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D_4 DOPAMINE RECEPTOR-SELECTIVE COMPOUNDS FROM THE CHINESE PLANT *PHOEBE CHEKIANGENSIS*

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Abstract: 5-Hydroxy-indoline (**1**), tyramine (**2**), N-norarmepavine (**3**), and a novel glycosylated tetrahydroisoquinoline analog SCH 71450 (**4**) were all isolated from a methanol extract from the fruit of the Chinese plant *Phoebe chekiangensis* based on their activity in displacing D_4 dopaminergic receptor ligand binding. These compounds and related natural products were evaluated for D_4 receptor selectivity relative to D_2 receptor binding displacement. Compounds **1** and **4** exhibited better D_4 selectivity than standard dopaminergic antagonists including the clinically useful compound clozapine. GppNHp shifts in displacement curves indicated that compound **1** is an agonist while compound **4** is an antagonist. © 1997 Elsevier Science Ltd.

Schizophrenia is a disabling mental illness which affects almost 1% of the worldwide population.[1] Effective antipsychotic drugs for treatment of schizophrenia may be divided into two classes. The butyrophenone haloperidol is representative of typical neuroleptics that induce motor toxicities, including tardive dyskinesia as the predominant side effect.[2] Clozapine, an atypical neuroleptic, lacks tardive dyskinesia and associated motor toxicities, but is limited clinically by the side effect of agranulocytosis.[3]

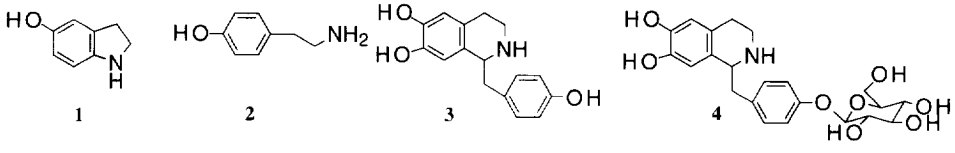
Several lines of evidence suggest that the antipsychotic activities of both classes of drugs are mediated by the blockade of the D_4 subtype of dopaminergic receptors, while blockade of D_2 dopaminergic receptors may mediate the motor toxicities of typical neuroleptics. Therapeutic concentrations of clozapine correlate with D_4 potency, but do not block D_2 receptors.[4] In addition, D_4 receptors are concentrated in cortical and limbic brain regions that are thought to be involved in schizophrenia,[5] and D_4 receptor density is elevated in schizophrenia.[5,6] Finally, antipsychotic drugs that lack D_4 vs. D_2 selectivity induce motor toxicities, whereas drugs selective for D_4 vs. D_2 receptors lack motor toxicities.[2,3] Therefore, we screened natural products to find novel dopaminergic antagonists selective for D_4 receptors relative to D_2 receptors.

Isolation and Identification of Active Compounds

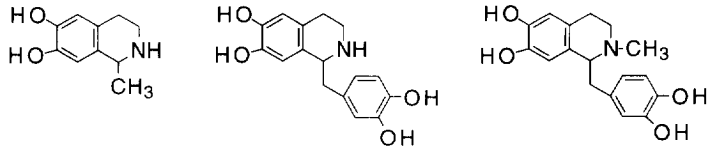
We have isolated four active plant metabolites from the methanolic extract from the fruit of the Chinese plant *Phoebe chekiangensis*. The detanninized extract (120 mg) using a polyamide column and methanol was subjected to bioassay guided fractionation by gel filtration over Sephadex LH-20 (methanol: water, 75: 25). The freeze-dried active fractions (78.2 mg) were run on semi-preparative HPLC on a Deltapak column (2.5 X 30 cm) and eluted with a gradient mixture of acetonitrile (ACN) and water with 0.05% trifluoroacetic acid (TFA) (0-35 min - 0.05% TFA to 10% ACN; 35-60 min - 10% ACN in 0.05% TFA; and 60-120 min 10 to 80% ACN). The active peak eluates on removal of solvent and freeze drying yielded 3.4, 2.5, 3.1, and 3.2 mgs of **1** (5-hydroxy-indoline), **2** (tyramine), **3** (N-norarmepavine) and **4** (SCH 71450) respectively. Compound **1** was the most active and relatively abundant. Chemical ionization mass spectrum using methane as carrier gas showed a molecular ion at 136 indicating a molecular weight of 135, and an odd number of nitrogens in the molecule. Examination of the ^{1}H spectrum of **1** revealed three aromatic proton singlets: two of them were *ortho*-coupled and the third one was

