

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Abogada
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ASUNTO	Radicación	09 149 335
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	15166
	Folios	006

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional			
Vía PCT (Fase Nacional)	☒	Capítulo I	☐ Capítulo II
No. Solicitud Internacional	PCT/DK2008/050214		
Fecha de Presentación Internacional	28/Agosto/2008		

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 87 a 175	30/Diciembre/2009
Reivindicaciones:	Folios 176 a 186	30/Diciembre/2009
	Folios 211 a 212	29/Septiembre/2011
Dibujos(figuras 1 y 2):	Folios 188 a 189	30/Diciembre/2009
Prioridad:	PA200701250	31/Agosto/2007

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486), y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "DERIVADOS DE CATECOLAMINA ÚTILES PARA EL TRATAMIENTO DE ENFERMEDAD DE PARKINSON".

El problema planteado en esta solicitud consiste en la necesidad de suministrar

compuestos derivados de catecolaminas útiles en el tratamiento de las enfermedades neurodegenerativas tales como enfermedad de Parkinson y de Huntington.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de la fórmula (I) y la composición que lo contiene.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D 491/004.

4. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

En las Reivindicaciones 1 y sus dependientes, se sugiere una razonable generalización del tipo de compuestos que ha sido sintetizado ó probado, con la actividad biológica que se les atribuye, teniendo en cuenta que R3 es H, metilo, etilo o n-propilo y no existe soporte para cuando R3 es ciclo-propilo, propargilo, alilo, ciclobutilo, hidroxietilo, 3-fluoropropilo y 2-fluoroetilo.(según los ejemplos de la descripción).

5. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la Fecha de Presentación de la Solicitud de Prioridad: Agosto 31 de 2007, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Nº	Documento	Título	Fecha de Publicación*
D1	Artículo	A novel synthesis and pharmacological evaluation of a potential dopamine D1/D2 agonist: 1-Propyl-1,2,3,4,4a,5,10,10a,octahydrobenzo[g]quinoline-6,7-diol	Junio 23 de 2007
D2	US2004/0167163	Optically pure paroxetine precursors	Agosto 26 de 2004

D1¹ divulga el compuesto 4, 1-Propyl-1, 2, 3, 4, 4a, 5, 10, 10a, octahydrobenzo [g] quinoline -6,7- diol con actividad como agonista de dopamina D1/D2.

D2² divulga el compuesto paroxetina con actividad como antiparkinsoniano.

6. Examen de Patentabilidad (Art 14 D486)

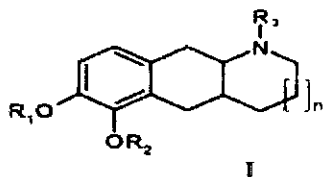
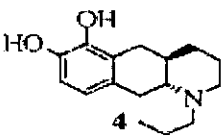
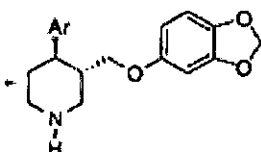
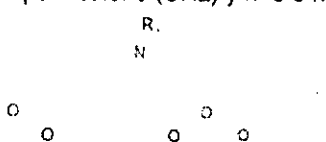
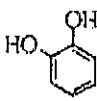
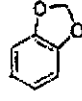
Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en un compuesto que tiene la estructura I.

1 A novel synthesis and pharmacological evaluation of a potential dopamine D1/D2 agonist: 1-Propyl-1,2,3,4,4a,5,10,10a,octahydrobenzo[g]quinoline-6,7-diol, Junio 23 de 2007; págs. 3438-3444

2 http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20040826&CC=US&NR=2004167163A1&KC=A1.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
 I	 4	 paroxetina
R1 y R2 se fusionan para formar un grupo metileno (CH2) y n=0 o n=1 		
R3=n-propilo, H	n-propilo	H

Los documentos D1 y D2 se consideran el estado de la técnica cercano porque D1 divulga el compuesto: 1-Propyl-1, 2, 3, 4, 4a, 5, 10, 10a, octahydrobenzo [g] quinoline -6,7- diol con actividad como agonista de dopamina D1/D2.

Los compuestos reivindicados difieren de los de D1 en que tienen un anillo 1,3 dioxol al fusionarse R1 y R2.

De acuerdo a esta diferencia no se evidencia un efecto logrado por el hecho de formar un anillo 1,3 dioxol al fusionar o sustituyentes R1 y R2.

Por lo tanto, el Problema técnico objetivo a resolver consiste en “cómo modificar el compuesto N°4 1-Propyl-1, 2, 3, 4, 4a, 5, 10, 10a, octahydrobenzo [g] quinoline -6,7- diol, para obtener un compuesto alternativo, con la misma actividad.

