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Design, synthesis and pharmacological evaluation of Enone prodrugs

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Liu, D. (2006). *Design, synthesis and pharmacological evaluation of Enone prodrugs: orally active precursors of catecholamines with antiparkinsonian activity*. s.n.

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Chapter 3

A novel synthesis and pharmacological evaluation of a dopamine D_1/D_2 agonist: TL-334

Abstract

We have demonstrated in Chapter 2, those enone prodrugs of dopaminergic catecholamines represent a new type of dopamine (DA) agonist. *trans*-1-Propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinoline-6,7-diol (TL-334, **1.26d**) is the active metabolite of GMC-6650 (**2.2a**) *in vivo*. Therefore, investigation of **1.26d** is important for the further research of the enone prodrug. In this chapter, a novel synthesis and a pharmacological evaluation of **1.26d** are reported. **1.26d** displayed behavioural and biochemical effects in microdialysis studies after administration of 10 nmol kg^{-1} *sc* and 100 nmol kg^{-1} *po*. The potency of **1.26d** observed in this model was greater than that displayed by *N*-propyl-norapomorphine (NPA, **1.36**) and apomorphine (APO, **1.23**).

This chapter is based on the work of Danyang Liu, Jan de Vries, Durk Dijkstra and Håkan Wikström in Groningen University; Dr. Claus T. Christoffersen, Division of Molecular and Cellular Pharmacology, Lundbeck A/S.

3.1 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 D_2 agonists,[1] which mimic the action of DA and possibly counteract the development of dyskinesias.[2] However, compared with *L*-dopa, these D_2 agonists showed weaker dopaminergic activity. It will make significant sense to make a drug having both virtues, strong dopaminergic activity, together with lower possibility of dyskinesias.

In vivo, GMC-6650 (**2.2a**) 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 (D_1-D_5).[3] According to the analysis of brain tissue and blood plasma samples after the rat was given **2.2a**, its catechol form TL-334 (**1.26d**) was found in brain, which was known as a D_2 agonist for decades.[4] Therefore, it is of interest to investigate **1.26d** and to compare its dopaminergic effects with **2.2a**.

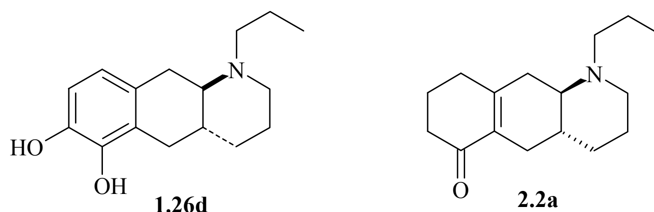


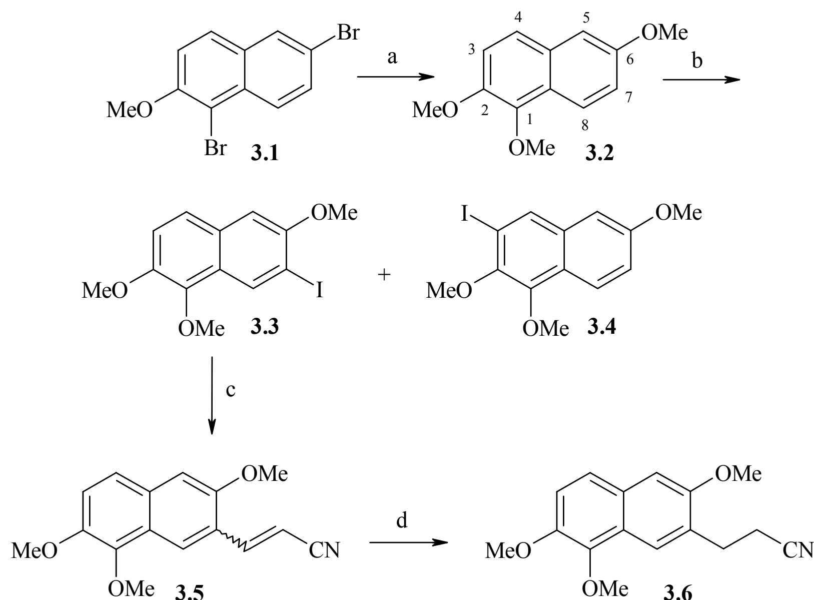
Figure 3.1 *Structures of TL-334 (**1.26d**) and GMC-6650 (**2.2a**).*

Over the last 20 years, a number of test models have been used for the evaluation of centrally acting DA receptor agonists and antagonists.[5,6,7,8] In the models aimed at testing DA agonists, the inhibition of motoric behaviour seen at low agonist doses is supposed to result from a stimulation of presynaptic DA receptors,[9,10] whereas the locomotor facilitation at higher doses is consequence of a postsynaptic DA agonist stimulation.[11,12] Compound **1.26d** has been shown to be one of the most effective agents, comparing with other catechol-containing DA agonists in these type of models. Microdialysis is mainly

an *in vivo* model suitable for investigating the pharmacological effects of DA agonists. Here, we report our results on the microdialysis experiments of **1.26d**.

