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Original article

D_1 Agonist and/or D_2 antagonist dopamine receptor properties of a series of ergoline derivatives: a structure–activity study

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Abstract – A series of (3,5-dioxopiperazin-1-yl)ergoline derivatives has been synthesised and evaluated in vitro and in vivo for their dopaminergic D_1 and D_2 components. The structural contributions to the pharmacological profile of the ergoline skeleton, its substituents on positions 1, 2, 6, 9, and the 3,5-dioxopiperazin-1-yl portion of the molecule were examined. Structure–activity relationships within this series suggested that substitution on the ergoline skeleton in position 1 or 2 and on the 3,5-dioxopiperazin-4-nitrogen generated compounds with a spectrum of dopamine agonistic/antagonistic activity sensitive to both the nature and position of substituents. © Elsevier, Paris

ergoline derivatives / structure–affinity relationship / dopaminergic / antidopaminergic activity

1. Introduction

Semi-synthetic ergoline derivatives possess a wide and divergent spectrum of pharmacological activities including central, peripheral and neurohumoral effects, due mainly to their capability to bind unselectively to adrenergic, dopaminergic and serotonergic receptor sites [1, 2]. Compounds of this class may act not only as agonists or antagonists at the receptor sites of biogenic amine neurotransmitters, but they may also assume a partial agonist and antagonist role. Of particular significance for drug discovery and development is the dopamine agonist activity of ergolines, which has many important clinical implications [3–6]. Structure–activity studies have shown how the side chain at C-8 and suitable modifications of the ergoline skeleton were determinant for the pharmacological profile of this class of compounds [7, 8]. Some semisynthetic ergolines are used to treat a number of clinical conditions. The selective dopamine agonists pergolide and cabergoline, for example, are used in the management of hyperprolactinemic states and Parkinson's disease [9–12] respectively, while nicergoline is used for the treatment of senile mental impairment [13, 14] *(figure 1)*.

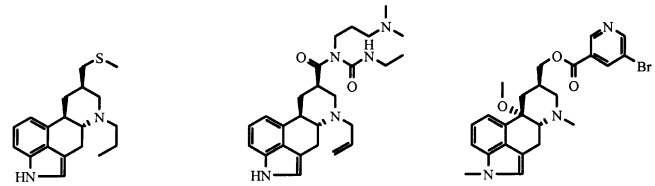

Figure 1.

In identifying antihypertensive ergolines, we have observed that **PNU 160425** had a very promising antihypertensive activity [15, 16]. This prompted us to synthesise analogues to discover more potent and selective compound. Replacement of the perhydro-2,4-dioxopyrimidin-1-yl by the isomer 3,5-dioxo-piperazin-1-yl moiety led to **1** (**PNU 160260**) *(figure 2)*.

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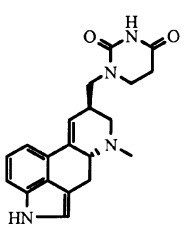
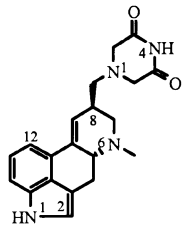


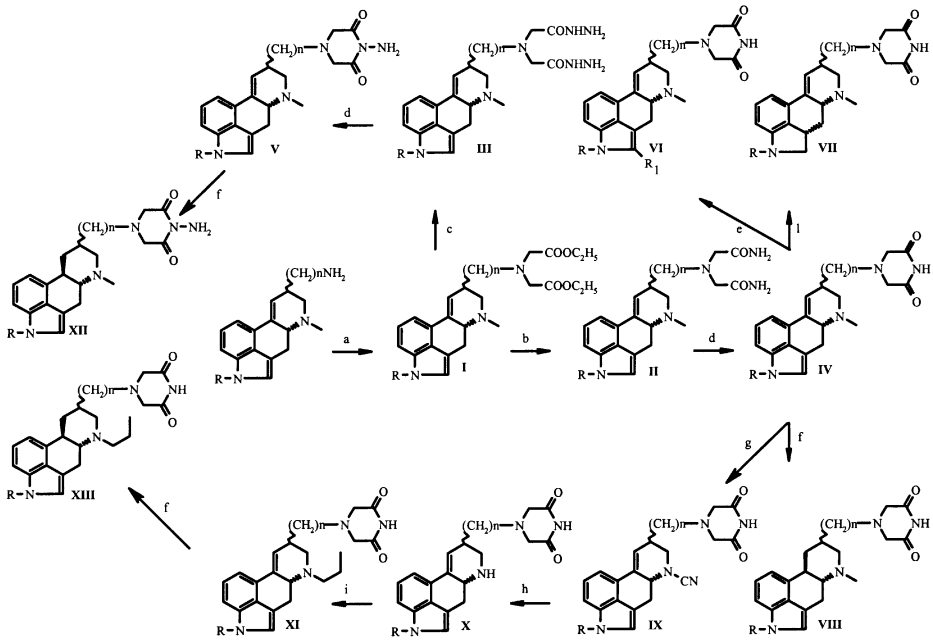
PNU 160425



1 PNU 160260

Figure 2.

Thus, it was found that **1** was devoid of antihypertensive activity but showed a unique central pharmacological profile. This compound behaves as a full dopamine antagonist in normal animals, but shows full agonist properties in denervated models in the same dose range. In normal animals, **1** impairs Sidman avoidance in rats, reduces spontaneous locomotion in mice and monkeys. Compound **1** also antagonises apomorphine-induced climbing behaviour in mice, yawning in rats, emesis in dogs and amphetamine-induced toxicity in grouped mice. However, after severely depleting central dopamine experimentally, **1** behaves as a powerful dopamine D_1 receptor agonist. This compound induces contralateral turning behaviour in 6-hydroxydopamine-lesioned rats and reverses 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced akinesia in monkeys and reserpine-induced hypokinesia in mice. Consequently **1** acts as a D_2 receptor antagonist under normal physiological conditions but is able to stimulate the D_1 receptors preferentially when endogenous dopamine is no longer available. Mixed agonist/antagonist activity on brain dopamine receptors has been reported for other ergot derivatives



a) $BrCH_2COOC_2H_5$, K_2CO_3, DMF, rt b) NH_3, DMF, rt c) NH_2NH_2, EtOH, reflux d) phenol, 160°
e) NBS, THF, rt, or CH_3SCl, CH_2Cl_2, 0°C, or SO_2Cl_2, $BF_3{\cdot}Et_2O$, CH_3CN, 0°C f) 3 atm H2, 10% Pd/C, AcOH, rt
g) BrCN, DMF, rt h) Zn, AcOH, rt i) C_3H_7I, DMF, TEA, rt l) $NaBH_4$, TFA