Cabe anotar que las pruebas farmacológicas aportadas (págs. 82 a 86, Fls. 168 a 172) muestran que el compuesto reivindicado puede estimular o inhibir los receptores D1 y D2 (al igual que el conocido en D1), además que algunos compuestos aquí incluidos muestran un perfil similar a la apomorfina y L-Dopa que tienen estructura similar al compuesto de D1.

Sin embargo, el compuesto de D2 que tiene actividad como antidepresivo y para tratar la enfermedad de Parkinson (párrafo 1, pág.1) enseña un anillo 1,3 dioxol fusionado al benceno (Figura 8).

De tal manera que tomando en cuenta todo lo anterior, la persona normalmente versada en la materia que pretenda obtener un compuesto alternativo con la misma actividad, al igual que los conocidos y que conozca el estado de la técnica, estaría motivada a realizar la sustitución de los H de los grupos OH en el compuesto de D1 e incluir un grupo CH2, para llegar al objeto de la reivindicación 1 de la solicitud, por lo cual ésta no tiene nivel inventivo.

Puesto que las reivindicaciones dependientes 2 a 13 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

7. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Nº	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Artículo	1-13	Nivel Inventivo
D2	US2004/0167163	1-13	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 20 AGO. 2012

Elaborado por: Lyna del Carmen Mordecay Dunoyer
Revisado Por: Gloria Jacqueline Alfonso Rozo *OK*
Aprobado por: José Luis Salazar López.



A novel synthesis and pharmacological evaluation of a potential dopamine D₁/D₂ agonist: 1-Propyl-1,2,3,4,4a,5,10,10a-octahydrobenzofgluquinoline-6,7-diol

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Abstract—Previously, we have demonstrated that enone prodrugs of dopaminergic catecholamines represent a new type of dopamine (DA) agonist. *Trans*-1-propyl-1,2,3,4,5,10,10a-octahydrobenzofgluquinoline-6,7-diol (TL-334), the active form of *trans*-1-propyl-2,3,4,4a,5,7,8,9,10,10a-decahydro-1H-benzofgluquinolin-6-one (GMP-4650), *in vivo* showed an extremely potent dopaminergic activity. Here, we report a novel synthesis and a pharmacological evaluation of TL-334 by means of microdialysis. © 2007 Elsevier Ltd. All rights reserved.

1. Introduction

Parkinson's disease (PD) is a progressive neurological disorder, which is characterized by the degeneration of nigrostriatal dopaminergic neurons. Currently, the most commonly used medicine for the treatment of PD is L-dopa, a bio-precursor of DA. However, the potential neurotoxicity and the long-term complications, such as dyskinesia associated with the use of L-dopa, encourage the early use of DA agonists,¹ which mimic the action of DA and possibly counteract the development of dyskinesia.²

We have previously described that (S)-6-(*N,N*-di-*n*-propylamino)-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one [(S)-PD148903, 1] is bioactivated *in vivo* to its corresponding catecholamine [(S)-5,6-di-OH-DPAT, 2] which acts as a potent mixed DA D₁/D₂ full agonist. Similarly, *in vivo*, 3 was found to display an extremely potent dopaminergic activity with a long duration of action. However, *in vitro* it does not display any DA receptor binding affinity (D₁ + D₂).³ According to the analysis of brain tissue and blood plasma samples after the *rat* was given 3, its catechol form 4, a well-known D₂ agonist,⁴ was found in brain

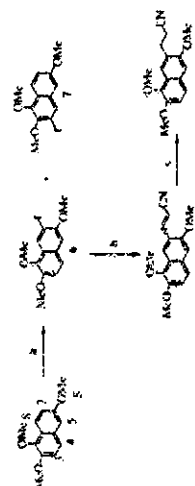
Keywords: Catecholamine; Enone prodrug; Microdialysis; Parkinson's disease.
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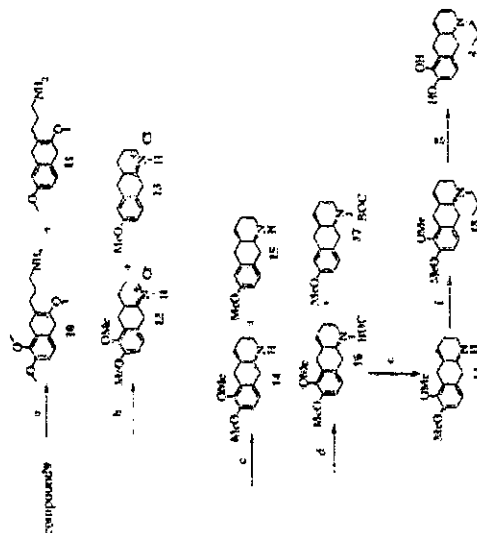