meta-coupled. The ^{13}C spectrum of **1** showed eight carbon signals, two for the methylenes and others for the aromatic carbons. Based on these data and UV spectrum, the structure was established as an indoline with a hydroxy group on the 5- or 6- position. The position of the hydroxyl group in **1** was established to be at C-5 by NOE effect between 3-H and 4-H.

Compound **2** showed a molecular ion peak in EIMS at m/z 137 and in CIMS at m/z 138, indicating a molecular weight of 137. Examination of the ^{1}H and ^{13}C NMR spectra of **2** revealed the presence of a p-hydroxy substituted phenyl ring and two vicinal methylenes. Accomodation of these spectral data along with the presence of a nitrogen suggested it to be the known compound tyramine, as in structure **2**.



1 **2** **3** **4**



5 Salsolinol **6 Tetrahydropapaveroline** **7 Laudanosoline**

Compound **3** showed a characteristic UV pattern with maxima at 200, 222, and 283 nm. The molecular weight was determined by the Cs^{+} ion liquid secondary ionization mass spectrum (SIMS) which showed an intense peak at *m/z* 272, suggesting a molecular weight of 271. Examination of the ^{1}H and ^{13}C NMR spectra of **3** revealed the presence of a *p*-di-substituted and a tetra-substituted aromatic rings. Of the four remaining carbon atoms three were methylenes, of which two were vicinally positioned and the fourth carbon signal was a methine. The characteristic UV spectrum and the position of the methine proton signal revealed the presence of a 1-benzyl substituted tetrahydroisoquinoline type structure. Upon further analysis of ^{1}H and ^{13}C NMR and COSY the structure was established as **3**. Compound **3** has been previously isolated from plants as N-norarmepavine.[7-9]

Compound **4** exhibited a similar UV profile with maxima at 200, 222, 292 nm, and a specific rotation of -2.3° (c 0.44 mg/ml MeOH). FABMS in n-butyl alcohol-thioglycerol-glycerol matrix showed an intense peak at *m/z* 434 indicating a molecular weight of 433, 162 mass units larger than **3**. The molecular formula was determined by SIMS peak matching and suggested the molecular formula to be $C_{22}H_{27}O_8N$, containing an additional $C_5H_{10}O_5$ compared to **3**. The readily notable feature of both ^{1}H and ^{13}C NMR of **4**, as compared to that of **3**, was that it contained most of the signals and some additional oxygenated proton and carbon signals. Exhaustive NMR analysis revealed the presence of a basic unit as in **3** and a sugar unit. The sugar was identified by acid

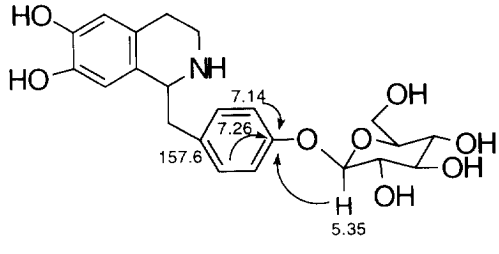


Figure 1
HMBC correlations to establish sugar attachment

hydrolysis of **4** followed by sugar analysis as D-glucose. The NMR spectra in pyridine-d_5 separated the anomeric proton of the sugar and HMBC experiments provided clear evidence that the anomeric carbon of the glucose is attached to the *p*-hydroxy benzyl group in a β-glycosidic fashion. Figure 1 shows the ^{1}H and ^{13}C long range correlations observed in the HMBC which establish the sugar linkage to the p-hydroxybenzylic group of the alkaloid moiety. Compound **4** is a novel glycoside of a tetrahydroisoquinoline alkaloid with a sugar attached to the hydroxy group of the benzyl unit of the tetrahydroisoquinoline moiety.

