conformer of $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 168.9, 145.8, 144.2, 143.9, 142.4, 141.4, 128.6, 129.5, 128.1, 127.8, 127.7, 127.5, 127.2, 126.8, 125.7, 114.5, 113.8, 110.8, 110.4, 60.2, 55.9, 54.4, 40.4, 37.6, 28.6, 27.4, 22.1, 21.7; MS (EI) 297 (M^+), 254, 239, 220, 178 (100), 163. HRMS (EI) *m/z* calcd for $C_{18}H_{19}NO_3$ $[M]^+$ 297.1365; found 297.1367.

*4.1.3. Procedures for the preparation of **9a–g***

*4.1.3.1. N-[2-(4-Hydroxy-3-O-methylphenyl)ethyl]amine hydrochloride (**6**).* To a solution of **3** (1.3 g, 6.4 mmol) in chloroform (20 mL) was added *tert*-butyloxycarbonyl anhydride (1.67 g, 7.6 mmol) and Et_3N (1.93 g, 19.5 mmol) (Scheme 2). After 24 h, the reaction was quenched by NH_4Cl solution. Organics were extracted by CH_2Cl_2 and washed by additional H_2O (100 mL × 2). Column chromatography (1:1, EtOAc:hexane) gave a white solid (1.32 g, 59%): m.p. 117–119 °C (lit. 115 °C [26]). Registry: 23699-77-6. It was dissolved in acetone (20 mL). After addition of K_2CO_3 and benzylbromide, the reaction mixture was refluxed for 12 h. Solvent was evaporated under reduced pressure and water was poured. Organics were extracted by ethyl acetate and dried over sodium sulfate. Column chromatography (3:7, EtOAc:hexane) gave white solid (1.57 g, 95%): m.p. 74–75 °C (lit. 68–70 °C [26]). Registry: 23428-81-1. The white solid (16.8 mmol, 6.0 g) in CH_2Cl_2 (20 mL) was treated with trifluoroacetic acid (20 mL) slowly under N_2 at 0 °C. The reaction was stirred for 40 min. After the reaction, the reaction mixture was slowly poured to



7a (90%), **8a** (18%), **9a** (58%), R = CH_2CH_3
7b (94%), **8b** (77%), **9b** (45%), R = $CH_2CH_2CH_3$
7c (96%), **8c** (70%), **9c** (67%), R = $CH(CH_3)_2$
7d (63%), **8d** (63%), **9d** (94%), R = CH_2Ph
7e (93%), **8e** (67%), **9e** (73%), R = $CH_2CH(CH_3)_2$
7f (88%), **8f** (58%), **9f** (72%), R = cyclopropyl
7g (79%), **8g** (81%), **9g** (80%), R = cyclobutyl

Scheme 2. (a) $(Boc)_2O$, Et_3N, $CHCl_3$, rt, 24 h; (b) benzyl bromide, K_2CO_3, acetone, reflux, 12 h; (c) TFA, CH_2Cl_2, 0 °C, 40 min; (d) acyl chloride, TEA, CH_2Cl_2, rt, 30 min−1 h; (e) $POCl_3$, CH_3CN, reflux, 2−5 h; (f) $NaBH_4$, 0 °C−rt, 24 h; (g) Pd/C, H_2, HCl, MeOH, rt, 12 h; (h) Ac_2O, CH_2Cl_2, 0 °C−rt, 2 h.

saturated sodium bicarbonate solution containing ice. Excess of diethyl ether was poured and extracted. Solvent was evaporated and the mixture was dissolved in chloroform, and washed with saturated $NaHCO_3$. Solvent was evaporated. The mixture was dissolved in diethyl ether. The mixture was solidified as HCl salt by pouring 1 M HCl ether (20 mL) and stirring. Filtration gave **6** as a white solid (1.32 g, 64%): m.p. 170−172 °C (lit. 173−174 °C [27]); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.24 (br s, 2H), 7.28−7.45 (m, 5H), 6.96 (d, $J = 8.0$ Hz, 1H), 6.90 (d, $J = 1.8$ Hz, 1H), 6.73 (dd, $J = 8.0$, 1.8 Hz, 1H), 5.04 (s, 1H), 3.77 (s, 3H), 2.96−3.03 (m, 2H), 2.80−2.88 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 149.3, 146.6, 137.2, 130.3, 128.2, 127.6, 127.5, 120.5, 114.1, 113.0, 70.1, 55.6, 32.4; MS (CI) 258 (M^+), 241 (100), 228, 91. Registry: 1860-57-7.

*4.1.3.2. N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]propionamide (**7a**).* To a solution of **6** (3.5 mmol, 0.9 g) in CH_2Cl_2 were added Et_3N (11 mmol, 1.1 g) and propionyl chloride (4.6 mmol, 0.42 g) dropwise at 0 °C. Ice bath was removed and stirred for 30−60 min. After removal of solvent by evaporation, H_2O was added and then extracted with ethyl acetate. The organic layer was washed with brine and water, and dried over Na_2SO_4. Solvent was evaporated and the product was purified by flash column chromatography (3:2:5, EtOAc:CH_2Cl_2:-hexane). Compound **7a** (0.98 g, 90%) was obtained as a white foam: m.p. 88−89 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.26−7.46 (m, 5H), 6.81 (d, $J = 8.0$ Hz, 1H), 6.73 (d, $J = 2.2$ Hz, 1H), 6.64 (dd, $J = 8.2$, 1.9 Hz, 1H), 5.65 (br s, 1H), 5.12 (s, 2H), 3.86 (s, 3H), 3.46 (q, $J = 6.6$ Hz, 2H), 2.73 (t, $J = 7.0$ Hz, 2H), 2.14 (q, $J = 7.5$ Hz, 2H), 1.11 (t, $J = 7.7$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.7, 149.6, 146.7, 137.1, 132.1, 128.4, 127.7, 127.2, 120.5, 114.2, 112.4, 71.0, 55.8, 40.5, 35.2, 29.6, 9.8; MS (EI) 313 (M^+), 240, 149, 137, 91 (100), 65, 57, 30. HRMS (EI) *m/z* calcd for $C_{19}H_{23}NO_3$ $[M]^+$ 313.1678; found 313.1675.

