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Asymmetric Total Synthesis of Tetrahydroprotoberberine Derivatives and Evaluation of Their Binding Affinities at Dopamine Receptors

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Abstract: Cocaine addiction remains a serious challenge for clinical and medical research because there is no effective pharmacological treatment. L-THP, a natural product isolated from *Corydalis yanhusuo* W.T. Wang, is one of the most frequently used traditional herbs to treat drug addiction in China. Our laboratory first reported that its demethylated metabolites L-ICP, L-CD, and L-CP had high affinity at dopamine D1, D2, and D5 receptors. Here we report the chemical synthesis of these metabolites and other derivatives and their binding affinities at dopamine receptors. The synthesis of these bioactive metabolites will allow further *in vivo* study of their potential in treating cocaine addiction.

Keywords: tetrahydroprotoberberine; cocaine; dopamine receptor

Cocaine is a highly addictive psychostimulant associated with intense craving during periods of abstinence. In the US, an estimated 1.4 million individuals aged 12 years or older were current (past month) users in 2013 (SAMHSA, 2014). Cocaine use disorder (CUD) is likely to continue, and there is no FDA-approved pharmacologic treatment[1-3], so it remains a major challenge for clinical and medical research[4].

L-THP is a natural product isolated from *Corydalis yanhusuo* W.T. Wang, one of the most frequently used traditional herbs to treat drug addiction in China[5]. It has been used in clinical practice in China for >40 years for its analgesic, sedating, and hypnotic effects (commercial name: Rotundine™)[6, 7]. Structurally, it resembles two dopamine molecules fused into a tetracyclic alkaloid (Figure 1). It has low toxicity and a high therapeutic index. Extensive studies, carried out mostly by Chinese scientists, show that it has no affinity for opioid receptors, does not change levels of prostaglandins, binds with moderate affinity to dopamine D1, D2, and D3 receptors[8], and has dual pharmacological properties of D1 partial agonism and D2 antagonism, with some affinity for α-adrenergic and serotonin receptors[9].

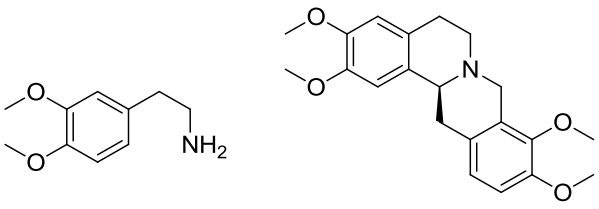
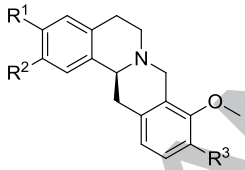


Figure 1. Structure of dopamine and tetrahydroprotoberberine

Recently we identified the monodemethylated metabolites L-corypalmine (L-CP), L-corydalmine (L-CD), and L-isocorypalmine (L-ICP) in the serum of mice and rats 30 min and 60 min after administration of L-THP 20 mg/kg. It is noteworthy that the partial structures (A, B ring and C, D ring) of these metabolites resemble the structure of dopamine. We have found that the C2-demethylated metabolite L-ICP binds to D1 and D5 receptors with high affinity and to D2, D3, and D4 with moderate affinity, and that it is functionally a partial agonist at D1 and D5 and an antagonist at D2, D3, and D4. As L-CP and L-CD are structurally analogous to L-ICP, they are likely to have similar novel pharmacological profiles. We thus hypothesize that L-THP is a pro-drug and exerts its behavioral effects via demethylated metabolites.

In a previous study, we synthesized L-ICP and L-SPD by treating L-THP with MsOH and methionine (1.2 eq) at room temperature[10]. But L-CP and L-CD were the minor products in this reaction. So total synthesis is the best method to get the tetrahydroprotoberberine derivatives (Table 1).