III **1** R=H, 8β-CH_2
2 R=CH_3, 8β-CH_2
3 R=CH_3CH_2, 8β-CH_2
4 R=$(CH_3)_2CH$, 8β-CH_2
11 R=H, 8a-CH_2
12 R=H, 8α
13 R=H, 8β
14 R=H, 8β-CH_2CH_2

V **19** R=H, 8β-CH_2

VI **5** R=H, 8β-CH_2, R_1=Br
6 R=H, 8β-CH_2, R_1=Cl
7 R=H, 8β-CH_2, R_1=CH_3S

VII **8** R=H, 3β-H, 8β-CH_2

VIII **20** R=H, 8β-CH_2
21 R=CH_3, 8β-CH_2

IX R=H, 8β-CH_2

XI **10** R=H, 8β-CH_2

X **9** R=H, 8β-CH_2

XII **25** R=H, 8β-CH_2

XIII **22** R=H, 8β-CH_2

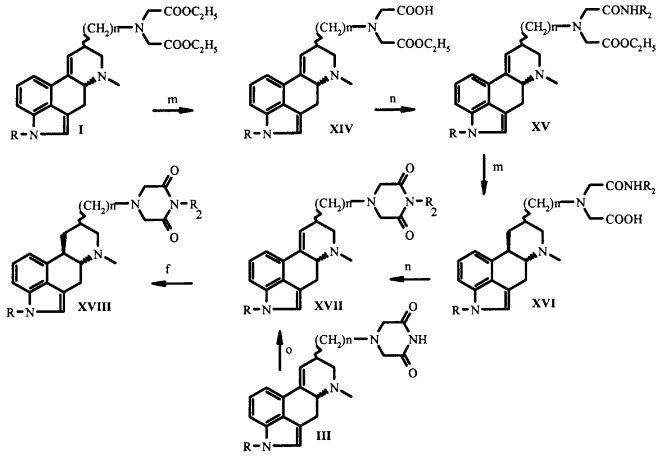
Figure 3.

with different pharmacological profile, but to our knowledge none of these change from antagonist to agonist depending on the functional state of the substrate [17, 18]. These interesting findings prompted us to investigate the biological properties of a series of analogues of **1**. Through systematic alteration of the parent molecule it was hoped to define the features of the structure that may be critical for the appearance of the unusual biological activities endowed by the whole molecule. In order to achieve this goal, a series of analogues presented in this paper was prepared and characterised in vitro and in vivo. The synthesis and the pharmacological properties of new compounds are described and the structure–activity relationship explored [19].

2. Chemistry

Compounds in *tables I* and *II* were prepared as outlined in *figures 3* and *4*.

Condensation of Δ-9,10-ergolenylamines **27**–**35** with ethylbromoacetate in dimethylformamide in the presence of potassium carbonate yielded iminodiacetic acid diethyl ester derivatives **I** as major product accompanied by a small amount of N-(ethoxycarbonylmethyl)-N-(ethoxycarbonylmethoxycarbonyl)-lysergamine derivatives, stemming from carbonatation and subsequent alkylation of the glycine ester intermediates. Reaction of the intermediates **I** with ammonia in dimethylformamide or hydrazine hydrate in refluxing ethanol, led almost quantitatively to the dicarbamides **II** or to the diacylhydrazide derivatives **III** respectively. These can be easily converted into the 3,5-dioxopiperazin-1-yl derivatives **IV**, **1**–**4**, **11**–**14** and **V**, **19**, in high yield, by heating in phenol at 160 °C in a flow of nitrogen. Electrophilic substitution into the most reactive position 2, with N-bromosuccinimide in tetrahydrofuran [20], or with methylsulphenylchloride in dichloromethane [21], or with sulphurylchloride in presence of catalytic amount of borotrifluoride in acetonitrile [22], converted **1** into **5**–**7** in almost quantitative yield, whilst reduction with sodium borohydride in trifluoroacetic acid at low temperature afforded, in acceptable yield, the indoline **8** accompanied by a low amount of its 3α-H stereoisomer and by dimeric products [23, 24]. Catalytic hydrogenation of **1**, **2**, **10**, **15**, **16** in acetic acid mainly provided the 10 α saturated analogues **20**–**24**, accompanied by their 10β-isomers present in small amount. These cis and trans isomers were readily separated using medium-pressure liquid chromatography or fractional crystallisation from acetone. According to the von Braun methodology, the 6-desmethyl derivative **9** was obtained in good yield by treatment of **1** with cyanogen bromide in dimethylformamide at room temperature providing 6-cyano analogue **IX**, that was subsequently reduced by Zn powder in acetic acid [25]. The propyl derivative **10** was prepared through alkylation of **9** with propyliodide in dimethylformamide in presence of triethylamine. A more versatile and efficient method that allows the preparation of a wide variety of (3,5-



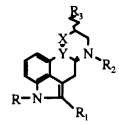
m) 0.1 M KOH, EtOH, rt n) DIC, Dioxane, reflux o) DEAD, Ph_3P, R_2OH, THF, rt, R_2 different from Aryl

XVII **15** R=H, R_2=CH_3, 8β-CH_2
16 R=H, R_2=C_6H_5, 8β-CH_2
17 R=H, R_2=(2, 6-CH_3)C_6H_3, 8β-CH2
18 R=H, R_2=C_6H_{11}, 8β-CH2

XVIII **23** R=H, R_2=CH_3, 8β-CH_2
24 R=H, R_2=C_6H_5, 8β-CH_2

Figure 4.