*4.1.3.3. N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]butyramide (**7b**).* Same procedure as for **7a** was performed with **6** (3.5 mmol, 0.9 g), butyryl chloride (4.2 mmol, 0.45 g), and Et_3N (10.5 mmol, 1.06 g) to obtain **7b** (1.1 g, 94%) as a white foam: m.p. 100−101 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.46−7.26 (m, 5H), 6.81 (d, $J = 8.0$ Hz, 1H), 6.73 (d, $J = 1.8$ Hz, 1H), 6.64 (dd, $J = 8.0$, 1.8 Hz, 1H), 5.56 (s, 1H), 5.12 (s, 2H), 3.86 (s, 3H), 3.47 (q, $J = 6.6$ Hz, 2H), 2.74 (t, $J = 7.0$ Hz, 2H), 2.09 (t, $J = 7.6$ Hz, 2H), 1.61 (sext, $J = 7.4$ Hz, 2H), 0.91 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9, 149.7, 146.7, 137.2, 132.0, 128.4, 127.7, 127.2, 120.5, 114.2, 112.4, 71.1, 55.9, 40.5, 38.6, 35.3, 19.1, 13.7; MS (EI) 327 (M^+), 240, 149, 137, 91 (100), 43. HRMS (EI) *m/z* calcd for $C_{20}H_{25}NO_3$ $[M]^+$ 327.1834; found 327.1831.

*4.1.3.4. N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]isobutyramide (**7c**).* Same procedure as for **7a** was performed with **6** (3.50 mmol, 0.9 g), isobutyryl chloride (4.2 mmol, 0.45 g), and Et_3N (11 mmol, 1.1 g) to obtain **7c** (1.1 g, 96%) as a white solid: m.p. 110−112 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.46−7.26 (m, 5H), 6.82 (d, $J = 8.2$ Hz, 1H), 6.73 (d, $J = 1.8$ Hz, 1H), 6.64 (dd, $J = 8.0$, 1.4 Hz, 1H), 5.56 (br s, 1H), 5.13 (s, 2H), 3.87 (s, 3H), 3.47 (q, $J = 6.6$ Hz, 2H), 2.74 (t, $J = 6.9$ Hz, 2H), 2.28 (quin, $J = 7.1$ Hz, 1H), 1.11 (d, $J = 7.0$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.8, 149.7, 146.7, 137.1, 132.1, 128.4, 127.7, 127.2, 120.6, 114.2, 112.4, 71.1, 55.9, 40.4, 35.5, 35.2, 19.5; MS (EI) 327 (M^+), 240, 149, 137, 91 (100), 43. HRMS (EI) *m/z* calcd for $C_{20}H_{25}NO_3$ $[M]^+$ 327.1834; found 327.1836.

*4.1.3.5. N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]-2-phenylacetamide (**7d**).* Same procedure as for **7a** was performed with **6** (3.4 mmol, 1.0 g), Et_3N (10 mmol, 1.0 g) and phenylacetyl chloride (3.8 mmol, 0.52 g) to obtain **7d** (0.8 g, 63%) as a white solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.71−7.47 (m, 10H), 6.65 (d, $J = 8.2$ Hz, 1H), 6.62 (d, $J = 1.8$ Hz, 1H), 6.44 (dd, $J = 8.2$, 2.2 Hz, 1H), 5.39 (br s, 1H), 5.12 (s, 2H), 3.82 (s, 3H), 3.51 (s, 2H), 3.42 (q, $J = 6.4$ Hz, 2H), 2.65 (t, $J = 6.7$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.8, 149.6, 146.7, 137.2, 134.7, 131.7, 129.4, 128.9, 128.5, 127.8, 127.2, 127.1, 120.5, 114.1, 112.2, 71.0, 55.9, 43.8, 40.6, 35.0; MS (EI) 375 (M^+), 240, 149, 137, 91 (100), 65. Registry: 4876-01-1.

*4.1.3.6. N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]-3-methylbutyramide (**7e**).* Same procedure as for **7a** was performed with **6** (3.5 mmol, 0.9 g), isovaleryl chloride (4.2 mmol, 0.51 g), and Et_3N (11 mmol, 1.1 g) to obtain **7e** (1.1 g, 93%) as a white solid: m.p. 114−115 °C (lit. 114−115 °C [28]); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.46−7.25 (m, 5H), 6.82 (d, $J = 8.0$ Hz, 1H), 6.73 (d, $J = 1.8$ Hz, 1H), 6.63 (dd, $J = 8.2$, 2.0 Hz, 1H), 5.51 (br s, 1H), 5.13 (s, 2H), 3.87 (s, 3H), 3.49 (q, $J = 6.7$ Hz, 2H), 2.74 (t, $J = 6.9$ Hz, 2H), 2.15−1.95 (m, 3H), 0.90 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.4, 149.7, 146.7, 137.1, 132.0, 128.5, 127.7, 127.2, 120.5, 114.2, 112.4, 71.1, 55.9, 46.1, 40.4, 35.3, 22.4; MS

(EI) 341 (M^+), 240, 149, 137, 91 (100), 65, 57, 30. Registry: 95290-23-6.

*4.1.3.7. N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]-2-cyclopropylacetamide (**7f**).* Same procedure as for **7a** was performed with **6** (3.9 mmol, 1.0 g), cyclopropanecarbonyl chloride (5.5 mmol, 0.59 g), and Et_3N (12 mmol, 1.2 g) to obtain **7f** (1.12 g, 88%)as a white solid: m.p. 115–116 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.45–7.26 (m, 5H), 6.82 (d, $J = 8.0$ Hz, 1H), 6.74 (d, $J = 1.8$ Hz, 1H), 6.58 (dd, $J = 8.0$, 1.8 Hz, 1H), 5.77 (br s, 1H), 5.13 (s, 2H), 3.87 (s, 3H), 3.48 (q, $J = 6.6$ Hz, 2H), 2.74 (t, $J = 7.0$ Hz, 2H), 1.85–1.19 (m, 1H), 0.95 (quin, $J = 3.8$ Hz, 2H), 0.74–0.64 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.4, 149.6, 146.7, 137.2, 132.1, 128.4, 127.7, 127.2, 120.5, 114.2, 112.4, 71.1, 55.9, 40.8, 35.3, 14.6, 7.0; MS (EI) 325 (M^+), 240, 149, 137, 91 (100), 69, 41. HRMS (EI) *m/z* calcd for $C_{20}H_{23}NO_3$ $[M]^+$ 325.1678; found 325.1678.