Figure 1. The structures of enones and the corresponding catecholamines (1 and 2, 3 and 4).

intermediates and were not able to reproduce all the steps of that synthesis, we developed a novel synthetic route, based on the work reported by Mellin and Hackel.¹⁴ The final compound 4 was synthesized according to the strategy displayed in Schemes 1 and 2. Compound 5 was synthesized according to the literature.¹⁵ Lithiation of 5 occurred at both C3 and C7 positions and resulted in a 1:1 mixture of anions. The lithium anions were quenched with iodine to produce in equal amounts the isomers 6 and 7. These two isomers were separated



Scheme 1. Synthesis of 4. Reagents and conditions: (a) *n*-BuLi, 2THF, 78 °C to rt, overnight; 78 °C, 1/2, 10 min; (b) ClCH₂CH=CN, Pd(PPh₃)₄, Et₃N, CH₂Cl₂, 120 °C, 3 days; (c) 10% Pd/C, 4 bar H₂, 3 overnight.



Scheme 2. Synthesis of 4. Reagents and conditions: (a) NaOEt, EtOH, reflux, 2 h; (b) MeOH, 17% NaCl, 48 °C, 1 h; (c) NaCN, 10% NaCN, 48 °C, 1 h; (d) TFA, CH₂Cl₂; (e) CH₂Cl₂, 1 bar H₂, 10% Pd/C, *n*-propanol, rt, 2 h; (f) 31% H₂O₂, 45 °C, 3 h.

(Scheme 2). It was found that 10% of the dimethoxy-substituted ring was also reduced; subsequently C1 methoxy group was cleaved and the mono-methoxy by-product 11 was formed. Separation of compounds 10 and 11 was found to be very difficult and tedious; therefore, the ring-closure, yielding compounds 12 and 13, was performed with this mixture. Reduction of these two compounds with NaCNBH₃ in MeOH at pH 4 produced exclusively the *trans*-isomers 14 and 15. These two compounds were separated after protection of the amine with a BOC-group (16 and 17). After column chromatography, the BOC-group was cleaved with TFA. The propylation of 14, 18 was performed via reductive amination with *n*-propionylaldehyde under the condition of 1 bar H₂ and 10% Pd/C in *n*-propanol. The methyl group was cleaved with freshly distilled aqueous 48% HBr and gave the target compound 4 (Scheme 2).

2.2. Pharmacology

2.2.1. In vitro function assay. Table 1 shows that the forskolin-stimulated cAMP accumulation in CHO cells transfected with the human D₂ receptor was completely inhibited by 4, with an IC₅₀ of 0.65 nM. Furthermore, a stimulation of cAMP accumulation in CHO cells stably expressing the human recombinant D₁ receptor was found by 4, with an IC₅₀ of 25 nM. The results of these functional assays showed that 4 is a full DA agonist on both D₁ and D₂ receptor subtypes.

2.2.2. Microdialysis study. The biochemical effects of 4 were studied by measuring their effects on the DA levels in the corpus striatum, the brain area of interest in PD, using microdialysis in freely moving rats.¹⁷ The normal DA release before administration of the test compound was set to 100%, and the changes in output are expressed in relation to the basal DA levels.

Compound 4 was administered *sc* (1 nmol and 10 nmol kg⁻¹, Fig. 2) and *po* (10 nmol kg⁻¹, Fig. 3) to male Wistar rats. A maximum of 30% DA decrease was found after *sc* injection of 1 nmol kg⁻¹ at 90 min post-administration. At this concentration no stereotyped behaviour (a typical DA D₂ receptor stimulation behaviour) was observed. A significant decrease of DA to 30% of basal levels was observed after administration of 10 nmol kg⁻¹ *sc*. The 10 nmol kg⁻¹ *sc* injection maintained decreased DA levels in the range of 30–70% for 6 h. During the experiment with 10 nmol kg⁻¹ *sc*, 4 induced strong

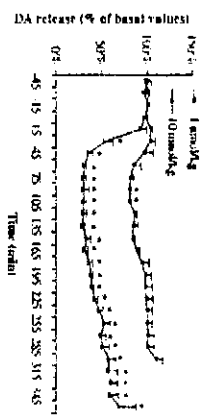


Figure 2. Effect of 4 (1 and 10 nmol kg⁻¹ *sc*, ● and ○, resp.) on striatal DA release in freely moving rats. The results are the mean (±SD) of data obtained from four rats (*n* = 4) (*p* < 0.05, Student's *t* test).