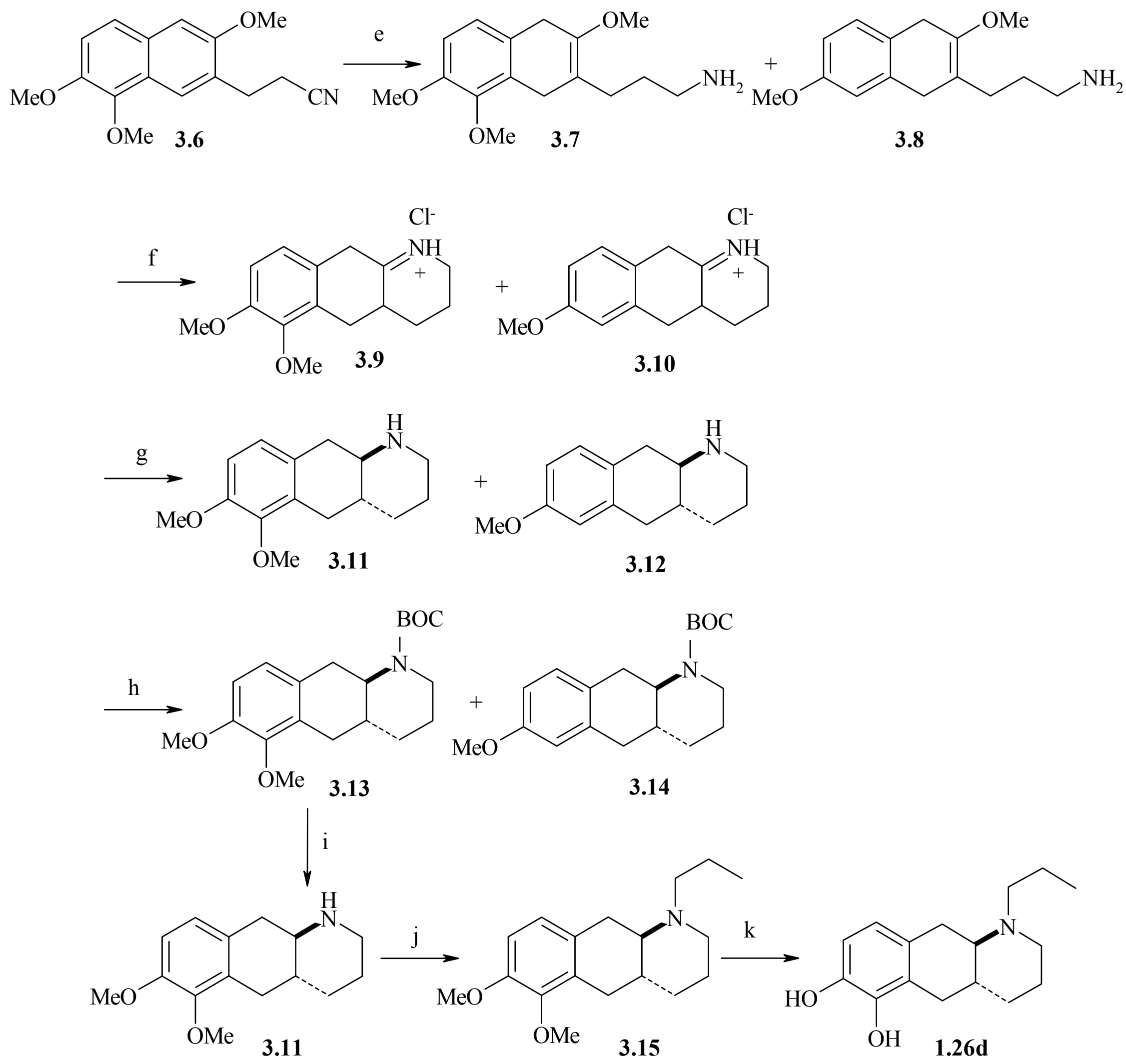
3.2 Chemistry

The synthetic route of TL-334 (**1.26d**) was first performed by Cannon and co-workers.[4] Since we obtained low yields for some of the intermediates and were not able to reproduce all the steps of that synthesis, we developed a novel synthetic route, we developed a novel synthetic route, based on the work reported by Mellin.[13] The final compound **1.26d** was synthesized according to the following strategy. Methoxylation of 1,6-dibromo-2-methoxynaphthalene (**3.1**)[14] with sodium methoxide in the presence of copper (I) iodide gave varying (50%-86% yield) results. The lithiation of the trimethoxynaphthalene (**3.2**) 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 of the isomers **3.3** and **3.4**. These two isomers were separated by column chromatography. The palladium catalyzed Heck reaction[15] of **3.3** using acrylonitrile and a catalytic amount of $Pd(OAc)_2$ gave a mixture of *E* and *Z*-isomers of **3.5** in 70% yield. After hydrogenation of this mixture with 10% Pd/C, the saturated nitrile **3.6** was formed which was used for next step without further purification.



Scheme 3.1 *Synthesis of **1.26d**. Reagents and conditions: a) $NaOCH_3$, CuI, 2,4,6-tri-methylpyridine, reflux; b) n-BuLi, THF, -78°C to RT, overnight; -78°C, I_2, 10 min; c) $CH_2CH{=}CN$, $Pd(OAC)_2$, Et_3N, CH_3CN, 120°C, 3 days; d) 10% Pd/C, 4 bar H_2 , RT overnight.*

The Robinson reduction of **3.6** with sodium in EtOH mainly occurred in the nitrile-substituted ring, simultaneously, the cyano group was reduced to the primary amine **3.7**. It was found that 10% of the dimethoxy-substituted ring was also reduced; subsequently C1 methoxy group was cleaved to form mono-methoxy by-product **3.8**. Separation of compounds **3.7** and **3.8** was very difficult and tedious; therefore, the mixture was used in the next step to form **3.9** and **3.10**.



Scheme 3.2 *Synthesis of **1.26d**. Reagents and conditions: e) Na/EtOH, reflux, 1 h; f) MeOH, 37% HCl, reflux, 4 h; g) $NaCNBH_3$; h) $(BOC)_2O$, Et_3N, DMAP; i) TFA, CH_2Cl_2; j) CH_3CH_2CHO, 1 bar H_2, 10% Pd/C, n-propanol, RT, 2 h; k) HBr 48%, 3 h.*