*4.1.3.8. N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]-2-cyclobutylacetamide (**7g**).* Same procedure as for **7a** was performed with **6** (3.5 mmol, 0.9 g), cyclobutanecarbonyl chloride (4.6 mmol, 0.54 g), and Et_3N (10.5 mmol, 1.06 g) to obtain **7g** (0.74 g, 79%) as a white solid: m.p. 104–105 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.46–7.25 (m, 5H), 6.81 (d, $J = 8.0$ Hz, 1H), 6.73 (d, $J = 1.8$ Hz, 1H), 6.64 (dd, $J = 8.2$, 2.2 Hz, 1H), 5.49 (br s, 1H), 5.13 (s, 2H), 3.87 (s, 3H), 3.47 (q, $J = 6.6$ Hz, 2H), 2.92 (quin, $J = 8.5$ Hz, 1H), 2.73 (t, $J = 7.0$ Hz, 2H), 2.37–1.98 (m, 4H), 1.94–1.78 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.9, 149.7, 146.7, 137.2, 132.1, 128.4, 127.7, 127.2, 120.6, 114.2, 112.4, 71.1, 55.9, 40.4, 39.9, 35.2, 25.3, 18.1; MS (EI) 339 (M^+), 240, 149, 137, 91 (100), 55. HRMS (EI) *m/z* calcd for $C_{22}H_{27}NO_3$ $[M]^+$ 339.1834; found 339.1838.

*4.1.3.9. 1-Ethyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**8a**).* $POCl_3$ (9.0 mmol, 1.38 g) was added to a solution of **7a** (3.0 mmol, 0.9 g) in anhydrous acetonitrile under N_2. The reaction mixture was refluxed for 2 h and then cooled to room temperature. Solvent was evaporated and vacuumed. The crude mixture was dissolved in anhydrous methanol and $NaBH_4$ (15 mmol, 0.59 mg) was slowly added under ice bath under nitrogen atmosphere. Ice bath was removed and the reaction was stirred for 24 h. After reaction, the mixture was quenched by 0.1 M NaOH solution and filtered with silica gel and Na_2SO_4 fitted drying tube. Saturated sodium bicarbonate solution was poured and then extracted with CH_2Cl_2 two times. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude, a slightly yellow solid, was dissolved in methanol and 10% palladium charcoal (0.1 g) was added. Hydrogenation was performed with H_2 balloon with vigorous stirring for 12 h. After reaction, the mixture was filtered with celite, and solvent was evaporated. Flash column chromatography (3–5% MeOH/CH_2Cl_2) was performed and the product was crystallized as hydrochloride salt of **8a** (128 mg, 18%) from 1.0 M hydrogen chloride in diethyl ether as a white powder: m.p. 150–152 °C (lit. 218–220 °C [29]); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (br s, 1H), 9.07 (br s, 1H), 9.02 (s, 1H), 6.73 (s, 1H), 6.66 (s, 1H), 4.23 (br s, 1H), 3.74 (s, 3H), 3.16 (br s, 1H), 3.01–2.93 (m, 1H), 2.86–2.80 (m, 1H), 1.97–1.87 (m, 2H), 1.01 (t, $J = 3.7$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.1, 145.2, 124.5, 122.5, 113.0, 112.0, 55.6, 55.0, 54.6, 26.2, 24.5, 9.8; LC MS 208.2 ($M^+ + 1$, 100). Registry: 19886-92-1.

*4.1.3.10. 7-Hydroxy-6-methoxy-1-propyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (**8b**).* Same procedure as for **8a** was followed with **7b** (3.0 mmol, 1.0 g), $POCl_3$ (9.0 mmol, 1.38 g), $NaBH_4$ (24 mmol, 0.91 g), and 10% palladium charcoal (0.1 g) to obtain **8b** (0.59 g, 77%) as a white solid: m.p. 209–211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80–9.20 (br s, 1H), 9.04 (br s, 1H), 6.72 (s, 1H), 6.66 (s, 1H), 4.26 (t, $J = 3.2$ Hz, 1H), 3.74 (s, 3H), 3.36–3.30 (m, 1H), 3.00–2.93 (m, 1H), 2.85–2.78 (m, 1H), 1.88–1.81 (m, 2H), 1.52–1.42 (m, 2H), 0.92 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.1, 145.2, 124.9, 122.5, 123.0, 112.0, 55.6, 53.5, 35.6, 24.5, 18.2, 13.7; LC MS (ESI) 222.2 ($M^+ + 1$, 100). HRMS (FAB) *m/z* calcd for $C_{13}H_{20}NO_2$ $[MH]^+$ 222.1494; found 222.1495.

*4.1.3.11. 7-Hydroxy-1-isopropyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**8c**).* Same procedure as for **8a** was followed with **7c** (3.0 mmol, 0.98 g), $POCl_3$ (9.0 mmol, 1.38 g), $NaBH_4$ (24 mmol, 0.91 g), 10% palladium charcoal (0.1 g) to obtain **8c** (536 mg, 70%) as a white solid: m.p. 194–196 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (br s, 1H), 9.04 (br s, 1H), 8.66 (br s, 1H), 6.69 (s, 1H), 4.26 (br t, $J = 3.4$ Hz, 1H), 3.75 (s, 3H), 3.37 (br s, 1H), 3.10–3.08 (m, 2H), 2.78–2.73 (m, 1H), 2.37–2.29 (m, 1H), 1.08 (d, $J = 7.2$ Hz, 3H), 0.84 (d, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.9, 145.3, 123.7, 123.4, 113.0, 111.9, 58.9, 55.5, 43.3, 30.8, 24.6, 19.2, 16.2; LC MS (ESI) 222.2 ($M^+ + 1$, 100). HRMS (FAB) *m/z* calcd for $C_{13}H_{20}NO_2$ $[MH]^+$ 222.1494; found 222.1489.

*4.1.3.12. 1-Benzyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**8d**).* Same procedure as for **8a** was followed with **7d** (1.6 mmol, 0.6 g), $POCl_3$ (13 mmol, 2.0 g), $NaBH_4$ (15 mmol, 0.59 mg), and 10% palladium charcoal (0.1 g) to obtain **8d** (0.1 g, 74%) as a white solid: m.p. 153–156 °C (lit. 115–119 °C w/HCl [30]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.36 (m, 5H), 6.62 (s, 1H), 6.57 (s, 1H), 4.04–4.10 (m, 1H), 3.82 (s, 3H), 3.13–3.22 (m, 2H), 2.70–2.90 (m, 3H), 2.62–2.70 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 145.1, 143.6, 139.1, 131.2, 129.3, 128.6, 126.6, 126.4, 112.0, 111.1, 56.8, 55.9, 42.4, 40.9, 29.5; MS (CI) 270 ($M^+ + 1$), 178 (100). Registry: 57256-36-7.