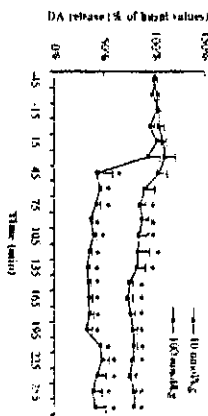
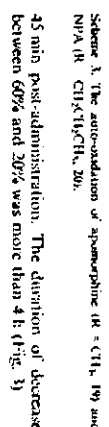


Figure 3. Effect of 4 (10 nmol kg⁻¹ *po*, ●) on striatal DA release in freely moving rats. The results are the mean (±SD) of data obtained from four rats (*n* = 4) (*p* < 0.05, Student's *t* test).

stereotyped behaviour like locomotor activities, peak grooming, yawning, sniffing, rearing, which is consistent with the postsynaptic effects of a centrally acting DA agonist. This phenomenon demonstrated that at low doses presynaptic DA receptors are stimulated, while high doses stimulate both the pre- and postsynaptic DA receptors.

The oral administration of 4 with the dose of 10 nmol kg⁻¹ showed a slight decrease in DA release. Comparing with *sc* administration, this result indicated a low oral bioavailability. However, these results showed that 4 is still 100 times more potent than apomorphine (19).¹⁷ Stereotyped behaviour was shown at the dose up to 100 nmol kg⁻¹ *po*. At this dose, 4 induced a decrease of the DA levels to 40% of basal values after



high resolution mass spectrometry (HRMS), performed on a JEOL MS (note PMS-6001) by the Department of Chemistry, University of Groningen.

All the reagents and solvents were commercially available and were used without further purification with the exception of MeOH, which was distilled from magnesium and dried over 4 Å molecular sieves, and THF, which was distilled from sodium/benzophenone and dried on sodium wire.

4.2. Chemistry

4.2.1. 7-Iodo-1,2,6-trimethoxy-naphthalene (6). A solution of *n*-BuLi in benzene (1.6 M, 11.3 mL, 18.0 mmol) was added to a solution of 5 (13.27 g, 15.0 mmol) in dry THF (25 mL) under a N₂ atmosphere at 78 °C. After addition, the mixture was allowed to warm to rt and was stirred overnight. The reaction mixture was cooled to 78 °C and a solution of 1₁ (4.19 g, 16.5 mmol) in dry THF (6 mL) was added dropwise. After the addition was complete, a saturated aqueous solution of NH₄Cl (11 mL) was added, followed by a saturated aqueous Na₂SO₃ (6 mL). The volatiles were evaporated. The residue was partitioned between ether and H₂O. The combined organic layers were washed with brine (3x 30 mL), dried over MgSO₄. After filtration and evaporation of the solvent, a residue was obtained which was purified by column chromatography (9-bead-eluent, gradient), yielding 7 as a white solid (1.8 g, 35% yield). Mp 128–130 °C; ¹H NMR (CDCl₃) δ 8.29 (s, 1H), 7.39–7.43 (d, 1H, *J* = 8.8 Hz), 7.21–7.26 (d, 1H, *J* = 9.0 Hz), 6.98 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H) ppm; ¹³C NMR (CDCl₃) δ 153.4, 145.3, 140.7, 131.6, 128.9, 124.3, 121.0, 115.1, 103.7, 87.6, 59.7, 55.5, 54.8 ppm; MS (EI) *m/z*: 344 (M⁺), 118 MS 343.99149 (calcd) Caled for C₁₁H₁₁O₃ 343.99093.

4.2.2. 3-Iodo-1,2,6-trimethoxy-naphthalene (7). The compound was obtained as the second fraction (1.5 g, 31.6% yield). Mp 95–95 °C; ¹H NMR (CDCl₃) δ 8.02, 7.98 (m, 2H), 7.12–7.18 (dd, 1H, *J* = 2.4 Hz, *J* = 9.3 Hz), 6.94–6.95 (d, 1H, *J* = 2.4 Hz), 4.02 (s, 3H), 3.94 (s, 3H), 3.87 (s, 3H) ppm; ¹³C NMR (CDCl₃) δ 156.2, 145.7, 144.6, 135.5, 130.6, 123.1, 121.9, 117.7, 101.0, 92.0, 59.7, 50.4, 53.8 ppm; MS (EI) *m/z*: 344 (M⁺).