Dopamine Receptor Binding Activity

Potency and selectivity of these natural products were determined by measurement of displacement of [^{3}H]-spiperone binding to D_2 and D_4 dopaminergic receptors. Crude extracts of *Phoebe chekiangensis* fruit were chosen for isolation of active components based on selective inhibition of D_4 receptor binding. Following purification, compounds **1** (5-hydroxy indoline) and **4** (SCH 71450) both showed selective inhibition of D_4 receptor binding relative to D_2 binding (Figure 2). Compound **1** exhibited IC_{50} values of 1.03 and 39.2 μM for D_4 and D_2 receptor membrane preparations, respectively. Compound **4** exhibited IC_{50} values of 2.42 and 47.5 μM for D_4 and D_2 receptors, respectively.

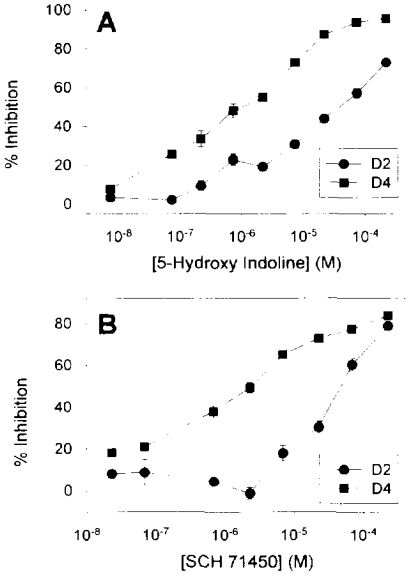


Figure 2: Selectivity of compounds 1 and 4. Potencies were assessed by displacement of the binding of [^{3}H]-spiperone to membranes from cell lines stably expressing human D_2 or $D_{4.2}$ receptors (Receptor Biology, Baltimore, MD). Test compounds were mixed in deep well 96-well plates with 0.5 nM [^{3}H]-spiperone in a buffer containing 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$ and 1 mM EDTA. Binding was initiated by addition of 100 μl membrane and allowed to proceed for 1 hr at room temperature. Nonspecific binding was defined in the presence of 10 μM haloperidol. Reaction mixtures were rapidly filtered through GF/B filters presoaked in 0.5% polyethyleneimine on a Brandel harvester, and washed 3x with ice-cold 50 mM Tris-HCl, pH 7.4. K_i values were determined from observed IC_{50} values using K_d values of 157 pM and 464 pM for [^{3}H]-spiperone which we determined for D_2 and D_4 membranes, respectively.

To determine whether compounds **1** and **4** are agonists or antagonists, we measured the ability of GppNHp (γ-imidoguanosine 5'-triphosphate) to shift the potencies of these compounds in [^{3}H]-spiperone binding assays. GppNHp, a nonhydrolyzable analog of guanosine triphosphate, has previously been shown to decrease the affinity of agonists for dopaminergic receptors, with no effect on antagonist affinity.[10] We chose to conduct

these GppNHp shift analyses with D_2, rather than D_4 receptor membranes, since we and others have found more significant shifts in agonist potency with D_2 membranes than with D_4 membranes.[11,12] Figures 3A and 3B show that 200 μM GppNHp increased the apparent IC_{50} value for dopamine from 729 nM to 2867 nM with no effect on the IC_{50} for the antagonist, haloperidol. These data confirm the observation that GppNHp-induced shifts in potency only occur with agonists. Figures 3C and 3D show that 200 μM GppNHp decreased the potency of compound **1** as with dopamine, but no shift was observed with compound **4**. These results indicate that 5-hydroxy indoline (**1**) is an agonist and SCH 71450 (**4**) is an antagonist at dopaminergic receptors.

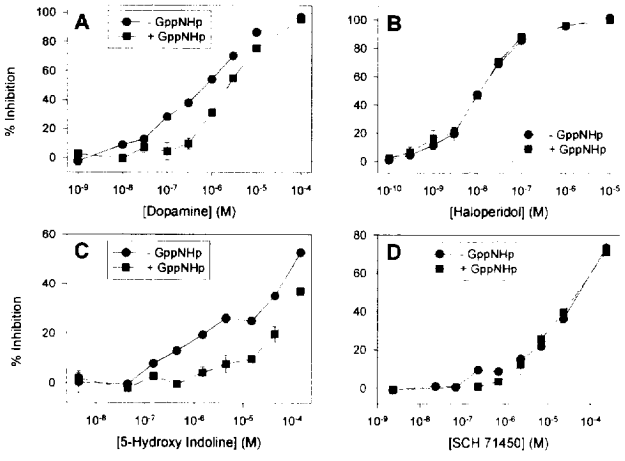


Figure 3: GppNHp-induced potency shifts to determine agonist vs. antagonist activity. Binding experiments were conducted with D_2 receptor membrane preparations as described for figure 2.