*4.1.3.13. 7-Hydroxy-1-isobutyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**8e**).* Same procedure as for **8a** was followed with **7e** (3.1 mmol, 1.1 g), $POCl_3$ (9.4 mmol, 1.44 g), $NaBH_4$ (25 mmol, 0.95 mg), and 10% palladium charcoal (0.1 g) to obtain **8e** (567 mg, 67%) as a white solid: m.p. 231–233 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (br

s, 1H), 9.36 (br s, 1H), 9.06 (s, 1H), 6.72 (s, 1H), 6.64 (s, 1H), 4.27 (br d, $J = 3.6$ Hz, 1H), 3.74 (s, 3H), 3.37–3.11 (m, 1H), 3.20–3.08 (m, 1H), 3.02–2.90 (m, 1H), 2.90–2.78 (m, 1H), 2.00–1.89 (m, 1H), 1.88–1.77 (m, 1H), 1.65–1.55 (m, 1H), 0.98 (d, $J = 6.4$ Hz, 3H), 0.95 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.0, 145.2, 125.4, 122.5, 113.0, 112.0, 55.6, 51.8, 43.3, 38.7, 24.4, 23.8, 23.0. 21.6; LC MS (ESI) 236.2 ($M^+ + 1$, 100). HRMS (FAB) *m/z* calcd for $C_{14}H_{22}NO_2$ $[MH]^+$ 236.1651; found 236.1650.

*4.1.3.14. 1-Cyclopropyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**8f**).* Same procedure as for **8a** was followed with **7f** (3.1 mmol, 1.0 g), $POCl_3$ (9.1 mmol, 1.40 g), $NaBH_4$ (25 mmol, 0.93 g), and 10% palladium charcoal (0.1 g) to obtain **8f** (458 mg, 58%) as a white solid: m.p. 210–213 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (br s, 2H), 9.09 (s, 1H), 6.97 (s, 1H), 6.73 (s, 1H), 3.74 (s, 3H), 3.55 (d, $J = 9.6$ Hz, 1H), 3.41–3.36 (m, 1H), 3.14–2.97 (m, 2H), 2.86–2.77 (m, 1H), 1.18–1.09 (m, 1H), 0.88–0.81 (m, 1H), 0.81–0.72 (m, 1H), 0.72–0.64 (m, 1H), 0.60–0.52 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.3, 145.2, 125.1, 122.4, 113.1, 111.9, 58.9, 55.6, 39.8, 24.5, 14.6, 5.8, 2.8; LC MS (ESI) 220.2 ($M^+ + 1$, 100), 203.2. HRMS (FAB) *m/z* calcd for $C_{13}H_{18}NO_2$ $[MH]^+$ 220.1338; found 220.1336.

*4.1.3.15. 1-Cyclobutyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**8g**).* Same procedure as for **8a** was followed with **7g** (3.0 mmol, 1.0 g), $POCl_3$ (8.8 mmol, 1.36 g), $NaBH_4$ (24 mmol, 0.89 g), and 10% palladium charcoal (0.1 g) to obtain **8g** (645 mg, 81%) as a white solid: m.p. 269–271 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90–9.00 (br s, 2H), 9.07 (s, 1H), 6.73 (s, 1H), 6.62 (s, 1H), 4.20 (d, $J = 9.2$ Hz, one conformer of C1–H), 4.14 (br d, $J = 4.8$ Hz, one conformer of C1–H), 3.73 (s, 3H), 3.31–3.25 (m, 1H), 3.16 (d, $J = 4.0$ Hz, 1H), 3.13–3.07 (m, 1H), 2.98–2.91 (m, 1H), 2.85–2.76 (m, 1H), 2.78–2.67 (m, 1H), 2.17–2.02 (m, 3H), 2.02–1.92 (m, 1H), 1.89–1.70 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.1, 145.1, 124.1, 122.4, 112.9, 112.1, 57.5, 55.5, 48.6, 38.3, 26.8, 25.2, 24.5, 17.6; LC MS (ESI) 234.2 ($M^+ + 1$, 100). HRMS (FAB) *m/z* calcd for $C_{14}H_{20}NO_2$ $[MH]^+$ 234.1494; found 234.1497.

*4.1.3.16. 2-Acetyl-1-ethyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**9a**).* To a solution of **8a** (0.36 mmol, 85 mg) in CH_2Cl_2 (10 mL) were added acetic anhydride (0.35 mmol, 36 mg) and Et_3N (1.8 mmol, 0.18 g) at room temperature. After stirring for 2 h, solvent was evaporated at reduced pressure. Flash column chromatography (1:1, EtOAc:hexane) was performed and recrystallization from CH_2Cl_2 and hexane gave **9a** (51 mg, 58%) as a white solid: m.p. 131–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (s, one conformer of C6–H or C8–H), 6.65 (s, one conformer of C6–H or C8–H), 6.58 (s, one conformer of C6–H or C8–H), 6.56 (s, one conformer of C6–H or C8–H), 5.46–5.40 (dd, $J = 8.8$, 6.0 Hz, one conformer of C1–H), 4.66–4.59 (m, one conformer of C3–H), 4.59–4.53 (t, $J = 7.2$ Hz, one conformer of C1–H), 3.859 (s, one conformer of OCH_3), 3.850 (s, one conformer of OCH_3), 3.78–3.73 (m, one conformer of C3–H), 3.55–3.42 (m, one conformer of C3–H), 3.06–2.97 (m, one conformer of C3–H), 2.94–2.79 (m, one conformer of C4–H, 1H), 2.79–2.70 (m, one conformer of C4–H), 2.65–2.57 (m, one conformer of C4–H), 2.17 (s, one conformer of $-NCOCH_3$), 2.16 (s, one conformer of $-NCOCH_3$), 1.88–1.70 (m, 2H), 0.96 (td, $J = 29.2$, 7.2 Hz, 3H). Anal. calculated ($C_{14}H_{19}NO_3$) C, 67.45; H, 7.68; N, 5.62; found C, 67.32; H, 7.84; N, 5.67.