Reduction of **3.9** and **3.10** with $NaCNBH_3$ in MeOH at pH 4 produced exclusively the *trans*-isomers **3.11** and **3.12**. These two compounds were separated after protection of the

amine with a BOC-group (**3.13** and **3.14**). After column chromatography, the BOC-group was cleaved with TFA. The propylation of **3.11** to **3.15** was performed *via* reductive amination with *n*-propionaldehyde under the condition of 1 bar H_2 and 10% Pd/C in *n*-propanol. The methyl group was cleaved with freshly distilled 48% HBr in water solution and formed the target compound **1.26d**.

3.3 Pharmacology

3.3.1 *In vitro* functional assay

A DA D_1 functional assay on stimulation of cAMP production in CHO cells stably expressing the human recombinant D_1 receptor was performed to investigate whether **1.26d** has any agonistic activity at the D_1 receptor. To determine the activity at the DA D_2 receptor, a DA D_2 functional assay was performed on inhibition of *c*AMP production in CHO cells transfected with the human D_2 receptor.

3.3.2 *In vivo* pharmacology

The potential pharmacological effects of **1.26d** were studied by measuring their effects on extracellular DA levels in the corpus striatum, the brain area of interest in PD, using microdialysis in freely moving rats.[16] The details were described in Chapter 2.

3.4 Results and discussion

3.4.1 *In vitro* functional assay

Table 3.1 shows the forskolin-stimulated *c*AMP accumulation in CHO cells transfected with the human D_2 receptor was completely inhibited by **1.26d**, with an EC_{50} of 0.65 nM. Furthermore, a stimulation of *c*AMP accumulation in CHO cells stably expressing the human recombinant D_1 receptor by **1.26d** by 95% was found, with an EC_{50} of 25 nM. The results of the functional assay showed that **1.26d** is a full DA agonist on both D_1 and D_2 receptor subtypes.

Table 3.1 *In vitro receptor functional assay (% intrinsic activity (EC_{50}, nM)) of **1.26d**.*

D_1 EC^d_{50} (nM)[a]	IA[c]	D_2 EC_{50} (nM)[b]	IA
25	95%	0.65	100%

a: DA D_1 functional assay on stimulation of cAMP production in CHO cells; b: DA D_2 functional assay on inhibition of cAMP formation in CHO cells transfected with the human D_2 receptor. c: IA: intrinsic activity; d: EC is the concentration (±S.D.) producing a half-maximal response.