Table I. Chemical data of the ergoline derivatives **1–13.**



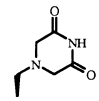
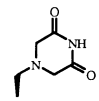
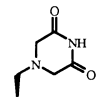
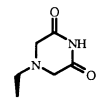
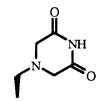
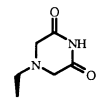
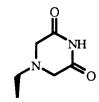
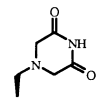
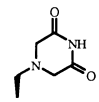
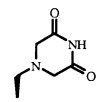
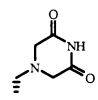
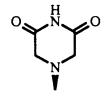
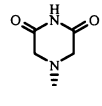
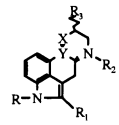
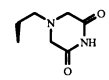
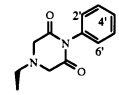
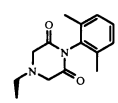
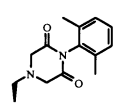
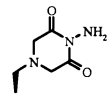
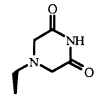
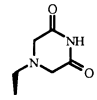
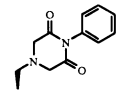
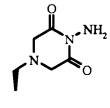
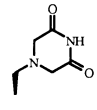
Compound	R	R_1	R_2	R_3	X–Y	Formula	M.p. (°C)
1	H	H	CH_3		CH=CH	$C_{20}H_{22}N_4O_2$	208–210
2	CH_3	H	CH_3		CH=CH	$C_{21}H_{24}N_4O_2$	216–218
3	CH_3CH_2	H	CH_3		CH=CH	$C_{22}H_{26}N_4O_2$	129–131
4	$(CH_3)_2CH$	H	CH_3		CH=CH	$C_{23}H_{28}N_4O_2$	137–140
5	H	Br	CH_3		CH=CH	$C_{20}H_{21}BrN_4O_2$	242–245
6	H	Cl	CH_3		CH=CH	$C_{20}H_{21}ClN_4O_2$	239–240
7	H	CH_3S	CH_3		CH=CH	$C_{21}H_{24}N_4O_2S$	206–208
8	H	2,3-H_2	CH_3		CH=CH	$C_{20}H_{24}N_4O_2$	135–140
9	H	H	H		CH=CH	$C_{19}H_{20}N_4O_2$	212–214
10	H	H	$CH_3CH_2CH_2$		CH=CH	$C_{22}H_{26}N_4O_2$	250–253
11	H	H	CH_3		CH=CH	$C_{20}H_{22}N_4O_2$	231–235
12	H	H	CH_3		CH=CH	$C_{19}H_{20}N_4O_2$	237–238
13	H	H	CH_3		CH=CH	$C_{19}H_{20}N_4O_2$	145–160

Table II. Chemical data of the ergoline derivatives **14–26.**



Compound	R	R_1	R_2	R_3	X–Y	Formula	M.p. (°C)
14	H	H	CH_3		CH=CH	$C_{21}H_{24}N_4O_2$	242–244
15	H	H	CH_3		CH=CH	$C_{21}H_{24}N_4O_2$	227–229
16	H	H	CH_3		CH=CH	$C_{26}H_{26}N_4O_2$	240–242
17	H	H	CH_3		CH=CH	$C_{28}H_{30}N_4O_2$	239–241
18	H	H	CH_3		CH=CH	$C_{26}H_{32}N_4O_2$	265–268
19	H	H	CH_3		CH=CH	$C_{20}H_{23}N_5O_2$	224–226
20	H	H	CH_3		CH_2–CH	$C_{20}H_{24}N_4O_2$	260–262
21	CH_3	H	CH_3		CH_2–CH	$C_{21}H_{26}N_4O_2$	268–270
22	H	H	$CH_3CH_2CH_2$		CH_2–CH	$C_{22}H_{28}N_4O_2$	276–278
23	H	H	CH_3		CH_2–CH	$C_{21}H_{26}N_4O_2$	235–237
24	H	H	CH_3		CH_2–CH	$C_{26}H_{28}N_4O_2$	260–262
25	H	H	CH_3		CH_2–CH	$C_{20}H_{25}N_5O_2$	210–212
26	H	H	CH_3			$C_{20}H_{24}N_4O_3$	278–280

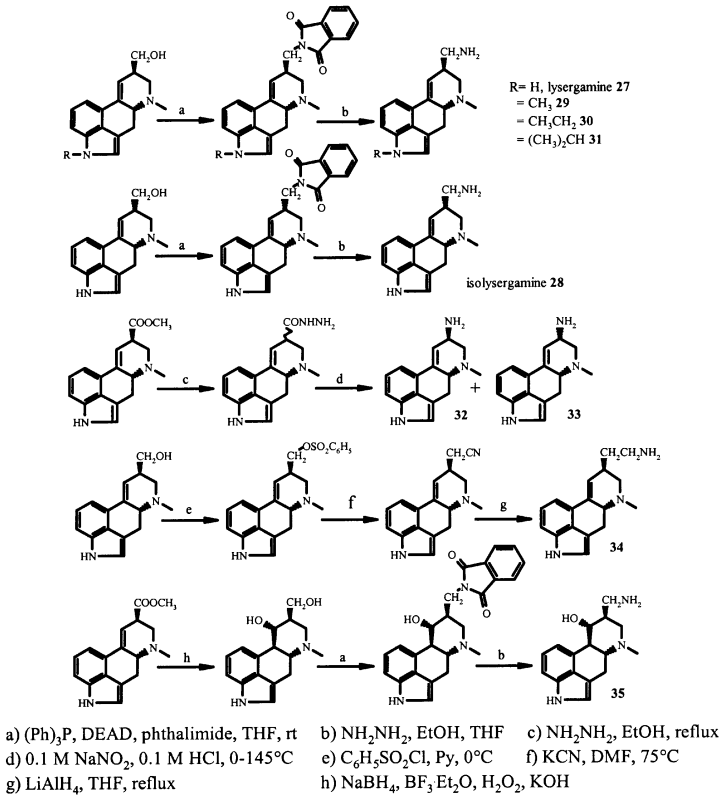


Figure 5.

dioxopiperazin-1-yl)ergoline derivatives from iminodiacetic acid derivative **V** and aliphatic or aromatic primary amines, under mild reactions condition, is shown in *figure 4*.