Table 1 shows K_i values for compounds **1**-**4** in comparison to some known compounds for displacement of [^{3}H]-spiperone binding to D_2 and D_4 dopaminergic receptors. Of the compounds isolated from *P. chekiangensis*, compound **1** showed the greatest fold selectivity for the D_4 receptor relative to the D_2 receptor (19-fold). The agonist activity suggested for compound **1** (Figure 3C) may not be surprising due to the structural similarity of **1** to the classical neurotransmitter agonists, serotonin and dopamine. Agonists at G-protein coupled receptors generally exhibit both high and low affinity binding interactions, corresponding to GDP-bound and GTP-bound states of the associated G-protein, respectively.[10] Since **1** is an agonist, the K_i values in Table 1 are likely to represent an average of K_i values for high and low affinity receptor states. Nevertheless, the apparent K_i values shown in Table 1 and the data in figure 2A clearly show that compound **1** is D_4 selective. Compound **2** was less active in binding to both receptor subtypes.

Tetrahydroisoquinoline Structure-Activity Relationships

The novel compound **4** was of the most interest as a potential lead for antipsychotic drug development, due to its ten-fold D_4 selectivity and apparent antagonist activity. Although less potent than the clinically useful drug, clozapine, compound **4** showed superior subtype selectivity (Table 1). In this study, clozapine showed only 3.5-fold selectivity for the D_4 receptor, in agreement with previously reported values.[13] The selectivity of **4**, but not **3**, for D_4 relative to D_2 receptor binding prompted us to extend tetrahydroisoquinoline structure-activity relationships to include related commercially available compounds. Salsolinol (**5**) was nonselective among dopaminergic receptor subtypes showing K_i values of approximately 5 μM. Compound **3**, which contains a *p*-

hydroxy phenyl substitution on the salsolinol nucleus, shows approximately a two-fold increase in potency for both receptor subtypes. As observed with tetrahydropapaveroline (**6**), an additional hydroxy substitution on the hydroxyphenyl group of **3** does not confer any change in selectivity or potency. However, substitution of a sugar on the hydroxyphenyl group of **3**, as in compound **4**, dramatically increases selectivity for the D_4 receptor to 10-fold. Similarly **7**, the N-methylated analog of **6**, has improved D_4 potency and selectivity relative to **6**. Thus, **7** showed a K_i value of 350 nM and 5.4-fold selectivity for the D_4 receptor relative to the D_2 receptor. These data suggest that both N-methylation and glycosylation improve potency and selectivity of tetrahydroisoquinolines for the D_4 receptor. Thus, it would be interesting to determine whether an N-methyl analog of compound **4** and a glycosylated analog of compound **7** would have still greater D_4 selectivity and potency.

Table 1: Binding Activity & Selectivity Against D_4 & D_2 Receptor Subtypes

Compound	Dopamine Receptor Binding K_i (μM)			Comments
	D_2	D_4	D_2/D_4	
5-Hydroxy-indoline (**1**)	9.4	0.5	19	Agonist
Tyramine (**2**)	>17	>35	--	N.D.
N-Norarmepavine (**3**)	3.9	3.1	1.3	N.D.
SCH 71450 (**4**)	11.4	1.2	10	Antagonist
Salsolinol (**5**)	5.4	6.4	0.85	N.D.
Tetrahydropapaveroline (**6**)	3.4	2.5	1.4	N.D.
Laudanosoline (**7**)	1.9	0.35	5.4	N.D.
Clozapine	0.25	0.072	3.5	Antagonist
Haloperidol	0.0036	0.0038	0.95	Antagonist
(+)-Butaclamol	0.0006	0.185	0.0034	Antagonist