3.4.2 Microdialysis study

The compound **1.26d** was administered *sc* (1 nmol and 10 nmol kg^{-1}, Figure 3.2) as well as *po* (10 nmol and 100 nmol kg^{-1}, Figure 3.3) to male Wistar rats.

A maximal DA decrease to 80% of controls was found 90 min after administration of 1 nmol kg^{-1} *sc* injection. 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 to 60% of control values for 6 h. During the experiment with 10 nmol kg^{-1} *sc*, **1.26d** induced locomotor activity, penile grooming, yawning, sniffing, rearing, which is consistent with the post-synaptic effects of a centrally acting DA agonist.

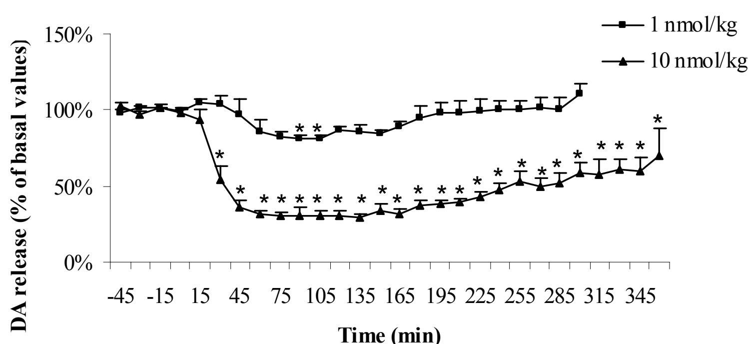


Figure 3.2 *Effect of **1.26d** (1 and 10 nmol kg^{-1} sc, ■, ▲ resp.) on striatal DA release in freely moving rats. The results are the mean (± SEM) of data obtained from 4 rats (* $p < 0.05$, Student's T test).*

Oral administration of **1.26d** in a dose of 10 nmol kg^{-1} induced a slight decrease in DA release. Comparing with *sc* administration, this result indicated a low oral bioavailability. However, these results show that **1.26d** is still 100 times more potent than apomorphine (**1.23**).[16] Stereotyped behaviour was shown after the administration of 100 nmol kg^{-1} *po*. At this dose, **1.26d** induced a decrease of the DA levels to 40% of basal values after 45 min. The duration of the decrease kept until the end of experiment (4 h) (Figure 3.3).

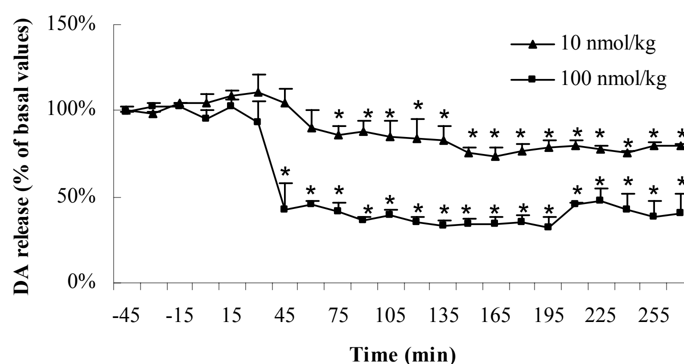
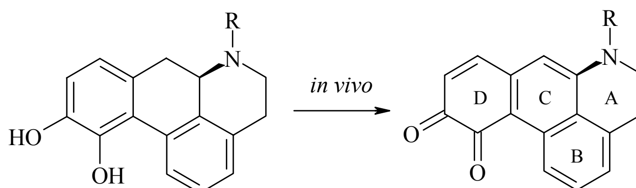


Figure 3.3 *Effect of* ***1.26d*** *(10 nmol kg^{-1} and 100 nmol kg^{-1} po, ▲ and ■ resp.) on striatal DA release in freely moving rats. The results are the mean (±SEM) of data obtained from 4 rats (* $p < 0.05$, student T test).*

3.5 Conclusions

The synthesis of **1.26d** (TL-334) was improved by a novel synthetic route. The reaction conditions for all the intermediates are easy to handle. The microdialysis study showed a dose-dependent decrease in the release of DA in the striatum after both *sc* and *po* administration of **1.26d**, and the results indicated that this compound acted as an extremely potent DA receptor agonist. Low dose (1 nmol kg^{-1}, *sc*) administration of **1.26d** induced a slight decrease of DA release in the striatum and no stereotyped behaviour (a typical DA D_2 receptor stimulation behaviour) was observed, whereas higher dose administration (10 nmol kg^{-1}, *sc*) induced a strong decrease in DA release in the striatum and a pronounced stereotyped behaviour. The lower dose of **1.26d** (10 nmol kg^{-1}) by *po* administration only slightly induced DA decrease and without any stereotyped behaviour. This phenomenon suggested that at low doses presynaptic DA receptor is stimulated, while high doses stimulate both the pre- and postsynaptic DA receptors. The results of microdialysis experiment also showed that **1.26d** was much