This method, based on the reaction of a acylimidazolide with a amide to form the imidic bond, gave rise in good yield to the 1-aryl or 1-heteroaryl-piperazin-2,6-dione derivatives which are quite inaccessible employing the former methodology. Mono saponification of **I** with potassium hydroxyde in ethanol gave the monoesters **V**. This intermediate was subsequently converted into the desired amido-esters **VI** by way of the acylimidazolide with 1,1'-carbonyldiimidazole in dioxane. Saponification of the ester function gave the acid-amides **VII** quantitatively. Ring closure of **VII,** after addition of 1 equivalent of 1,1'-carbonyldiimidazole, proceeded efficiently and cleanly in dioxane at reflux providing the piperazindiones **15**–**18** [26, 27]. Alternatively, 3,5-dioxopiperazin-4-nitrogen derivatives, such as **15** and **18**, can be prepared from **1** by reaction with methanol or cyclohexanol in presence of triphenylphosphine and diethylazodicarboxylate in accordance with the Mitsunobu methodology [28]. The amines required for compounds **1**–**4**, **11**–**14**, **26** listed in *tables I* and *II* were prepared as shown in *figure 5*.

6-Methyl-Δ-9,10-didehydroergolin-8β-methanamine (lysergamine) **27** and 6-methyl-Δ-9,10-didehydroergolin-8α-methanamine (isolysergamine) **28** were synthesised from lysergol and isolysergol, after conversion into the phthalimido derivatives by reaction with phthalimide, triphenylphosphine and diethylazodicarboxylate in tetrahydrofuran, followed by deblocking of the phthalimido group with hydrazine hydrate [28]. Analogously, 1,6-dimethyl-Δ-9,10-didehydroergolin-8β-methanamine **29**, 1-ethyl-6-methyl-Δ-9,10-didehydroergolin-8β-methanamine **30** and 1-isopropyl-6-methyl-Δ-9,10-didehydroergolin-8β-methanamine **31** were prepared as for **28**, starting from the corresponding 1-alkyl-lysergols, obtained by indole-nitrogen alkylation of lysergol with alkyl iodides in DMSO in presence of ground potassium hydroxyde [29]. 6-Methyl-Δ-9,10-didehydroergolin-8β-amine **32** and 6-methyl-Δ-9,10-didehydroergolin-8α-amine **33** were prepared through a Curtius degradation

Table III. In vitro characterization for the ergoline derivatives **1–26.** Affinities are expressed as IC_{50} in μM, standard errors are ±10 % of the mean reported values.

Compound	α_1	α_2	D_1	D_2	5-HT_1	5-HT_2
1	0.113	0.004	0.055	0.006	0.023	0.024
2	0.213	0.059	0.064	0.007	0.160	0.009
3	0.664	0.022	0.097	0.018	0.683	0.006
4	1.593	0.011	0.112	0.043	1.122	0.021
5	0.007	0.019	0.173	0.002	0.272	0.058
6	0.006	0.015	0.121	0.0008	0.040	0.074
7	0.031	0.004	0.231	0.001	0.404	0.071
8	6.404	0.612	1.015	0.284	0.058	0.862
9	0.175	0.351	0.234	0.046	0.026	4.121
10	0.732	0.012	0.090	0.004	0.023	0.154
11	0.634	0.073	0.632	0.122	0.014	0.087
12	2.450	0.231	2.467	> 10	4.876	> 10
13	1.80	0.355	5.13	0.254	0.251	0.153
14	0.157	0.031	0.643	0.210	0.036	0.143
15	0.507	0.042	0.418	0.030	0.031	0.075
16	0.453	0.031	0.286	0.030	0.021	0.142
17	0.786	0.021	> 10	0.784	0.579	0.347
18	0.321	0.078	0.145	0.019	0.167	0.047
19	2.407	0.342	> 10	0.132	0.213	0.518
20	1.705	0.503	0.493	0.065	> 10	0.392
21	0.302	0.364	0.143	0.022	> 10	0.065
22	0.795	0.071	0.333	0.004	0.853	0.572
23	1.302	1.311	1.804	0.133	> 10	0.222
24	1.20	0.377	> 10	0.062	> 10	5.554
25	4.40	0.201	0.461	0.088	1.23	0.282
26	3.46	0.568	0.574	0.697	2.54	0.763

carried out on the hydrazides of lysergic acid [30], whereas 6-methyl-Δ-9,10-didehydroergolin-8β-ethylamine **34** was provided by lithium aluminium hydride reduction of 6-methyl-Δ-9,10-didehydroergolin-8β-acetonitrile [31]. 6-Methyl-9α-hydroxy-dihydrolysergamine **35** was prepared from of 9α-hydroxy-dihydrolysergol, stemming from oxidative hydroboration of methyl lysergate [32], followed by conversion of hydroxymethyl into aminomethyl as described previously for the analogues.

3. Pharmacology

Screening in vitro was based on binding affinity studies for the receptors of neurotransmitters. This work was paralleled by in vivo evaluation of their antidopaminergic (antagonism of the apomorphine-induced climbing behaviour in normal rat) and dopaminergic component (contralateral turning behaviour in 6-hydroxydopamine lesioned rat). Classical structural modifications of the lead compound **1** were undertaken to investigate the role of the different functionalities present in the molecule.

3.1. In vitro characterization

The compounds described in this study were evaluated for their α_1, α_2, D_1, D_2, 5-HT_1 and 5-HT_2 receptor binding affinities assessed by measurement of the displacement of [^{3}H]-prazosin binding in rat frontal cortex [33], [^{3}H]-yohimbine binding in rat frontal cortex [34], [^{3}H]-SCH-23390 binding in rat striatum [35], [^{3}H]-spiroperidol binding in rat striatum [36], [^{3}H]-5-HT binding in rat hippocampus [37] and [^{3}H]-ketanserin binding in rat pre-frontal cortex [38] respectively, and the results are reported in *table III*.