*4.1.3.17. 2-Acetyl-7-hydroxy-6-methoxy-1-propyl-1,2,3,4-tetrahydroisoquinoline (**9b**).* Same procedure as for **9a** was followed with **8b** (0.78 mmol, 0.20 g), acetic anhydride (0.78 mmol, 79 mg) and Et_3N (2.3 mmol, 0.24 g) and it gave **9b** (92 mg, 45%) as a white solid: m.p. 121–122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (s, one conformer of C6–H or C8–H), 6.64 (s, one conformer of C6–H or C8–H), 6.58 (s, one conformer of C6–H or C8–H), 6.56 (s, one conformer of C6–H or C8–H), 5.54–5.48 (m, one conformer of C1–H), 4.70–4.56 (m, one conformer of C1–H and C3–H), 3.854 (s, one conformer of OCH_3), 3.845 (s, one conformer of OCH_3), 3.79–3.71 (m, one conformer of C3–H), 3.57–3.47 (m, one conformer of C3–H), 3.10–3.00 (m, one conformer of C3–H), 2.95–2.58 (m, one conformer of C4–H, 2H), 2.164 (s, one conformer of $-NCOCH_3$), 2.157 (s, one conformer of $-NCOCH_3$), 1.88–1.60 (m, 2H), 1.50–1.24 (m, 2H), 1.12–0.84 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 145.5, 144.1, 143.8, 130.8, 129.9, 125.5, 124.4, 113.3, 112.5, 110.9, 110.4, 57.1, 55.9, 51.9, 40.7, 29.4, 38.7, 35.4, 28.7, 27.6, 21.8, 21.7, 19.9, 19.6, 14.0. Anal. calculated ($C_{15}H_{21}NO_3$) C, 68.42; H, 8.04; N, 5.32; found C, 68.48; H, 8.04; N, 5.30.

*4.1.3.18. 2-Acetyl-7-hydroxy-1-isopropyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**9c**).* Same procedure as for **9a** was followed with **8c** (0.78 mmol, 0.20 g), acetic anhydride (0.78 mmol, 79 mg) and Et_3N (3.9 mmol, 0.39 g) and it gave **9c** (141 mg, 67%) as a white solid: m.p. 136–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.73 (s, one conformer of C6–H or C8–H), 6.67 (s, one conformer of C6–H or C8–H), 6.61 (s, one conformer of C6–H or C8–H), 6.60 (s, one conformer of C6–H or C8–H), 5.18 (d, $J = 8.8$ Hz, one conformer of C1–H), 4.54–4.44 (m, one conformer of C3–H), 4.16 (d, $J = 8.2$ Hz, one conformer of C1–H), 3.857 (s, one conformer of OCH_3), 3.850 (s, one conformer of OCH_3), 3.74–3.62 (m, one conformer of C3–H), 3.26–3.16 (m, one conformer of C3–H), 2.95–2.72 (m, one conformer of C4–H, 2H), 2.15 (s, 3H), 2.08–1.90 (m, 1H), 1.14–0.92 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.8, 145.7, 145.5, 143.5, 143.1, 130.0, 128.9, 125.6, 124.9, 114.6, 113.8, 111.0, 110.4, 63.6, 57.7, 55.9, 42.0, 36.5, 33.7, 33.4, 28.0, 26.8, 22.0, 21.98, 20.5, 20.2, 20.0, 19.8. Anal. calculated ($C_{15}H_{21}NO_3$) C, 68.42; H, 8.04; N, 5.32; found C, 68.17; H, 8.28; N, 5.35.

*4.1.3.19. 2-Acetyl-1-benzyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**9d**).* Same procedure as for **9a** was followed with **8d** (0.26 mmol, 69 mg) and acetic anhydride

(0.28 mmol, 29 mg) and it gave **9d** (75 mg, 94%) as a white foam: m.p. 66–70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–6.95 (m, 5H), 6.72 (s, one conformer of C6–H or C8–H), 6.58 (s, one conformer of C6–H or C8–H), 6.50 (s, one conformer of C6–H or C8–H), 6.48 (s, one conformer of C6–H or C8–H), 4.62–4.40 (m, one conformer of C1–H), 3.85 (s, one conformer of OCH_3), 3.82 (s, one conformer of OCH_3), 3.15–2.95 (m, one conformer of C3–H and one conformer of C4–H), 2.90–2.80 (m, one conformer of benzyl-H), 2.55–2.52 (m, one conformer of benzyl-H), 2.55–2.45 (m, one conformer of benzyl-H), 2.06 (s, one conformer of $-NCOCH_3$), 1.40 (s, one conformer of $-NCOCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 169.3, 145.7, 145.4, 144.0, 143.9, 138.0, 129.7, 129.4, 129.2, 128.9, 128.7, 128.0, 127.0, 126.4, 126.0, 125.2, 113.4, 112.6, 110.9, 110.2, 59.3, 55.9, 55.8, 53.6, 42.9, 42.0, 41.7, 34.9, 28.6, 28.0, 22.0, 20.8; MS (CI) 312 ($M^+ + 1$, 100), 220, 178. HRMS (FAB) *m/z* calcd for $C_{19}H_{21}NO_3$ $[MH]^+$ 312.1600; found 312.1597.

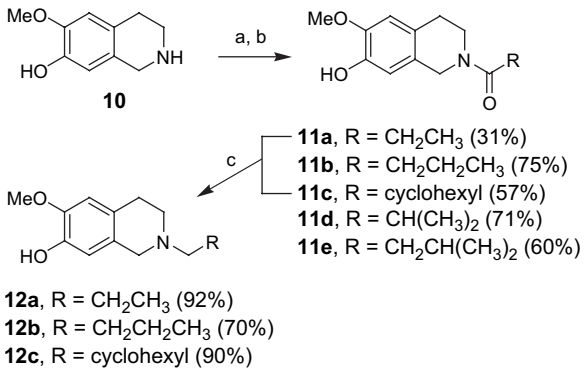
*4.1.3.20. 2-Acetyl-7-hydroxy-1-isobutyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**9e**).* Same procedure as for **9a** was followed with **8e** (0.73 mmol, 0.20 g), acetic anhydride (0.73 mmol, 75 mg) and Et_3N (3.7 mmol, 0.37 g) and it gave **9e** (148 mg, 73%) as a white solid: m.p. 136–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (s, one conformer of C6–H or C8–H), 6.61 (s, one conformer of C6–H or C8–H), 6.58 (s, one conformer of C6–H or C8–H), 6.54 (s, one conformer of C6–H or C8–H), 5.64–5.56 (m, one conformer of C1–H), 4.70–4.64 (m, one conformer of C1–H), 4.58–4.39 (m, one conformer of C3–H), 3.86 (s, one conformer of OCH_3), 3.84 (s, one conformer of OCH_3), 3.80–3.71 (m, one conformer of C3–H), 3.58–3.47 (m, one conformer of C3–H), 3.18–3.07 (m, one conformer of C3–H), 2.94–2.81 (m, one conformer of C4–H, 1H), 2.76–2.69 (m, one conformer of C4–H, 1H), 2.17 (s, one conformer of $-NCOCH_3$), 2.157 (s, one conformer of $-NCOCH_3$), 1.83–1.38 (m, 3H), 1.10–0.88 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 169.5, 145.5, 145.2, 144.1, 143.8, 131.1, 129.9, 125.5, 124.4, 113.3, 112.5, 110.9, 110.5, 55.9, 55.6, 50.4, 46.5, 46.0, 40.3, 35.8, 28.6, 27.4, 25.0, 24.7, 23.4, 23.1, 22.7, 22.4, 21.6, 21.4. Anal. calculated ($C_{16}H_{23}NO_3$) C, 69.29; H, 8.36; N, 5.05; found C, 69.26; H, 8.60; N, 5.02.