more potent than apomorphine (**1.23**).[16] Apomorphine is a "stand-by" medicine in the treatment of PD when "on-off" syndrome occurs after long-term *L*-dopa treatment.[17,18] In the structures of apomorphine (**1.23**) and NPA (**1.36**), a second aromatic moiety is present, which makes the C-ring more favorable to aromatization *in vivo*.[19] Comparing with apomorphine, on this point, **1.26d** is less sensitive to auto-oxidation, although the bioavailability is probably low due to the catechol moiety (Scheme 3.3).



Scheme 3.3 *The auto-oxidation of apomorphine (R = CH_3, **1.23**) and NPA (R = $CH_2CH_2CH_3$, **1.36**)*

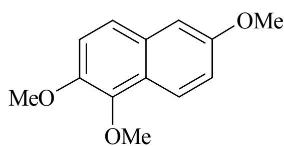
Taken together, although the catechol moiety in **1.26d** is responsible for a low bioavailability, it could also possess the same neuroprotective and neurotrophic effects as *R*-(-)-apomorphine (**1.23**).[20,21] This neuroprotective effect resides in the catechol moiety, which can act as a radical scavenger.[22] The high efficiency of **1.26d** makes it of interest to administer low dose either by *sc* (10 nmol kg^{-1}) or by *po* (100 nmol kg^{-1}), which could make **1.26d** a candidate for the treatment of PD.

3.6 Experimental section

3.6.1 Chemistry. General conditions see Chapter 2 for details.

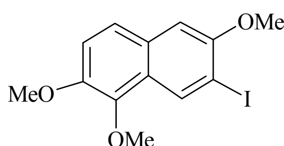
Materials. All the reagents and solvents were commercially available and were used without further purification with the exception of MeOH, which was distilled from magnesium and dried over 4Å molecular sieves, and THF which was distilled from sodium/bezophenone and dried on sodium wire.

1,2,6-Trimethoxynaphthalene (3.2). Sodium (6.28 g, 0.27 g atom) was added under N_2 to dry MeOH (80 mL). After addition, the warm solution was diluted with dry 2,4,6-trimethylpyridine (40 mL), subsequently dried CuI (7.84 g, 41.2 mmol) and 1,6-dibromo-

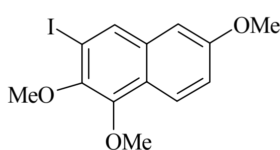


2-methoxynaphthalene **3.1** (11.4 g, 36.0 mmol) were added. The reaction mixture was diluted with an additional amount of dry 2,4,6-trimethylpyridine (80 mL) and was heated to reflux for 20 h under N_2. After cooling, the reaction mixture was filtered through Celite®, and the filtrate was acidified with 6N HCl (200 mL) and the resulting mixture was extracted with ether (5 x 120 mL). The combined ether layers were washed with 2N HCl (80 mL) and brine (2 x 100 mL) and dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by column chromatography on silica gel (*n*-hexane:ether, gradient) to obtain **3.2** as a white solid (6.8 g, 86.4% yield). Mp 54-56°C. (lit. 55°C[23]); ^{1}H-NMR ($CDCl_3$) δ 7.97-8.00 (d, 1H, *J* = 9.2 Hz), 7.41-7.44 (d, 1H, *J* = 9.2 Hz), 7.03-7.22 (m, 3H), 3.95 (s, 3H), 3.92 (s, 3H), 3.85 (s, 3H) ppm; ^{13}C-NMR ($CDCl_3$) δ 155.1, 145.5, 142.0, 129.3, 122.5, 121.5, 121.2, 117.6, 114.7, 104.0, 59.7, 55.6, 53.7 ppm; MS (EI) m/z 218 (M^+).

7-Iodo-1,2,6-trimethoxynaphthalene (3.3). A solution of *n*-BuLi in hexane (1.6 M, 11.3 mL, 18.0 mmol) was added to a solution of **3.2** (3.27 g, 15.0 mmol) in dry THF (28 mL) under a N_2 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 I_2 (4.19 g, 16.5 mmol) in dry THF (6 mL) was added dropwise. After the addition was complete, a saturated aqueous solution of NH_4Cl (11 mL) was added, followed by a saturated aqueous $Na_2S_2O_3$ (6 mL). The volatiles were evaporated. The residue was partitioned between ether and H_2O. The combined organic layers were washed with brine (3 x 30 mL), dried over $MgSO_4$. After filtration and evaporation of the solvent, a residue was obtained which was purified by column chromatography (*n*-hexane:ether, gradient), yielding **3.3** as a white solid (1.8 g, 35% yield). Mp 128-130°C; ^{1}H-NMR ($CDCl_3$) δ 8.59 (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; ^{13}C-NMR ($CDCl_3$) δ 152.4, 145.5, 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^+). HRMS 343.99149 (obsd). calcd for $C_{13}H_{13}O_3I$ 343.99095.