3.2. In vivo characterization

The functional evaluation of the antidopaminergic or dopaminergic component present in the compounds examined was assessed as follows, and the results are reported in *table IV*.

3.2.1. Antagonism of apomorphine-induced climbing behaviour in rats

The inhibition of apomorphine-induced climbing behaviour was employed as a method for detecting potential

Table IV. In vivo characterization of the ergoline derivatives **1–26.** Number of turns are the mean values of five experiments. APO: apomorphine; 6-OH-DA: 6-hydroxydopamine: turning/treated: 6-OH-DA lesioned rats.

Compound	Antagonism of APO-induced climbing behaviour		Contralateral turning behaviour in 6-OH-DA lesioned rats		Turning/treated rats
	mg/kg s.c.	% Inhib.	mg/kg s.c.	Turns in 6 h	
1	0.4	89	0.1	2423	10/10
2	0.2	100	0.5	1668	4/4
3	0.4	61	0.5	391	1/4
4	0.4	42	0.5	1102	3/5
5	0.4	89	0.5	2075	4/4
6	0.4	91	0.05	3351	7/7
7	0.4	76	0.05	3567	5/5
8	0.4	26	0.5	–	0/4
9	0.4	17	0.5	–	0/4
10	0.4	26	0.05	1146	3/4
11	0.4	41	0.5	1058	5/5
12	0.4	0	0.5	–	0/5
13	0.4	0	0.5	–	0/5
14	0.4	0	0.5	–	0/4
15	0.4	84	0.5	1841	4/4
16	0.4	0	0.5	3056	4/4
17	0.4	12	0.5	1588	5/5
18	0.4	15	0.5	–	0/5
19	0.4	10	1	1687	5/5
20	0.4	0	1	312	1/5
21	0.4	10	1	710	4/4
22	0.4	11	0.5	2475	4/4
23	0.4	0	1	1828	4/4
24	0.4	0	0.5	1716	4/4
25	0.4	0	1	345	2/4
26	0.4	5	0.5	–	0/4

antipsychotic agents. In fact, both typical and atypical antipsychotic agents antagonise the response [39]. Experiments suggested that D_2 receptors appear to be primarily responsible for the mediation of climbing in the rats, while the D_1 receptors would appear to have a role in potentiating the climbing response induced by D_2 stimulation. The requirement for both D_1 and D_2 receptor stimulation to produce climbing behaviour is experimentally supported by the fact that both D_1 and D_2 antagonists will inhibit apomorphine-induced climbing [40–42].

3.2.2. Contralateral turning behaviour in 6-OHDA-lesioned rats

The compounds were evaluated in rats with unilateral 6-OHDA-induced lesions of the nigro-striatal DA pathway. This model is commonly used for in vivo screening of potential dopamine agonists. In this preparation, either directly acting non selective or selective D_1- and D_2-agonists induce a rotational turning behaviour towards the non-lesioned side (contralateral rotation), which is brought about by the development of postsynaptic DA receptor supersensitivity in the denervated striatum [43–45]. This action can be blocked by their respective antagonists [46]. Moreover, taking into account only dopaminergic mechanism, experimental results indicate a physiological interaction between D_1 and D_2 receptors [47]. The result of this test is predictive for a potential anti-Parkinson activity, inasmuch as the denervation of catecholaminergic pathway, loss of neurotransmitters from nerve terminals, and the development of postsynaptic DA receptor supersensitivity, as a consequence of injection of 6-OHDA, resemble the pathological and biochemical manifestation of Parkinson's disease.

4. Results

*4.1. Modifications on the indole nitrogen **2–4***

The D_2 component was slightly decreased when the indole nitrogen was substituted by a linear group, a methyl or ethyl as in **2**, **3**, and more significantly by a branched alkyl group, an isopropyl as in **4**. On the

contrary, the 5-HT_2 component was enhanced particularly in **3** reaching nanomolar values. An appreciable increase in the antidopaminergic activity was observed for **2**, whilst the dopaminergic activity was diminished or almost suppressed as in the case of **3**.

*4.2. Modifications on the position 2 of the indole ring **5–8***

Substitution by a halogen atom (chlorine or bromine), or by a thiomethyl strongly enhanced the α_1 and D_2 affinities. A subnanomolar D_2 affinity was revealed for **6**. Conversely, these substitutions appear to be detrimental for the 5-HT_1 and 5-HT_2 serotonergic components. These substitutions have virtually no influence on the in vivo antidopaminergic activity, whilst the dopaminergic activity was remarkably increased in particularly the case of **6** and **7**. Reduction of the 2, 3-indole double bond strongly affected the affinities for all the receptor sites considered, leading to the inactive compound **8**.

*4.3. Modifications on position 6 of the ergoline skeleton **9, 10***

Replacement of the 6-methyl by hydrogen was detrimental in term of in vitro and as well as in vivo activity. In fact, the nor analogue **9** was completely inactive. On the contrary, replacement with the n-propyl group seemed not to have significantly affected the dopaminergic activity when **10** was compared to **1**, whereas the antidopaminergic activity was strongly reduced.

*4.4. Modifications of the length or of the stereochemistry of the chain in position 8 **11–14***

A series of analogues which probes the influence of the spacing between the ergoline skeleton and the 3,5-dioxopiperazin-1-yl functionalities was examined. By shortening or lengthening of one methylene unit the spacer in position 8 as in the case of **12**, **13** and **14**, led to compounds devoid of activity. A similar result was encountered by changing the stereochemistry as in the case of the 8-α analogue **11**.

*4.5. Modifications on the 3,5-dioxopiperazin-4-nitrogen **15–18***

The effects of changes to the 3,5-dioxopiperazin-1-yl functionality on binding profile as well as in vivo activity were assessed. As illustrated by **15**–**18**, substitution of the imidic hydrogen of the 3,5-dioxopiperazin moiety resulted in a decrease in binding affinities, in particular for **17**. A notable diminution in the antidopaminergic activity was also observed for **16**–**18**. As far as the dopaminergic activity is concerned, only **16** maintained a significant activity, albeit lower than **1**. This compound was devoid of the antidopaminergic component present in the parent. It is interesting to note that increasing the bulkiness of the substituent led to a substantial decrease in in vitro and in vivo dopaminergic activity as observed for **17**. Finally, the cyclohexyl derivative **18** was inactive.