*4.1.3.21. 2-Acetyl-1-cyclopropyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**9f**).* Same procedure as for **9a** was followed with **8f** (0.77 mmol, 0.20 g), acetic anhydride (0.77 mmol, 78 mg) and Et_3N (3.9 mmol, 0.39 g) and it gave **9f** (145 mg, 72%) as a white solid: m.p. 175–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, one conformer of C6–H or C8–H), 6.74 (s, one conformer of C6–H or C8–H), 6.59 (s, one conformer of C6–H or C8–H), 6.56 (s, one conformer of C6–H or C8–H), 4.95 (d, $J = 8.8$ Hz, one conformer of C1–H), 4.72–4.63 (m, one conformer of C3–H), 4.17 (d, $J = 8.0$ Hz, one conformer of C1–H), 3.858 (s, one conformer of OCH_3), 3.848 (s, one conformer of OCH_3), 3.85–3.78 (m, one conformer of C3–H), 3.72–3.63 (m, one conformer of C3–H), 3.30–3.18 (m, one conformer of C3–H), 2.96–2.60 (m, one conformer of C4–H, 2H), 2.17 (s, one conformer of $-NCOCH_3$), 2.12 (s, one conformer of $-NCOCH_3$), 1.30–1.10 (m, 1H), 0.77–0.48 (m, 3H), 0.44–0.34 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 145.5, 144.0, 143.7, 129.9, 128.5, 125.7, 124.5, 114.6, 113.3, 112.7, 110.8, 110.4, 60.4, 55.9, 55.5, 41.2, 36.1, 28.8, 27.7, 21.7, 21.5, 18.0, 17.8, 5.31, 5.25, 2.9, 2.5. Anal. calculated ($C_{15}H_{19}NO_3$) C, 68.94; H, 7.33; N, 5.36; found C, 68.94; H, 7.47; N, 5.35.

*4.1.3.22. 2-Acetyl-1-cyclobutyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**9g**).* Same procedure as for **9a** was followed with **8g** (0.88 mmol, 0.24 g), acetic anhydride (0.88 mmol, 89 mg) and Et_3N (4.4 mmol, 0.44 g) and it gave **9g** (195 mg, 80%) as a white solid: m.p. 123–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (s, one conformer of C6–H or C8–H), 6.65 (s, one conformer of C6–H or C8–H), 6.58 (s, one conformer of C6–H or C8–H), 6.56 (s, one conformer of C6–H or C8–H), 5.46 (d, $J = 9.6$ Hz, one conformer of C1–H), 4.68–4.60 (m, one conformer of C3–H), 4.50 (d, $J = 9.2$ Hz, one conformer of C1–H), 3.85 (s, one conformer of OCH_3), 3.84 (s, one conformer of OCH_3), 3.77–3.69 (m, one conformer of C3–H), 3.58–3.44 (m, one conformer of C3–H), 3.04–2.95 (m, one conformer of C3–H), 2.92–2.68 (m, one conformer of C4–H, 2H), 2.66–2.55 (m, 1H), 2.21 (s, one conformer of $-NCOCH_3$), 2.15 (s, one conformer of $-NCOCH_3$), 2.10–1.90 (m, 3H), 1.89–1.68 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 169.3, 145.6, 145.4, 143.8, 143.5, 129.5, 128.4, 125.2, 124.1, 113.0, 112.2, 111.1, 110.6, 61.5, 56.0, 55.9, 41.4, 41.1, 41.0, 35.7, 28.6, 27.8, 27.6, 27.4, 26.1, 25.5, 21.9, 21.7, 17.6. Anal. calculated ($C_{16}H_{21}NO_3$) C, 69.79; H, 7.69; N, 5.09; found C, 70.01; H, 7.81; N, 5.06.

*4.1.4. Procedures for the preparation of **11a–e***

*4.1.4.1. 2-Ethylcarbonyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**11a**).* To a solution of **10** in CH_2Cl_2 (10 mL) were added propionic anhydride and Et_3N and stirred for 1 h at room temperature (Scheme 3). The reaction was quenched by addition of water and the organic layer was extracted with additional CH_2Cl_2 (5 mL). After CH_2Cl_2 was evaporated at reduced pressure, residual oil was dissolved in methanol (10 mL) and K_2CO_3 was added. The reaction was refluxed for 3 h. The solution was filtered and excess CH_2Cl_2 was poured. The organic layer was washed with 1.0 M HCl solution (20 mL) and brine (10 mL). The organic solution was dried over Na_2SO_4 and concentrated by evaporation. Flash column chromatography (1:1, EtOAc:hexane) gave **11a** as a white solid: m.p. 95–96 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.71 (s, one conformer of C5–H or C8–H), 6.66 (s, one conformer of C5–H or C8–H), 6.62 (s, one conformer of C5–H or C8–H), 6.60 (s, one conformer of C5–H or C8–H), 6.10 (s, one conformer of O–H), 5.96 (s, one conformer of O–H), 4.62 (s, one conformer of C1–H), 4.50 (s, one conformer of C1–H), 3.86 (s, O CH_3, 3H), 3.95–3.49 (m, C4–H, 2H), 2.85–2.70 (m, C3–H, 2H), 2.46 (q, $J = 7.4$ Hz, 2H), 1.15–1.05 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.8, 145.5, 144.6, 144.3,



Scheme 3. (a) Propionic anhydride, butyryl chloride, cyclohexanecarbonyl chloride, isobutyryl chloride, or 3-methylbutyryl chloride, TEA, CH_2Cl_2, rt, 1 h; (b) K_2CO_3, MeOH, reflux, 2 h; (c) LAH, THF, reflux, 3–5 h.

126.12, 126.11, 112.5, 111.8, 111.0, 110.6, 56.0, 46.8, 43.8, 43.2, 39.8, 29.1, 28.1, 26.9, 26.7, 9.4; MS (EI) 235 (M^+, 100), 220, 178, 163, 150, 135. HRMS (EI) *m/z* calcd for $C_{13}H_{17}NO_3$ $[M]^+$ 235.1208; found 235.1208.