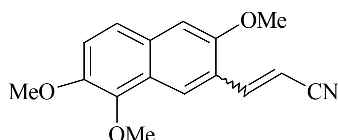


3-Iodo-1,2,6-trimethoxynaphthalene **3.4** was obtained as the second fraction (1.5 g, 31.6% yield). Mp 93-95°C; ^{1}H-NMR ($CDCl_3$) δ 8.02-7.98 (m, 2H), 7.12-7.18 (dd, 1H, *J* = 2.4 Hz, *J* = 9.3 Hz), 6.94-6.95 (d, 1H, *J* = 2.4 Hz), 4.02 (s, 3H), 3.94 (s, 3H), 3.87 (s, 3H) ppm; ^{13}C-NMR ($CDCl_3$) δ 156.2, 145.7, 144.6, 132.5, 130.6, 123.1, 121.9, 117.7,

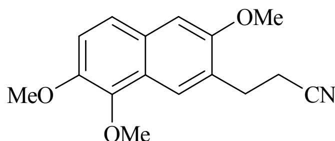


103.0, 92.0, 59.7, 59.4, 53.8 ppm; MS (EI) m/z 344 (M^+).

(*E*)-3-(3,7,8-trimethoxy-2-naphthyl)-2-propenenitrile (3.5). A mixture of compound **3.3** (1.8 g, 5.2 mmol), acrylonitrile (0.42 g, 7.9 mmol), $Pd(OAc)_2$ (24 mg, 0.1 mmol), triethylamine (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®, CH_2Cl_2 (100 mL) was added and the solution was washed with 1M HCl (3 x 20 mL), brine (3 x 20 mL) and dried over $MgSO_4$. After filtration and evaporation of the solvent, a dark residue was obtained which was purified by column chromatography (*n*-hexane:ether = 4:1), yielding **3.5** as a yellow solid (980 mg, 70% yield). Mp. 97°C-100°C; ^{1}H-NMR ($CDCl_3$) 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), 6.18-6.26 (d, 1H, J = 16.8 Hz), 3.65 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H) ppm; ^{13}C-NMR ($CDCl_3$) δ 153.0, 145.6, 142.2, 129.9, 122.9, 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 $C_{16}H_{15}NO_3$ 269.10518.

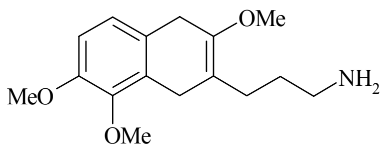


3-(3,7,8-Trimethoxy-2-naphthyl)propanenitrile (3.6). **3.5** (300 mg, 1.1 mmol) was dissolved in a mixture of CH_2Cl_2 /MeOH (1 mL: 4 mL) and was hydrogenated over 10% Pd/C at 3 bar H_2 for 4 h at RT. The reaction mixture was filtered over Celite®. The filtrate was concentrated to afford **3.6** as a straw yellow solid (280 mg, 93% yield). Mp 67-70°C; ^{1}H-NMR ($CDCl_3$) 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 ($CDCl_3$) 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 $C_{16}H_{17}NO_3$ 271.12083.



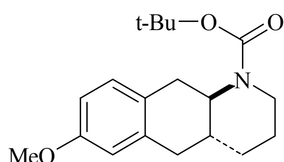
3-(3,7,8-Trimethoxy-1,4-dihydro-2-naphthalenyl)-1-propanamine (3.7). Slices of Na (1.4 g, 0.06 g atom) were added to a refluxing solution of **3.6** (380 mg, 1.4 mmol) in EtOH (25 mL) under a N_2 atmosphere. After the dissolution of Na was completed (1 h), the heating was interrupted, water (4 mL) and NH_4Cl (3.2 g) were added slowly. The mixture was filtered and the volatiles were evaporated. The residue was partitioned

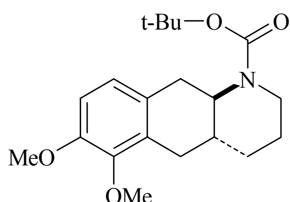
between water and ether. The combined organic layers were washed with water (3 x 10 mL), dried over K_2CO_3. After filtration and evaporation of the solvent, **3.7** (320 mg) was obtained as a colorless semi-solid. In this mixture, 10% of by-product **3.8** was observed by GC. This mixture was used in the next step without further purification.