Table 1. Structure of tetrahydroprotoberberine derivatives



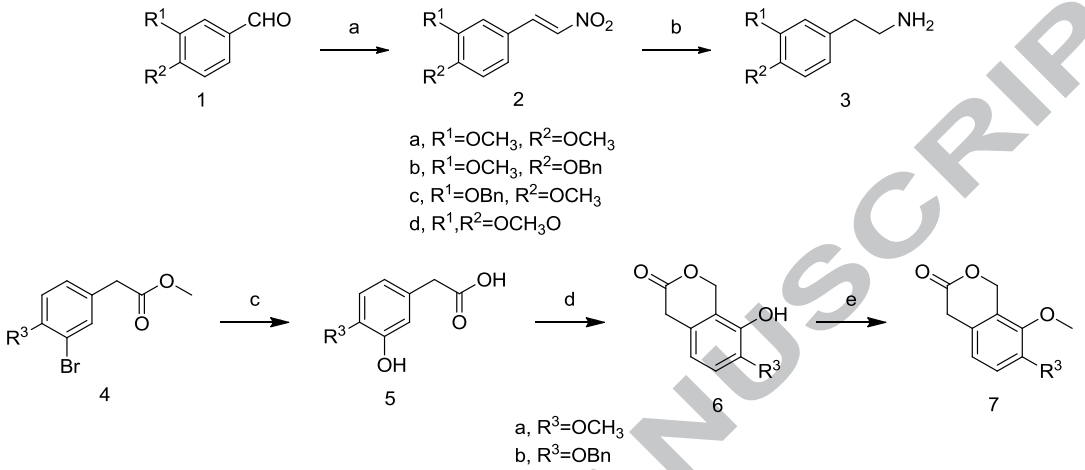
	R1	R2	R3
L-THP	CH_3O	CH_3O	CH_3O
L-ICP (14a)	CH_3O	OH	CH_3O
L-CP (14b)	OH	CH_3O	CH_3O
L-CD (14c)	CH_3O	CH_3O	OH
L-SPD (14d)	CH_3O	OH	OH
L-THB (14e)	OCH_2O		CH_3O
L-DTHB (14f)	OCH_2O		OH

Total synthesis requires the two key intermediates 3 and 7, which were synthesized using the route described in Scheme 1 below. Phenylethylamine 3 was readily synthesized from vanillin or isovanillin; the overall yield was 50-52%. To prepare the intermediates 7, bromination of 2-(4-hydroxyphenyl) acetate with bromine was followed by protection with benzyl bromide or methyl iodide in acetone to give the intermediate 4. Phenols 5 were synthesized from halogen benzene 4 with (bis(8-quinolinolate)copper(II)) catalyst with a chemical yield of 90%. Intermediate 5 was subjected to hydroxymethylation with phenylboric acid and paraformaldehyde to afford the lactone 6. Finally, the lactone 6 was methylated with iodomethane to provide the intermediates 7.

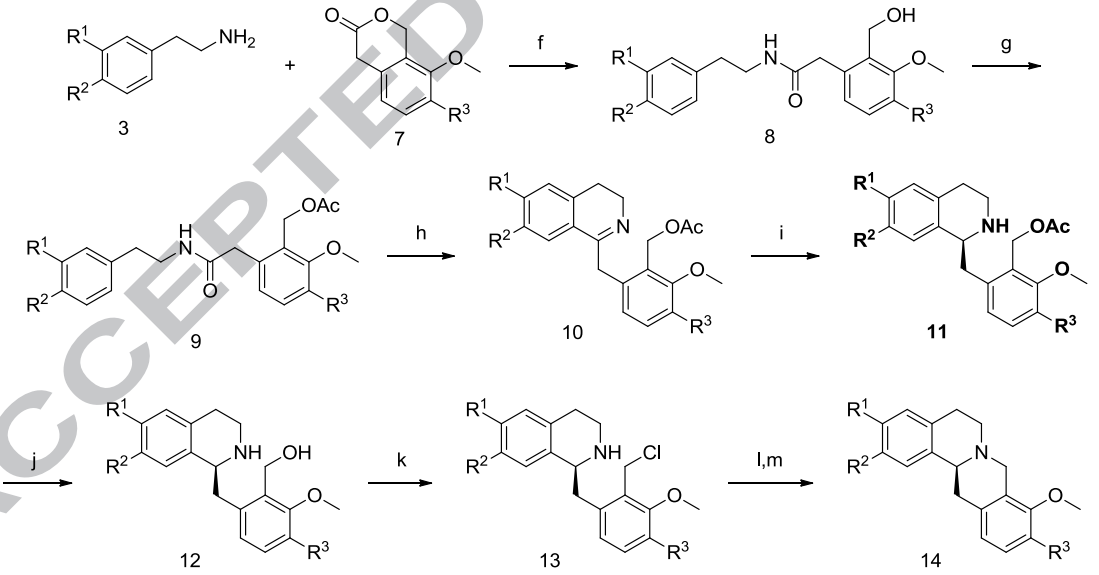
The method for the preparation of Intermediate 5 has been reported by Weller et al.[11], but the reaction conditions require concentrated sodium hydroxide as base and $CuSO_4$ as a catalyst, reacted at 150℃ for 36 hours in stainless steel cannula. The change was made to simplify the reaction conditions by choosing bis(8-quinolinolate)copper(II) as the catalyst. The reaction was done at 120°C for 6 hours without nitrogen protection in sealed stainless steel cannula, and the yield was increased to 85–90%. At the same time, the bis(8-quinolinolate)copper (II) catalyst was recovered by filtration and washing.