*4.1.4.2. 7-Hydroxy-6-methoxy-2-propylcarbonyl-1,2,3,4-tetrahydroisoquinoline (**11b**).* Same procedure as for **11a** was followed with **10** (1.0 mmol, 0.22 g), butyryl chloride (1.0 mmol, 93 mg), Et_3N (4.5 mmol, 0.45 g), and K_2CO_3 (0.1 g) and it gave **11b** (187 mg, 75%) as a white solid: m.p. 115–116 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.71 (s, one conformer of C5–H or C8–H), 6.66 (s, one conformer of C5–H or C8–H), 6.61 (s, one conformer of C5–H or C8–H), 6.60 (s, one conformer of C5–H or C8–H), 6.21 (s, one conformer of O–H), 6.03 (s, one conformer of O–H), 4.62 (s, one conformer of C1–H), 4.50 (s, one conformer of C1–H), 3.85 (s, O CH_3, 3H), 3.95–3.60 (m, C4–H, 2H), 2.90–2.70 (m, C3–H, 2H), 2.46 (t, $J = 7.4$ Hz, 2H), 1.80–1.60 (m, 2H), 1.05–0.90 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1, 145.8, 144.7, 144.5, 126.2, 125.2, 112.7, 111.9, 111.2, 110.8, 56.1, 47.1, 43.9, 43.5, 39.9, 35.8, 35.7, 29.3, 28.2, 18.7, 14.1; MS (EI) 249 (M^+, 100), 220, 178, 163, 150. HRMS (EI) *m/z* calcd for $C_{14}H_{19}NO_3$ $[M]^+$ 249.1365; found 249.1361.

*4.1.4.3. 2-Cyclohexylcarbonyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**11c**).* Same procedure as for **11a** was followed with **10** (2.0 mmol, 0.43 g), cyclohexanecarbonyl chloride (4.0 mmol, 0.59 mg), Et_3N (0.9 mL), and K_2CO_3 (0.32 g) and it gave **11c** (302 mg, 57%) as a white solid: m.p. 160–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (s, one conformer of C5–H or C8–H), 6.68 (s, one conformer of C5–H or C8–H), 6.61 (s, one conformer of C5–H or C8–H), 6.59 (s, one conformer of C5–H or C8–H), 5.83 (s, one conformer of O–H), 5.68 (s, one conformer of O–H), 4.61 (s, one conformer of C1–H), 4.54 (s, one conformer of C1–H), 3.861 (s, one conformer of O CH_3), 3.857 (s, one conformer of O CH_3), 3.79 (t, $J = 6.0$ Hz, one conformer of C4–H), 3.68 (t, $J = 6.0$ Hz, one conformer of C4–H), 2.81 (t, $J = 5.6$ Hz, one conformer of C3–H), 2.74 (t, $J = 5.6$ Hz, one conformer of C3–H), 2.59–2.49 (m, 1H), 1.90–1.64 (m, 5H), 1.64–1.44 (m, 2H), 1.38–1.28 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 174.8, 145.4, 145.3, 144.4, 144.1, 126.3, 126.3, 125.2, 125.0, 112.4, 111.6, 110.9, 110.42, 56.0, 46.9, 44.0, 43.2, 41.1, 41.0, 40.0, 29.6, 29.4, 29.3, 28.1, 26.0, 25.9; MS (EI) 289 (M^+, 100), 274, 178, 163, 150. HRMS (EI) *m/z* calcd for $C_{17}H_{23}NO_3$ $[M]^+$ 289.1678; found 289.1682.

*4.1.4.4. 7-Hydroxy-2-isopropylcarbonyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**11d**).* Same procedure as for **11a** was followed with **10** (1.0 mmol, 0.22 g), isobutyryl chloride (2.0 mmol, 21 μL), Et_3N (0.5 mL), and K_2CO_3 (0.16 g) and it gave **11d** (178 mg, 71%) as a white solid: m.p. 115–117 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.76 (s, one conformer of C5–H or C8–H), 6.67 (s, one conformer of C5–H or C8–H), 6.61 (s, one conformer of C5–H or C8–H), 6.20 (s, one conformer of O–H), 6.00 (s, one conformer of O–H), 4.61 (s, one conformer of C1–H), 4.55 (s, one conformer of C1–H), 3.86 (s, O CH_3, 3H), 3.95–3.60 (m, C4–H, 2H), 3.00–2.65 (m, 3H), 1.45 (t, $J = 6.6$ Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.8, 145.5, 144.4, 126.3, 125.0, 112.6, 111.7, 111.0, 110.7, 56.0, 46.8, 44.0, 43.1, 40.1, 30.4, 29.4, 28.0, 19.3; MS (EI) 249 (M^+, 100), 234, 206, 178, 163, 150. HRMS (EI) *m/z* calcd for $C_{14}H_{19}NO_3$ $[M]^+$ 249.1365; found 249.1368.

*4.1.4.5. 7-Hydroxy-2-isobutylcarbonyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**11e**).* Same procedure as for **11a** was followed with **10** (1.0 mmol, 0.22 g), 3-methylbutyryl chloride (2.0 mmol, 25 μL), Et_3N (0.5 mL), and K_2CO_3 (0.16 g) and it gave **11e** (158 mg, 60%) as a white solid: m.p. 141–142 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.71 (s, one conformer of C5–H or C8–H), 6.66 (s, one conformer of C5–H or C8–H), 6.61 (s, one conformer of C5–H or C8–H), 6.60 (s, one conformer of C5–H or C8–H), 6.21 (s, one conformer of O–H), 6.04 (s, one conformer of O–H), 4.63 (s, one conformer of C1–H), 4.51 (s, one conformer of C1–H), 3.85 (s, O CH_3, 3H), 3.95–3.60 (m, C4–H, 2H), 2.85–2.67 (m, 2H), 2.38–2.05 (m, 3H), 1.10–0.95 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.4, 145.7, 145.5, 144.6, 144.3, 126.2, 126.1, 125.0, 112.5, 111.7, 111.0, 110.6, 55.9, 47.2, 43.8, 43.6, 42.5, 42.3, 39.8, 29.2, 28.1, 25.6, 22.7; MS (EI) 263 (M^+), 220 (100), 206, 178, 163, 150. HRMS (EI) *m/z* calcd for $C_{15}H_{21}NO_3$ $[M]^+$ 263.1521; found 263.1523.