4.2.3. (E)-1,3,7,8-Tetramethoxy-2-naphthyl-2-propionate (8). A mixture of compound 6 (1.8 g, 5.2 mmol), acrylonitrile (0.42 g, 7.9 mmol), Pd(OAc)₂ (24 mg, 0.1 mmol), methylaniline (0.53 g, 5.3 mmol) and MeCN (4 mL) was heated in a capped Pyrex flask at 120 °C for 3 days. The cooled reaction mixture was filtered through Celite® (CH₂Cl₂ (100 mL) was added and the solution was washed with 1N HCl (3x 20 mL), brine (3x 20 mL) and dried over MgSO₄. After filtration and evaporation of the solvent, a dark residue was obtained which was purified by column chromatography (980 mg, 70% yield). Mp 97–100 °C; ¹H NMR (CDCl₃) δ 8.07 (s, 1H), 7.62–7.70 (d, 1H, *J* = 16.9 Hz), 7.36–7.44 (d, 1H, *J* = 8.8 Hz), 7.20–7.27 (d, 1H, *J* = 8.8 Hz), 7.00 (d, 1H, *J* = 3.7 Hz), 6.15–6.26 (d, 1H, *J* = 16.8 Hz), 3.65

Table 1. In vitro receptor functional assay (%-intrinsc activity) (IC₅₀, nM) of 4

Compound 4	D ₁ (IC ₅₀ , nM)	D ₂ (IC ₅₀ , nM)	D ₃ (IC ₅₀ , nM)
Dopamine	25	55	100
Apomorphine	35	100	100
(S)-1,3,7,8-Tetramethoxy-2-naphthyl-2-propionate	52	85	100
(R)-1,3,7,8-Tetramethoxy-2-naphthyl-2-propionate	>10 000	5	100

^aDA D₁ functional assay on stimulation of cAMP production in CHO cells.

^bDA D₂ functional assay on stimulation of cAMP formation in CHO cells transfected with the human D₂ receptor.

^cDA D₃ functional assay.

^dIC₅₀ is the concentration (±SD) producing a half maximal response.

3. Conclusion

The results of microdialysis experiments also showed that 4 was comparable with apomorphine (19), which is a 'stand-by' medicine in the treatment of PD when 'on-off' syndrome occurs after long-term L-dopa treatment.¹⁸ In the structures of apomorphine (19) and NPA (20), there is a second aromatic moiety, which makes the C-ring more favorable to aromatization *in vivo*.²⁰ Comparing with apomorphine, on this point, 4 is less sensitive to be auto-oxidized (Scheme 3); however, the bioavailability is still low due to the catechol moiety.

The results of the functional assay showed that 4 is a full DA agonist on both D₁ and D₂ receptor subtypes. The microdialysis study showed a dose-dependent decrease in the release of DA in the striatum after both *sc* and *po* administration of 4, and the results indicated that this compound behaves as an extremely potent DA receptor agonist. Although the catechol moiety in 4 is decisive for the low bioavailability, on the other hand, it could also possess the same neuroprotective effects as *p*-4-*β*-apomorphine (19).^{3,22} This neuroprotective effect resides in the phenolic moiety, which can act as a radical scavenger.²³ The high efficacy of 4 results in the possibility of administering low dose either by *sc* (10 nmol kg⁻¹ or by *po* (100 nmol kg⁻¹), which could make 4 a candidate for the treatment of Parkinson's disease.

4. Experimental

4.1. General

Melting points were determined in open glass capillaries on an electrothermal digital melting point apparatus and are uncorrected. ¹H NMR spectra were recorded at 300 MHz on a Varian-VXR 300 spectrometer and ¹³C NMR spectra were recorded at 50.5 MHz on a Varian Gemini 200 spectrometer. The chemical shifts are given in units (ppm) relative to TMS; the splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Coupling constants are given in Hz. The spectra recorded were consistent with the proposed structures of intermediates and final compounds. Electronic ionization (EI) mass spectra were obtained on Shimadzu QP 5000 GC/MS system equipped with a 17 A GC. Chemical ionization (CI) mass spectra were recorded by the mass spectrometry unit of the University of Groningen. Compounds that were obtained as oil, or as solid in a very small amount, were analysed by

(s, 3H), 3.90 (s, 3H), 3.87 (s, 3H) ppm; ^{13}C NMR (CDCl₃) δ 153.0, 145.6, 142.2, 129.9, 122.4, 122.3, 120.8, 117.5, 116.5, 104.4, 96.7, 59.8, 55.5, 54.0 ppm; MS (EI) m/z : 269 (M⁺), HRMS 269.10596 (obsd). Calcd for C₁₆H₁₇NO₂: 269.10518.