*4.6. Modification on the Δ-9,10 double bond **20–25***

When the Δ-9,10 double bond was satured, the affinities were strongly decreased with respect to the unsaturated analogues **1**, **2**, **10**, **15**, **16** and **19**. The antidopaminergic activity was abolished in all the compounds considered. Conversely, the dopaminergic activity was in some way retained as in the case of **22**–**24**, albeit lower than the unsaturated analogues.

*4.7. Modification on position 9 of the ergoline skeleton **26***

The introduction of a 9α-hydroxy group was very deleterious for in vitro and in vivo activity. In fact, **26** was totally inactive when compared to the unsaturated **1** and saturated analogue **20**.

The subset of compounds **2**, **5**–**7**, and **16**, having high affinity for dopaminergic receptor sites and potent in vivo antidopaminergic and dopaminergic activity was evaluated in more details at lower doses. In addition, interaction studies using the selective D_1 antagonist R-(+)-SCH-23390 (R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine hydrochloride) and D_2 antagonist S-(–)-sulpiride (S-(–)-5-(aminosulphonyl)-N-[(1-ethyl-2-pyrrolodinyl)-methyl]-2-methoxy-benzamide), were carried out to identify the dopamine receptor site mediating contralateral turning behaviour for these compounds, as reported in *table V*.

5. Discussion

The ergoline derivatives of the present study illustrate many key points relevant for the structural requirements necessary for the dopaminergic or antidopaminergic activity within this series. The structural requirement for retaining the antidopaminergic and dopaminergic activity present in the parent appears to be quite strict. In fact, only substitution in position 2 allows the maintenance of the uncommon pharmacological profile presented by **1**. Compounds such as **5**–**7** exert a more pronounced agonist and antagonist dopaminergic activity than the parent. The turning behaviour induced by **5**–**7** was antagonised

Table V. Evaluation of the ergoline derivatives **2, 5–7, 16.** The number of turns are the mean values of five experiments. Turn./treat. = Turning/treated 6-OH-DA lesioned rats.

Compound	Anti-climbing	Turning behaviour			Interaction study		
	ED_{50} mg/kg s.c.	Dose mg/kg s.c.	Turns in 6 h	Turn./treat. animals	Dose mg/kg s.c.	% inhibit. SCH-23390	% inhibit. S-sulpiride
1	0.18	0.1	2423	10/10	0.1	95	21
2	0.03	0.1	927	5/5	0.1	15	89
5	0.02	0.1	2600	5/7	0.025	25	64
6	0.06	0.1	2770	5/5	0.1	22	69
7	0.14	0.1	2874	5/5	0.05	22	97
16	–	0.1	1227	6/6	0.1	34	86

mainly by the D_2 antagonist S-(–)-sulpiride and not significantly by the selective D_1 antagonist R-SCH-(+)-23390. These results indicate that after a severe dopamine depletion, **5**–**7** behave as D_2 agonist, as opposed to **1** that acts as D_1 agonist. Of equal importance to the achievement of dopaminergic activity was the substitution of the imidic proton of the 3,5-dioxopiperazinyl moiety with phenyl group as in **16**. This compound revealed to be a potent D_2 agonist as shown by the interaction experiment, devoid of any antagonistic component. The considerable reduction in dopaminergic activity in **17** relative to **16** suggests that the D_2 receptor is sensitive to steric bulk in agonists of this structural type. Interestingly, the cyclohexyl analogue **18** was completely devoid of activity, notwithstanding displaying higher D_1 and D_2 affinity than **16** and **17**. The results show that the dopaminergic component is strongly dependent on structural modifications on the ergoline skeleton and on the heterocyclic moiety, and the exert of this activity is not always paralleled by in vitro the dopaminergic affinity. These results suggest, in first instance, the involvement of other receptor components or a different central bioavailability. In conclusion, in this study, we have reported a series of compounds related to **1**, whose dopamine antagonistic/agonistic activity can be strongly affected by the choice of the substituent in position 1 or 2 of the ergoline skeleton leading to in vivo potent dopamine antagonists/agonists, or by the 3,5-dioxopiperazin-4-nitrogen substituent that mainly afforded in vivo potent D_2 agonists.

6. Experimental protocol

6.1. Chemistry

Analytical and spectroscopic data were consistent with the structure of the corresponding compounds. Electron-impact mass spectra were recorded in form of *m/z* (intensity to base = 100) on a Finnigan MAT SSQ 7000 mass spectrometer. ^{1}H NMR were recorded on a Bruker AC 200 spectrometer at 200 MHz and Varian VXR 400 MHz. Chemical shifts are reported as δ values in part per million (ppm) relative to tetramethylsilane (δ 0.00) used as internal standard. Microanalyses were performed on Carlo Erba autoanalyser and were within ±0.4% of the calculated values.

*6.1.1. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-9,10-didehydro-6-methylergoline **1***