Racemic-*trans*-*tert*-butyl-6,7-dimethoxy-3,4,4a,5,10,10a-hexahydrobenzo[*g*]quino-line-1(2*H*)-carboxylate (3.13). A solution of **3.7** (280 mg, 1.0 mmol) in MeOH (5 mL) and 37% HCl (1 mL) was heated to reflux under N_2. After refluxing for 4 h, the solution was concentrated to dryness; an orange oil was obtained (285 mg). The imine **3.9** (together with by-product **3.10**) was used in the next step without any purification.

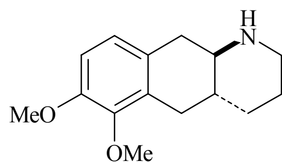
This crude oil (**3.9** and **3.10**) was dissolved in MeOH (5 mL) under N_2. The solution was adjusted to pH 4 with the addition of acetic acid. $NaCNBH_3$ (63 mg, 1.0 mmol) was added in portions to the solution. After stirring at RT for 2 h, the mixture was cooled to 0°C and acidified to pH < 2 with conc. HCl. The solvent was removed *in vacuo* and the residue was partitioned between 1 M NaOH and ether. The combined organic layers were washed with brine, dried over K_2CO_3. After filtration and evaporation of the solvent, a crude oil was obtained (220 mg), which was purified by column chromatography (Al_2O_3, CH_2Cl_2/MeOH, gradient), yielding a light yellow oil **3.11** (contaminated with **3.12**), 150 mg (60% yield). This yellow oil was dissolved in CH_2Cl_2 (20 mL) under N_2 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 CH_2Cl_2 (5 mL) was added to the mixture dropwise, and the reaction was stirred at RT overnight. The reaction was cooled with ice and 2N HCl was added until PH < 3. The acid layer was washed with CH_2Cl_2 (3 x 30 mL). The combined organic layers were washed with saturated $NaHCO_3$ (3 x 30 mL), dried over $MgSO_4$. 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, **3.14** was obtained as a semi- solid (15 mg, 0.05 mmol), ^{1}H-NMR ($CDCl_3$) 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 (td, 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,



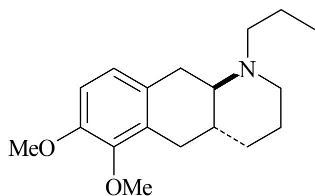


J = 16.5 Hz), 2.44-2.64 (m, 2H) 1.49-1.92 (m, 4H), 1.42 (s, 9H), 1.15-1.40 (m, 1H) ppm; ^{13}C-NMR ($CDCl_3$) δ 156.2, 154.0, 135.3, 128.9, 126.3, 111.8, 110.5, 77.8, 55.8, 53.7, 35.7, 35.1, 33.1, 32.5, 27.1, 23.9, 20.5 ppm; MS (EI) m/z 317 (M^+); The second fraction as the product **3.13** (140 mg, 66% yield) ^{1}H-NMR ($CDCl_3$) 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), 2.20-2.29 (m, 1H), 1.59-1.99 (m, 4H), 1.42 (s, 9H), 1.13-1.40 (m, 1H), ^{13}C-NMR ($CDCl_3$) 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, 32.74, 32.71, 29.8, 27.1, 24.1, 20.6 ppm; MS (EI) m/z 347 (M^+). HRMS 347.21046 (obsd). calcd for $C_{20}H_{29}NO_4$ 347.20963.

Racemic-*trans*-6,7-dimethoxy-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinoline (3.11). To the stirred solution of **3.13** (120 mg, 0.35 mmol) in CH_2Cl_2 (1 mL) at 0°C, trifluoroacetic acid (1 mL, 5.70 mmol) was added dropwise. The reaction was allowed to warm to RT, and further stirred for 2.5 h. After evaporation *in vacuo*, the residue was cooled on ice and H_2O (5 mL) was added. The water layer was neutralized with saturated $NaHCO_3$, extracted with CH_2Cl_2 (3 x 30 mL) and ther organic layer was dried over K_2CO_3. After filtration and evaporation of the solvent, a light yellow oil **3.11** (85 mg, 98% yield) was obtained. ^{1}H-NMR ($CDCl_3$) 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 ($CDCl_3$) 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^+).