4.2.4. 3-(4,7,8-Trimethoxy-1,4-dihydro-2-naphthalenyl)-1-propanamine (9). Compound 8 (300 mg, 1.1 mmol) was dissolved in a mixture of CH₂Cl₂/MeOH (1:4 mL) and was hydrogenated over 10% Pd/C at 3 bar H₂ for 4 h at r.t. The reaction mixture was filtered over Celite®. The filtrate was concentrated to afford 9 as a straw-yellow solid (280 mg, 93% yield). Mp 67–70 °C; ^1H NMR (CDCl₃) δ 7.91 (s, 1H), 7.44–7.48 (d, 1H, J = 9.0 Hz), 7.21–7.26 (d, 1H, J = 9.0 Hz), 3.91–3.99 (m, 9H), 3.09–3.16 (t, 2H, J = 7.3 Hz), 2.67–2.74 (t, 2H, J = 7.3 Hz) ppm; ^{13}C NMR (CDCl₃) δ 153.2, 145.5, 141.7, 128.7, 127.0, 122.6, 121.2, 120.9, 118.1, 114.5, 103.6, 59.7, 55.6, 53.8, 26.2, 16.2 ppm; MS (EI) m/z : 271 (M⁺), HRMS 271.11996 (obsd). Calcd for C₁₆H₁₇NO₂: 271.12083.

4.2.5. 3-(4,7,8-Trimethoxy-1,4-dihydro-2-naphthalenyl)-1-propanamine (10). Slices of Na (1.4 g, 0.06 g-atom) were added to a refluxing solution of 9 (180 mg, 1.4 mmol) in EtOH (25 mL) under a N₂-atmosphere. After the dissolution of Na was completed (1 h), the heating was interrupted, water (4 mL) and NH₄Cl (3.2 g) were added slowly. The mixture was filtered and the volatiles were evaporated. The residue was partitioned between water and ether. The combined organic layers were washed with water (3 × 10 mL) and dried over K₂CO₃. After filtration and evaporation of the solvent, 10 (320 mg) was obtained as a colourless semi-solid. In this mixture, 10% of by-product 11 was observed by GC. This mixture was used in the next step without further purification.

4.2.6. Racemic-trans-*trans*-4-*tert*-butyl-6,7-dimethoxy-3,4,4a,5,10,10a-hexahydrobenz[*b*]quinoline-12(11*H*)-carboxylate (16). A solution of 10 (280 mg, 1.0 mmol) in MeOH (5 mL) and 37% HCl (1 mL) was heated to reflux under a N₂-atmosphere. After refluxing for 4 h, the solution was concentrated to dryness; an orange oil was obtained (285 mg). The imine 12 (together with by-product 13) was used in the next step without any purification.

This crude oil (12 and 13) was dissolved in MeOH (15 mL) under N₂. The solution was adjusted to pH 4 with the addition of acetic acid. NaCNBH₃ (63 mg, 1.0 mmol) was added in portions to the solution. After stirring at r.t. for 2 h, the mixture was cooled to 0 °C and acidified to pH < 2 with conc HCl. The solvent was removed in vacuum and the residue was partitioned between 1 M NaOH and ether. The combined organic layers were washed with brine, dried over K₂CO₃. After filtration and evaporation of the solvent, a crude oil was obtained (220 mg), which was purified by column chromatography (Al₂O₃, CH₂Cl₂/MeOH, gradient), yielding a light yellow oil 14 (contaminated with 15) (150 mg (60% yield)). This yellow oil was dissolved in CH₂Cl₂ (20 mL) under N₂ with cooling. Triethylamine (130 mg, 1.28 mmol) and DMAP (15 mg, 0.12 mmol) were added at 0 °C. Di-*tert*-butyl dicarbonate (160 mg, 0.73 mmol) dissolved in CH₂Cl₂ (5 mL) was added to the mixture dropwise, and the reac-