A solution of ethylbromoacetate (67 g, 425 mmol) in dimethylformamide (150 mL) was added dropwise to a stirred solution of **27** (50 g, 197 mmol) and potassium carbonate (60.8 g, 440 mmol) in dimethylformamide (300 mL) at room temperature. After stirring for 3 h, the suspension was evaporated in vacuo and the residue taken up in ethylacetate was partitioned with water then washed with brine and dried over sodium sulphate. Evaporation of the solvent, filtration of the residue on a small pad of silica gel by elution with acetone/cyclohexane 1:3 afforded after crystallisation from diethylether, 2.4 g of N-(ethoxycarbonylmethyl)-N-(ethoxycarbonylmethyloxycarbonyl)-lysergamine, m.p. 124–126 °C. MS *m/z*: 469 ($C_{25}H_{31}N_3O_6$, 56, $[M]^{+\cdot}$), 236 (36), 235 (24), 222 (38), 221 (41), 202 (100), 192 (34), 174 (15), 154 (19), 130 (33). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.64 (t, *J* = 7.2 Hz, 6 H, $COOCH_2\mathit{CH}_3$), 2.23 (dd, *J* = 9.5, 11.4 Hz, 1 H, H-7ax), 2.32 (s, 3 H, CH_3N), 2.66 (m, 1 H, H-4ax), 2.87 (m, 1 H, H-8ax), 3.12 (m, 1 H, H-5ax), 3.16 (m, 1 H, H-7eq), 3.56 (ddd, *J* = 5.3, 14.6 Hz, 1 H, H-4eq), 3.66 (m, 2 H, CH_2-8), 4.15 (q, *J* = 7.2 Hz, 4 H, $COO\mathit{CH}_2CH_3$), 4.32 (s, 2 H, NCH_2COO), 4.55 (d, *J* = 3.3 Hz, 2 H, $NCOOCH_2COO$), 6.56 (bs, 1 H, H-9), 6.73 (m, 1 H, H-2), 7.32 (m, 3 H, H-12, H-13, H-14), 10.1 (bs, 1 H, NH-1).

Continuing the elution with acetone/cyclohexane 1:2, 65 g of N-[(9,10-didehydro-6-methylergoline-8β-yl)methyl]-iminodiacetic acid diethyl ester (77% yield), m.p.

156–158 °C, were recovered after crystallisation from a small volume of ethanol. MS *m/z*: 425 ($C_{24}H_{31}N_3O_4$, 38, $[M]^{+\cdot}$), 352 (22), 236 (47), 235 (26), 222 (42), 221 (56), 202 (100), 192 (24), 174 (23), 154 (17), 130 (41). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.27 (t, *J* = 7.1 Hz, 6 H, $COOCH_2CH_3$), 2.23 (dd, *J* = 9.7, 11.4 Hz, 1 H, H-7ax), 2.57 (s, 3 H, CH_3N), 2.66 (m, 1 H, H-4ax), 2.6–3.0 (m, 2 H, CH_2-8), 2.87 (m, 1 H, H-8ax), 3.12 (m, 1 H, H-5ax), 3.16 (m, 1 H, H-7eq), 3.52 (ddd, *J* = 5.4, 14.6 Hz, 1 H, H-4eq), 3.61 (s, 4 H, NCH_2COO), 4.25 (q, *J* = 7.1 Hz, 4 H, $COOCH_2CH_3$), 6.39 (bs, 1 H, H-9), 6.88 (m, 1 H, H-2), 7.15 (m, 3 H, H-12, H-13, H-14), 9.4 (bs, 1 H, NH-1).

Gaseous ammonia was bubbled into a solution of N-[(9,10-didehydro-6-methylergoline-8β-yl)methyl]-iminodiacetic acid diethyl ester (25 g, 59 mmol) in dimethylformamide (100 mL) cooled at –10 °C until saturation. After keeping at room temperature overnight, the solvent was removed in vacuo and the residue was crystallised from methanol to provide 17 g of N-[(9,10-didehydro-6-methylergoline-8β-yl)methyl]-iminodiacetamide (78% yield), m.p. 245–247 °C. MS-FD (EHC = 25 mA) *m/z*: 367 ($C_{20}H_{25}N_5O_2$, 100, $[M]^{+\cdot}$). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.25 (dd, *J* = 9.6, 11.4 Hz, 1 H, H-7ax), 2.53 (s, 3 H, CH_3N), 2.76 (m, 1 H, H-4ax), 2.5–3.1 (m, 2 H, CH_2-8), 2.89 (m, 1 H, H-8ax), 3.17 (m, 1 H, H-5ax), 3.21 (m, 1 H, H-7eq), 3.45 (s, 4 H, NCH_2CONH_2), 3.52 (ddd, *J* = 5.4, 14.6 Hz, 1 H, H-4eq), 6.33 (bs, 1 H, H-9), 6.91 (m, 1 H, H-2), 7.16 (m, 3 H, H-12, H-13, H-14), 7.31 (bs, 4 H, $CONH_2$), 9.4 (bs, 1 H, NH-1).

A stirred mixture of phenol (15 g) and N-[(9,10-didehydro-6-methylergoline-8β-yl)methyl]-iminodiacetamide (3 g, 8 mmol) was slowly heated at 160 °C in a flow of nitrogen and kept at this temperature until the evolution of ammonia has ceased. After cooling, the solid was taken up in diethyl ether and the suspension filtered. The precipitated was dissolved in a small amount of acetone/chloroform 1:1 and filtered on a small column of silica gel eluting with acetone. The fractions containing the product were pooled and evaporated to small volume providing 1.9 g of **1** (63% yield) as shiny crystals. MS *m/z*: 350 ($C_{20}H_{22}N_4O_2$, 95, $[M]^{+\cdot}$), 236 (18), 223 (100), 221 (97), 207 (31), 192 (79), 180 (31), 167 (34), 154 (34), 127 (46), 71 (21), 42 (21). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.07 (dd, *J* = 9.5, 11.0 Hz, 1 H, H-7ax), 2.44 (s, 3 H, CH_3N), 2.3–2.6 (m, 3 H, H-4ax, CH_2-8), 2.84 (m, 1 H, H-8ax), 2.95 (m, 2 H, H-5ax, H-7e), 3.40 (m, 4 H, $N(CH_2CO)_2$), 3.44 (dd, *J* = 5.5, 14.5 Hz, 1 H, H-4e), 6.26 (bs, 1 H, H-9), 7.0–7.2 (m, 4 H, H-12, H-13, H-14), 10.69 (bs, 1 H, NH-1), 11.18 (bs, 1 H, CONHCO).