*4.1.5. Procedures for the preparation of **12a–c** (Scheme 3)*

*4.1.5.1. 7-Hydroxy-6-methoxy-2-propyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (**12a**).* To a solution of **11a** (0.68 mmol, 0.16 g) in freshly distilled THF (10 mL) was added 1.0 M lithium aluminium hydride (0.6 mL) in THF solution. The reaction mixture was refluxed for 4 h and cooled to room temperature. The reaction was quenched by EtOAc, 1.0 M NaOH solution, and H_2O and the solution was filtered. Organics were extracted by CH_2Cl_2 (20 mL) and concentrated under reduced pressure. Flash column chromatography (5% MeOH/CH_2Cl_2) was performed and **12a** (138 mg, 92%) was

crystallized as a hydrochloride salt (off-white solid) from ethanol and 1.0 M hydrochloride diethyl ether: m.p. 203–205 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (br s, OH, 1H), 6.74 (s, 1H), 6.59 (s, 1H), 4.27 (d, $J = 12.6$ Hz, 1H), 4.06 (dd, $J = 16.0$, 8.0 Hz, 1H), 3.75 (s, 3H), 3.37 (br s, 1H), 3.25–3.00 (m, 4H), 2.95–2.90 (br m, 1H), 1.85–1.75 (m, 2H), 0.90 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.3, 145.4, 121.6, 120.1, 112.9, 111.8, 56.4, 55.6, 51.0, 48.7, 24.3, 16.8, 11.0; MS (EI) 221 (M^+), 192 (100), 150. HRMS (EI) *m/z* calcd for $C_{13}H_{19}NO_2$ $[M]^+$ 221.1416; found 221.1415.

*4.1.5.2. 2-Butyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**12b**).* Same procedure as for **12a** was performed with **11b** (0.56 mmol, 0.14 g) and 1.0 M lithium aluminium hydride (0.6 mL) and it gave **12b** (92 mg, 70%) as a hydrochloride salt (white solid): m.p. 105–110 °C dec.; ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.77 (s, 1H), 6.60 (s, 1H), 4.34 (d, $J = 13.6$ Hz, 1H), 4.06 (dd, $J = 15.2$, 8.0 Hz, 1H), 3.75 (s, 3H), 3.37 (br s, 1H), 3.25–3.00 (m, 4H), 2.95–2.90 (br m, 1H), 1.85–1.75 (m, 2H), 1.50–1.25 (m, 2H), 0.93 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 147.3, 145.4, 121.6, 120.1, 112.9, 111.8, 55.6, 54.6, 51.0, 48.7, 25.1, 24.3, 19.5, 13.5; MS (EI) 235 (M^+), 192 (100), 150. HRMS (EI) *m/z* calcd for $C_{14}H_{21}NO_2$ $[M]^+$ 235.1572; found 235.1571.

*4.1.5.3. 2-Cyclohexylmethyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**12c**).* Same procedure as for **12a** was performed with **11c** (0.6 mmol, 0.18 g) and 1.0 M lithium aluminium hydride (0.4 mL) and it gave **12c** (149 mg, 90%) as a hydrochloride salt (off-white solid): m.p. 193–195 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.76 (s, 1H), 6.61 (s, 1H), 4.34 (d, $J = 13.2$ Hz, 1H), 4.11 (dd, $J = 15.2$, 7.2 Hz, 1H), 3.74 (s, 3H), 3.65–2.65 (m, 6H), 2.00–1.65 (m, 6H), 1.40–1.80 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 147.3, 145.4, 121.5, 119.9, 113.1, 111.8, 60.6, 55.9, 55.6, 32.1, 30.7, 25.4, 25.0, 23.9, 18.5; MS (EI) 275 (M^+), 192 (100), 150. HRMS (EI) *m/z* calcd for $C_{17}H_{25}NO_2$ $[M]^+$ 275.1885; found 275.1885.

4.2. Biology

4.2.1. Cell culture

BV-2 cells, a murine microglial cell line, were grown and maintained in DMEM supplemented with 10% fetal calf serum and penicillin–streptomycin at 37 °C in a humidified incubator under 5% CO_2. For experiments, the cells were plated on polystyrene tissue culture dishes at a density of 2×10^5 cells/well in 24-well culture plates. After 24 h, the cells were changed into fresh medium and treated with bacterial LPS (0.2 μg/mL). The tetrahydroisoquinoline derivatives (at various concentrations dissolved in DMSO) were added at the same time with LPS treatment. After 24 h, the medium was taken to measure NO and BH4 and lactate dehydrogenase (LDH) activity.

4.2.2. NO production

Accumulated nitrite, a stable oxidation metabolite of NO, was measured by the Griess reaction [24]. Briefly, 200 μL aliquots of the culture medium were mixed with 100 μL of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, and 2.5% H_3PO_4) in a 96-well microtiter plate, and the absorbance was read at 540 nm. The effect of each compound on NO production was expressed as the percentage of NO produced by the LPS-treated control cells.

4.2.3. BH4 production

BH4 produced was determined according to the method reported previously [25]. To the 900 μL aliquot of culture medium, 100 μL of 1 M phosphoric acid and 200 μL of acidic iodine solution (0.5% I_2 and 1.0% KI in 0.2 M trichloroacetic acid) were added and incubated for 1 h in the dark. The oxidation reaction was terminated by addition of 0.1 mL of 0.1% ascorbic acid. The reaction mixture was then centrifuged for 15 min at $8000 \times g$ and the supernatant was diluted with distilled water. BH4 was separated isocratically with 5% methanol as mobile phase using HPLC and detected by a fluorescence detector (Waters, Boston, MA, USA). BH4 standard curve was prepared every time. The BH4 content was calculated using Waters 991 computerized integrator system as nanograms of BH4 per milligram of cellular protein and expressed as percentage of LPS-treated control.

4.2.4. Determination of cytotoxicity by LDH activity

Cytotoxicity of the newly synthesized compounds was assessed by determining the activity of LDH released into the culture medium. Aliquots (50 μL) of cell culture medium were incubated at room temperature in the presence of 0.26 mM NADH, 2.87 mM sodium pyruvate, and 100 mM potassium phosphate buffer (pH 7.4) in a total volume of 200 μL. The rate of NAD^+ formation was monitored for 5 min at 2-s intervals at 340 nm using a microplate spectrophotometer (SPECTRA MAX 340 pc; Molecular Devices, Menlo Park, CA). Cytotoxicity values were expressed as percentage of LDH released by the tetrahydroisoquinoline derivatives compared with that released from LPS-treated control cells and thus, numbers approaching 100 indicate no toxicity.

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