tion was stirred at r.t. overnight. The reaction was cooled with ice and 2N HCl was added until pH < 3. The compound was washed with CH₂Cl₂ (3 × 30 mL). The combined organic layers were washed with saturated NaHCO₃ (3 × 30 mL), dried over MgSO₄. After filtration and evaporation of the solvent, the obtained residue was purified by column chromatography (*n*-hexane/ethyl acetate 9:1) and the two fractions were separated. The first fraction, 17, was obtained as a semi-solid (15 mg, 0.05 mmol). ^1H NMR (CDCl₃) δ 6.94–6.97 (d, 1H, J = 8.4 Hz), 6.55–6.66 (m, 2H), 3.82–3.89 (dd, 1H, J = 7.5 Hz, J = 13.6 Hz), 3.72 (s, 3H), 3.51–3.60 (dd, 1H, J = 4.8 Hz, J = 11.0 Hz), 3.10–3.17 (dd, 1H, J = 4.8 Hz, J = 15.4 Hz), 2.92–3.01 (m, 1H), 2.74–2.80 (dd, 1H, J = 4.4 Hz, J = 16.5 Hz), 2.44–2.64 (m, 2H), 1.49–1.92 (m, 4H), 1.42 (s, 9H), 1.15–1.40 (m, 1H) ppm; ^{13}C NMR (CDCl₃) δ 156.2, 154.0, 135.5, 128.9, 126.3, 111.8, 110.5, 77.8, 55.8, 53.7, 35.7, 35.1, 33.1, 32.5, 27.1, 25.9, 20.5 ppm; MS (EI) m/z : 317 (M⁺). The second fraction, as the product 16 (140 mg, 69% yield). ^1H NMR (CDCl₃) δ 6.67–6.77 (m, 2H), 3.81–3.88 (dd, 1H, J = 7.3 Hz, J = 13.9 Hz), 3.78 (s, 3H), 3.74 (s, 3H), 3.41–3.52 (m, 1H), 2.92–3.16 (m, 3H), 2.56–2.65 (dd, 1H, J = 11.4 Hz, J = 15.0 Hz), 2.29–2.29 (m, 1H), 1.59–1.99 (m, 4H), 1.42 (s, 9H), 1.13–1.40 (m, 1H), ^{13}C NMR (CDCl₃) δ 154.0, 148.9, 144.8, 128.6, 127.5, 123.1, 109.0, 77.7, 58.4, 55.4, 54.3, 35.2, 34.7, 32.71, 29.8, 27.1, 23.1–20.6 ppm; MS (EI) m/z : 332 (M⁺). HRMS 332.21046 (obsd). Calcd for C₂₀H₂₅NO₄: 332.15963.

4.2.7. Racemic-trans-6,7-dimethoxy-1,2,3,4,4a,5,10,10a-octahydrobenz[*b*]quinoline-14(11*H*). To the stirred solution of 16 (120 mg, 0.35 mmol) in CH₂Cl₂ (1 mL) at 0 °C, trifluoroacetic acid (1 mL, 5.70 mmol) was added dropwise. The reaction mixture was allowed to warm to r.t. and further stirred for 2.5 h. After evaporation in vacuum, the residue was cooled on ice and H₂O (5 mL) was added. The water layer was neutralized with saturated NaHCO₃, extracted with CH₂Cl₂ (3 × 30 mL) and the organic layer was dried over K₂CO₃. After filtration and evaporation of the solvent, a light yellow oil 14 (85 mg, 98% yield) was obtained. ^1H NMR (CDCl₃) δ 6.67–6.76 (dd, 2H, J = 8.4 Hz, J = 17.9 Hz), 3.78 (s, 3H), 3.75 (s, 3H), 3.06–3.09 (d, 1H, J = 11.7 Hz), 2.93–2.98 (dd, 1H, J = 5.1 Hz, J = 17.2 Hz), 2.65–2.82 (m, 2H), 2.51–2.57 (m, 2H), 2.15–2.19 (dd, 1H, J = 11.7 Hz, J = 17.6 Hz), 1.92–1.95 (d, 1H, J = 12.8 Hz), 1.51–1.69 (m, 3H), 1.37–1.40 (m, 1H), 1.12–1.19 (m, 1H), ^{13}C NMR (CDCl₃) δ 148.9, 144.7, 128.9, 127.1, 122.4, 108.8, 58.4, 55.8, 54.3, 45.3, 36.7, 35.3, 30.7, 29.2, 25.4 ppm; MS (EI) m/z : 247 (M⁺).

4.2.8. Racemic-trans-6,7-dimethoxy-1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenz[*b*]quinoline-18(19). To a stirred solution of 14 (50 mg, 0.20 mmol) in *n*-propanol (3 mL) under a N₂ atmosphere, *n*-propionaldehyde (60 mg, 1.01 mmol) was added, followed by 10% Pd/C (20 mg). After stirring at r.t. for 3 h, the mixture was filtered over Celite®. The solvent was evaporated to remove the volatiles. The residue was purified by column chromatography (CH₂Cl₂/MeOH, gradient), a yellow oil 18 was obtained (44 mg, 0.15 mmol, 75% yield). ^1H NMR (CDCl₃) δ 6.71–6.84 (dd, 2H, J = 8.5 Hz,

J = 10.5 Hz), 3.83 (s, 3H), 3.79 (s, 3H), 2.94–3.12 (m, 2H), 2.23–2.81 (m, 6H), 1.06–2.13 (m, 8H), 0.85–0.92 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (CDCl₃) δ 148.8, 144.4, 128.6, 127.2, 122.6, 108.8, 59.8, 58.4, 54.3, 54.1, 51.4, 35.8, 33.0, 30.8, 29.7, 24.0, 16.0, 10.6 ppm; MS (EI) m/z : 289 (M⁺).