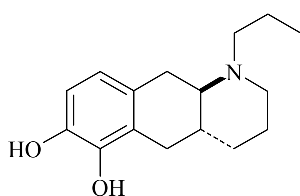
Racemic-*trans*-6,7-dimethoxy-1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinoline (3.15). To a stirred solution of **3.11** (50 mg, 0.20 mmol) in *n*-propanol (3 mL) under N_2, *n*-propionaldehyde (60 mg, 1.01 mmol) was added, followed by 10% Pd/C (20 mg). After stirring at RT for 3 h, the mixture was filtered over Celite®. The solvent was evaporated to remove volatiles. The residue was purified by column chromatography



(CH_2Cl_2: MeOH, gradient), a yellow oil **3.15** was obtained (44 mg, 0.15 mmol, 75% yield). ^{1}H-NMR (CDCl$_3$) 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 (CDCl$_3$) 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^+).

Racemic-*trans*-1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinoline-6,7-diol (1.26d).[4]

A solution of **3.15** (110 mg, 0.38 mmol) in freshly distilled 48% HBr was heated to 135°C under N_2 for 3 h. The volatiles were evaporated *in vacuo* to obtain **1.26d** (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; ^{1}H-NMR (CDCl$_3$) δ 6.51 (d, 1H, J = 8.0 Hz), 6.39 (d, 1H, J =8.0 Hz), 2.96-3.50 (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).



3.6.2 Pharmacology

Animals. Animals used for the biochemical and behaviour activity experiments were male rats of a Wistar derived strain (Harlan, the Netherlands) weighing 300-350 g. The rats were placed in a room at controlled environmental conditions (21°C, humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). Animals were not used during the first week after arrival in the laboratory. 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 Animal Care Committee.

Drugs. TL-334 (**1.26d**) was tested as it hydrobromide salt unless noted otherwise. The drug was dissolved in physiological (0.9%) saline immediately prior to use. All *in vivo* experiments were performed at the Animal Laboratory Unit of the University of Groningen, The Netherlands.

Surgery and brain microdialysis. On-line brain microdialysis in freely moving animals has previously been described.[24] Details see Chapter 2.

3.6.3 Receptor functional assay

The intrinsic activity of TL-334 at the DA D_1 and D_2 receptor were determined according to methods previously described[25,26] with some modification.

D_1 *c*AMP assay. The ability of TL-334 to inhibit the D_1 receptor mediated *c*AMP formation in CHO cells stably expressing the human recombinant D_1 receptor was measured as follows. Cells were seeded in 96-well plates at a concentration 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 $MgCl_2$, 0.9 mM $CaCl_2$, 1 mM IBMX in PBS) and the assay was initiated by addition of 100 µL of a mixture of 30 nM A68930 (selective DA D_1 receptor agonist) and TL-334 diluted in G buffer.

The cells were incubated for 20 minutes at 37°C and the reaction was stopped by the addition of 100 µL S buffer (0.1 M HCl and 0.1 mM $CaCl_2$) and the plates were placed at 4°C for 1 h. 68 µL N buffer (0.15 M NaOH and 60 mM NaAc) was added and the plates were shaken for 10 minutes. 60 µL of the reaction were transferred to *c*AMP FlashPlates (DuPont NEN) containing 40 µL 60 mM NaAc pH 6.2 and 100 µL IC mix (50 mM NaAc pH 6.2, 0.1 % NaAzid, 12 mM $CaCl_2$, 1% BSA and 0.15 µ Ci/ml ^{125}I-*c*AMP) were added. Following an 18h incubation at 4°C the plates were washed once and counted in a Wallac TriLux counter.

D_2 *c*AMP assay. The ability of TL-334 to inhibit the D_2 receptor mediated inhibition of *c*AMP formation in CHO cells transfected with the human D_2 receptor was measure as follows.

Cells were seeded in 96 well plates at a concentration 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 $MgCl_2$, 0.9 mM $CaCl_2$, 1 mM IBMX in PBS) and the assay was initiated by addition of 100 µL of a mixture of 1 µM quinpirole, 10 µM forskolin and TL-334 in G buffer.

The cells were incubated 20 minutes at 37°C and the reaction was stopped by the addition of 100 µL S buffer (0.1 M HCl and 0.1 mM $CaCl_2$) and the plates were placed at 4°C for 1 h. 68 µL N buffer (0.15 M NaOH and 60 mM NaAc) were added and the plates were shaken for 10 minutes. 60 µL of the reaction were transferred to *c*AMP FlashPlates (DuPont NEN) containing 40 µL 60 mM NaAc pH 6.2 and 100 µL IC mix (50 mM NaAc pH 6.2, 0.1 % NaAzid, 12 mM $CaCl_2$, 1% BSA and 0.15 µCi/mL ^{125}I-cAMP) were added.

Following 18h incubation at 4°C the plates were washed once and counted in a Wallac TriLux counter.

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