Intermediates 8 was prepared by the condensation of lactone 7 with phenethylamine 3 in ethanol. The benzyl alcohol group was converted to the corresponding acetate 9 with acetic anhydride/triethylamine. The Bischler-Napieralski reaction was done in the presence of phosphorus oxychloride in acetonitrile, and the imine 10 was obtained in approximately quantitative yield. Freshly prepared imine 10 was used directly for the next reduction without

further purification. Asymmetric transfer hydrogenation was done under argon in DMF, in the presence of Noyori's catalyst and formic acid/triethylamine (v/v = 5/2) as the hydrogen source. Intermediates 13 was prepared by the chlorination of the benzyl alcohol group in 12 with thionyl chloride in methylene chloride at 0℃. The ring closure reaction was accomplished by basifying the methylene chloride solution with saturated sodium bicarbonate. Deprotection was achieved by refluxing 14 in concentrated hydrochloric acid, with ee values up to 99.6%.



Scheme 1. Preparation of the amine and isochroman-3-one. Reagents and conditions: (a) CH_3NO_2, CH_3COOH, CH_3COONH_4, 100 ℃, 68–78%; (b) THF, $LiAlH_4$, reflux, 70–75%; (c) 30% NaOH，bis(8-quinolinolate)copper(II), 89–90%; (d) $C_6H_5B(OH)_2$, toluene, 110 ℃, 1 h; $(HCHO)_n$, 20 h, then H_2O, reflux, 2 h, 46–50%; (e) CH_3I, acetone, K_2CO_3, reflux, 84–88%.



Scheme 2. Synthesis of (-)-tetrahydroprotoberberine derivatives. Reagents and conditions: (f) C_2H_5OH, reflux, 46–57%; (g) CH_3COCl, pyridine, CH_2Cl_2, 62–70%; (h) $POCl_3$, CH_3CN, reflux; (i) (Ru[(R,R)-Tsdpen](g6-p-cymene), DMF, HCOOH/TEA, room temperature, 32–40%; (j) NaOH, $EtOH/H_2O$, 86–92%; (k) $SOCl_2$, CH_2Cl_2; (l) saturated $NaHCO_3$, 56–65%; (m) concentrated HCl, C_2H_5OH, reflux, 68–85%.