Tetrahydropapaveroline (**6**), the condensation product of dopamine and dopaldehyde, has previously been reported to displace ^{3}H-spiperone binding to anterior pituitary dopamine receptors with similar potency to that reported here.[14] In Parkinson's disease patients receiving L-dopa therapy[15] and in rats after acute ethanol administration,[16] endogenous tetrahydropapaveroline has been detected in the urine and brain, respectively. When infused directly into cerebral ventricles, tetrahydropapaveroline has been shown to markedly increase preference for alcohol consumption, and has been shown to localize to dopaminergic pathways projecting to the limbic forebrain.[17] Thus, it is interesting to speculate that tetrahydropapaveroline, and perhaps some of the D_4-selective tetrahydroisoquinolines, such as **4** and **7**, described here may play a role in the regulation of psychosis, alcohol consumption, and other dopamine receptor-regulated behaviors.

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Physicochemical Data: **1**: ^{1}H NMR ($CDCl_3$) δ 2.79 (dd, *J* = 8, 0.5 Hz, 2H), 3.1 (dd, *J* = 8, 0.5 Hz, 2H), 6.57 (dd, *J* = 8, 2.5 Hz, 1H), 6.68 (d, *J* = 2.5 Hz, 1H), 6.73 (d, *J* = 8Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 34.1, 42.3, 116.7, 121.0, 129.1, 130.8, 145.5, 146.8; **2**: ^{1}H NMR ($CDCl_3$) δ 2.85 (dd, *J* = 8, 0.5 Hz, 2H), 3.1 (dd, *J* = 8, 0.5 Hz, 2H), 6.75 (d, *J* = 8 Hz, 2H), 7.1 (d, *J* = 8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 34.0, 42.3, 116.7, 128.5, 130.8, 157.8; **3**: ^{1}H NMR ($CDCl_3$) δ 2.95 (dt, *J* = 2, 7 Hz, 2H), 3.2-3.4 (m, 2H), 3.2-3.5 (m, 2H), 4.47 (dd, *J* = 1, 3 Hz, 1H), 6.61(s, 1H), 6.63(s, 1H), 6.8(d, *J* = 8 Hz, 2H), 7.13 (d, *J* = 8 Hz, 2H), ^{13}C NMR ($CDCl_3$) δ 25.8, 40.5, 40.9, 58.0, 114.2, 116.2, 117.0, 123.8, 127.0, 131.7, 145.9, 146.9, 158.2; **4**: ^{1}H NMR (CD_3OD) δ 2.95 (dt, *J* = 2, 7 Hz, 2H), 3.4 (m, 1H), 3.45 (m, 2H), 3.5 (m, 3H), 3.4 - 3.6 (m, 2H), 3.67 (dt, *J* = 6.5,2 Hz, 1H), 3.9 (dd, *J* = 2, 10 Hz, 1H), 4.6 (m, 1H), 4.6(m, 1H), 6.58 (s, 1H), 6.62 (s, 1H), 7.1 (d, *J* = 8 Hz, 2H), 7.25 (d, *J* = 8 Hz, 1H); ^{1}H NMR (Pyridine-d_5) δ 2.9 (dt, 1H), 3.1 (dt, 1H), 3.4 (m, 2H), 3.5 (m, 2H), 3.6 - 3.4 (m, 2H), 4.1 (m,1H), 4.3 (m, 1H), 4.4 (m, 1H), 4.55 (dd,1H), 4.85 (t, 1H), 5.35 (m, 1H), 7.14 (d, *J* = 8 Hz, 2H), 7.2 (s, 2H), 7.26 (d, *J* = 8 Hz, 2H); ^{13}C NMR (CD_3OD) δ 25.9, 40.5, 40.9, 57.9, 62.6, 71.4, 74.9, 78.0, 78.3, 102.3, 114.2, 116.2, 118.4, 123.8, 125.7, 131.7, 145.9, 146.9, 158.7; ^{13}C NMR (Pyridine-d_5) δ 26.0, 39.8, 40.3, 56.7, 62.2, 74.7, 71.1, 78.7, 78.3, 102.1, 114.4, 116.4, 117.0, 123.0, 123.9, 130.6, 131.1, 145.9, 147.0, 157.6.

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