4.2.9. Racemic-trans-1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenz[*b*]quinoline-6,7-diol (4). A solution of 18 (110 mg, 0.38 mmol) in freshly distilled 48% HBr was heated to 155 °C under N₂ for 3 h. The volatiles were evaporated in vacuum to obtain 4 (120 mg, 0.35 mmol, 92% yield) as hydrobromide salt. An analytical amount was recrystallized from MeOH/ether and white crystals were obtained. Mp 309–312 °C; ^1H NMR (CDCl₃) δ 6.51 (d, 1H, J = 8.0 Hz), 6.39 (d, 1H, J = 8.0 Hz), 2.96–3.80 (m, 5H), 2.65–2.74 (m, 1H), 2.14–2.24 (m, 1H), 1.60–1.92 (m, 5H), 1.20–1.39 (m, 4H), 0.92–0.97 (t, 3H, J = 7.3 Hz); MS (CI) m/z : 262 (M⁺ + 1).

4.3. Pharmacology

4.3.1. Animals. Animals used in the biochemical and behaviour activity experiments were male rats of the Wistar strain (from Harlan, Netherlands) weighing 300–350 g. The rats were placed in a room with controlled environmental conditions (21 °C, humidity 60–65%, lights on at 8 a.m. and off at 5 p.m. At least for 1 week from arrival until used in the experiments. Animal procedures were conducted in accordance with guidelines published in the NIH guide for the care and use of laboratory animals and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

4.3.2. Drugs. All compounds were tested as their hydrochloride salts, except 4 was tested as hydrobromide salt. The drugs were dissolved in physiological (0.9%) saline immediately prior to use. All in vivo experiments were performed at the laboratory animal unit of the Rijksuniversiteit Groningen, the Netherlands, and in vitro experiments were performed at Division of Molecular and Cellular Pharmacology, Lundbeck A/S.

4.3.3. Surgery and brain microdialysis. On-line brain microdialysis in freely moving animals has been described.³⁴

4.4. Receptor functional assay

The intrinsic activity of 4 at the DA D₁ and D₂ receptor was determined according to methods previously described^{35,26} with some modification.

4.4.1. D₁ cAMP assay. The ability of the compounds to stimulate the D₁ receptor mediated cAMP formation in CHO cells stably expressing the human recombinant D₁ receptor was measured as follows.

Cells were seeded in 96-well plates at a density of 11000 cells/well 3 days prior to the experiment. On the day of the experiment the cells were washed once in preheated G buffer (1 mM MgCl₂, 0.9 mM CaCl₂, 1 mM BMAX

in PBS) and the assay was initiated by addition of 100 μL of the test compound diluted in G buffer.

The cells were incubated for 20 min at 37 °C and the reaction was stopped by the addition of 100 μL of 0.1 M HCl and 0.1 mM CaCl₂ and the plates were placed at 4 °C for 1 h. About 65 μL of N buffer (0.15 M NaOH and 60 mM NaAc) was added and the plates were shaken for 10 min. About 60 μL of the reaction mixture was transferred to cAMP FlashPlates (DuoPoint NEN) containing 40 μL 60 mM NaAc, pH 6.2, 100 μL IC mix (50 mM NaAc, pH 6.2, 0.1% NaAc, 12 mM CaCl₂, 1% HSA and 0.15 μM ^{125}I -cAMP) were added. Following an 18-h incubation at 4 °C the plates were washed once and counted in a Wallac TriLux counter.

4.4.2. D₂ cAMP assay. The ability of the compounds to whether stimulate the D₂ receptor mediated inhibition of cAMP formation in CHO cells transfected with the human D₂ receptor was measured as follows.

Cells were seeded in 96-well plates at a density of 8000 cells/well 3 days prior to the experiment. On the day of the experiment the cells were washed once in preheated G buffer (1 mM MgCl₂, 0.9 mM CaCl₂, 1 mM IBMX in PBS) and the assay was initiated by addition of 100 μL of a mixture of 10 μM forskolin and test compound in G buffer.

The cells were incubated for 20 min at 37 °C and the reaction was stopped by the addition of 100 μL of 0.1 M HCl and 0.1 mM CaCl₂ and the plates were placed at 4 °C for 1 h. 65 μL of N buffer (0.15 M NaOH and 60 mM NaAc) were added and the plates were shaken for 10 min. Reaction mixture (60 μL) was transferred to cAMP FlashPlates (DuoPoint NEN) containing 40 μL 60 mM NaAc, pH 6.2, 100 μL IC mix (50 mM NaAc, pH 6.2, 0.1% NaAc, 12 mM CaCl₂, 1% HSA and 0.15 μM ^{125}I -cAMP) were added. Following an 18-h incubation at 4 °C the plates were washed once and counted in a Wallac TriLux counter.

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