Dopamine receptor binding was performed according to published procedures[12-13]. For D1 and D5 receptors, [^{3}H]SCH23390 was used as the radiolabeled ligand and fluphenazine (10 μM) was used to define nonspecific binding. For D2, D3, and D4 receptors, [^{3}H]methylspiperone and

(+)-butaclamol (4 μM) were used, respectively. Membranes were prepared from transfected HEK293 or CHO cells as described previously[14]. Saturation binding of [^{3}H]SCH23390 or [^{3}H]methylspiperone to the D1 and D5 receptors or D2, D3, and D4 receptors, respectively, was done with at least 8 concentrations of [^{3}H]SCH23390 (ranging from 20 pM to 20 nM) or [^{3}H]methylspiperone (ranging from 20 pM to 10 nM). Binding was done in 50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.4) at room temperature for 1 h in duplicate in a volume of 250 μL with 10-200 μg membrane protein depending on receptor expression level. Incubations were terminated by filtration through Whatman GF/B filters under vacuum, and radioactivity on filters was measured. Competitive inhibition of [^{3}H]SCH23390 (2 nM) binding to the D1 and D5 receptors or [^{3}H]methylspiperone (1 nM) binding to the D2, D3, and D4 receptors by tetrahydroprotoberberine derivatives was performed with various concentrations (10^{-11} M to 10^{-5} M). Binding data were analyzed with the Prism program (GraphPad, San Diego, CA) and Kd, Bmax, and Ki values were determined.

Table 2: K_i values (nM) of tetrahydroprotoberberine derivatives binding to the human dopamine D1, D4, and D5 or D2 and D3 receptors stably expressed in HEK293 cells or CHO cells. Competitive inhibition by three compounds of [^{3}H]SCH23,390 binding to D1 and D5 receptors and [^{3}H]N-methylspiperone binding to D2, D3, and D4 receptors was done and the K_i values were determined. Each value represents mean ± s.e.m. of three experiments performed in duplicate.

	D1	D2	D3	D4	D5
L-THP*	153 ± 9	1125 ± 97	1371 ± 212	>1000	305 ± 65
L-ICP (14a)*	6.2 ± 0.1	41.8 ± 1.8	37.3 ± 3.4	77.4 ± 17.5	9.5 ± 1.4*
L-CP (14b)	199 ± 19	42.4 ± 3.9	262 ± 24	>1000	313 ± 50
L-CD (14c)	107 ± 16	533 ± 86	1713 ± 545	>1000	242 ± 33
L-THB (14e)	48.6 ± 4.4	80.2 ± 9.6	433 ± 45	486 ± 49	62.1 ± 6.0
L-DTHB (14f)	6.5 ± 0.7	49.2 ± 3.7	82.4 ± 0.5	1978 ± 70	15.1 ± 0.9

***** Data from [13]

Table 2 shows competitive inhibition by L-THP and its demethylated analogs of radioligand binding to dopamine receptors. L-THP has a low affinity for D1 and D5 (Ki of 140 nM and 305 nM) receptors and does not appear to bind to D2, D3, or D4 receptors (Ki >1,000 nM). L-THB, and L-DTHB showed high to moderate affinity for D1, D2 and D5, low affinity for D3 and D4. L-ICP showed high to moderate affinity for D1, D2, D3, D4 and D5. L-CP and L-CD have similar agonists activity with L-THP.

A great deal of research has focused on candidate medications to modulate dopaminergic mechanisms through which psychomotor stimulants produce addictive effects, i.e., drugs that expressly target the DA transporter, D1-like receptor, or D2-like receptors[15-17]. However, effective dopaminergic-based pharmacotherapies to manage the widespread use of psychomotor stimulant addiction have not yet been successful, suggesting that explicitly targeting the DA transporter or individual DA receptor subtypes may be insufficient to fully blunt the addiction-related effects of cocaine[15-20]. In this study, a series of metabolites of L-tetrahydroprotoberberine were sterospecifically synthesized following the procedure reported in the literature with minor modifications. The results of dopamine receptor binding revealed that all have stronger affinity than the parent L-THP. Further functional assay indicated that L-ICP, L-THB, and L-DTHB are partial antagonists at D1, antagonists at D2, and agonists at D5. In view of the prominent role of dopaminergic mechanisms in cocaine's abuse-related effects, an alternative, and perhaps more attractive approach for treating cocaine addiction may lie in the use of pharmacological agents that concurrently target both DA D1- and D2-like receptor subtypes. Their effects on cocaine self-administration and reinstatement of cocaine seeking following extinction are being

investigated.

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Graphical abstract