*6.1.2. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-9,10-didehydro-1,6-dimethyl methylergoline **2***

Compound **2** was synthesised from **29** following the procedure reported for **1**. MS *m/z*: 364 ($C_{21}H_{24}N_4O_2$, 100, $[M]^{+\cdot}$), 250 (11), 237 (72), 235 (46), 206 (34), 194 (13), 181 (12), 168 (13), 127 (12), 71 (17), 42 (92). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.03 (dd, *J* = 10.2, 10.2 Hz, 1 H, H-7ax), 2.3–2.5 (m, 3 H, H-4ax, CH_2-8), 2.42 (s, 3 H, CH_3N-6), 2.7–3.0 (m, 3 H, H-5ax, H-7e, H-8ax), 3.37 (m, 4 H, $N(CH_2CO)_2$), 3.43 (dd, *J* = 5.4, 14.8 Hz, 1 H, H-4e), 3.71 (s, 3 H, CH_3N-1), 6.25 (bs, 1 H, H-9), 6.9–7.2 (m, 4 H, H-12, H-13, H-14), 11.15 (bs, 1 H, CONHCO).

*6.1.3. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-1-ethyl-9,10-didehydro-6-methylergoline **3***

Compound **3** was synthesised from **30** following the procedure reported for **1**.

MS *m/z*: 378 ($C_{22}H_{26}N_4O_2$, 58, $[M]^{+\cdot}$), 263 (11), 251 (94), 249 (59), 235 (24), 221 (52), 220 (65), 208 (24), 195 (23), 182 (26), 127 (23), 71 (28), 42 (100). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.43 (t, *J* = 7.3 Hz, 3 H, NCH_2CH_3), 2.18 (dd, *J* = 10.2, 10.2 Hz, 1 H, H-7ax), 2.51 (m, 2 H, CH_2-8), 2.56 (s, 3 H, CH_3N), 2.69 (ddd, *J* = 1.6, 11.3, 14.3 Hz, 1 H, H-4ax), 2.93 (m, 1 H, H-8ax), 3.0–3.2 (m, 2 H, H-5ax, H-7e), 3.43 (m, 4 H, $N(CH_2CO)_2$), 3.50 (dd, *J* = 5.4, 14.3 Hz, 1 H, H-4e), 4.11 (q, *J* = 5.4, 14.3 Hz, 1 H, H-4e), 4.11 (q, *J* = 7.3 Hz, 2 H, NCH_2CH_3), 6.28 (bs, 1 H., H-9), 6.81 (d, *J* = 1.6 Hz, 1 H, H-2), 7.1–7.2 (m, 3 H, H-12, H-13, H-14), 8.54 (bs, 1 H, CONHCO).

*6.1.4. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-1-isopropyl-9,10-didehydro-6-methylergoline **4***

Compound **4** was synthesised from **31** following the procedure reported for **1**. MS *m/z*: 392 ($C_{23}H_{28}N_4O_2$, 32, $[M]^{+\cdot}$), 265 (49), 263 (23), 235 (23), 192 (19), 167 (13), 154 (12), 127 (15), 71 (25), 43 (83), 42 (100). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.50–1.51 (two d, *J* = 6.7 Hz, 6 H, $(CH_3)_2CHN$), 2.18 (dd, *J* = 10.3, 10.3 Hz, 1 H, H-7ax), 2.52 (m, 2 H, CH_2-8), 3.0–3.2 (m, 2 H, H-5ax, H-7e), 3.44 (m, 4 H, $N(CH_2CO)_2$), 3.50 (dd, *J* = 5.5, 14.4 Hz, 1 H, H-4e), 4.59 (m, 1 H, $(CH_3)_2CH$), 6.28 (bs, 1 H, H-9), 6.91 (d, *J* = 1.6 Hz, H-2), 7.1–7.2 (m, 3 H, H-12, H-13, H-14), 8.20 (bs, 1 H, CONHCO).

*6.1.5. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-2-bromo-9,10-didehydro-6-methylergoline **5***

A solution of N-bromosuccinimide (2.4 g, 14 mmol) in dioxane (45 mL) was added dropwise to a stirred solution of **1** (4.2 g, 12 mmol) in dioxane (60 mL) at 40 °C. After 1h, the solvent was removed and the residue taken up in chloroform was washed with 0.1 M of ammonium hydroxyde, then with brine and dried over sodium sul-

phate. The residue was chromatographed on silica gel eluting with acetone/cyclohexane 1:1 to give after crystallisation from acetone 3.1 of **5** (60% yield). MS *m/z*: 430 (56), 428 ($C_{20}H_{21}BrN_4O_2$, 56, $[M]^{+\cdot}$), 301 (47), 299 (23), 205 (49), 192 (55), 191 (52), 154 (23), 153 (32), 152 (30), 127 (100), 71 (27). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.04 (dd, *J* = 10.4, 10.4 Hz, 1 H, H-7ax), 2.3–2.4 (m, 3 H, H-4ax, CH_2-8), 2.41 (s, 3 H, CH_3N), 2.79 (m, 1 H, H-8ax), 2.9–3.0 (m, 2H, H-5ax, H-7e), 3.36 (m, 4 H, $N(CH_2CO)_2$), 6.26 (bs, 1 H, H-9), 7.0–7.1 (m, 3 H, H-12, H-13, H-14), 11.14 (bs, 1 H, CONHCO), 11.41 (bs, 1 H, NH-1).

*6.1.6. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-2-chloro-9,10-didehydro-6-methylergoline **6***

A solution of sulphurylchloride (1.3 g, 9.5 mmol) in acetonitrile (10 mL) was added dropwise) to a stirred solution of **1** (2.8 g, 8 mmol) in acetonitrile (50 mL) and borotrifluoride etherate (0.1 mL) at 0 °C. After stirring for 3 h, the solvent was removed in vacuo and the residue taken up in dichloromethane was washed with 0.1 M ammonium hydroxyde and dried over sodium sulphate. The solvent was removed, and the residue was twice crystallised from ethylacetate to provide 2.1 g of **6** (68% yield). MS *m/z*: 386 (29), 384 ($C_{20}H_{21}ClN_4O_2$, 86, $[M]^{+\cdot}$), 271 (11), 269 (19), 257 (85), 241 (19), 228 (26), 227 (41), 226 (32), 192 (26), 127 (24), 42 (37). ^{1}H-NMR (200 MHz, Py-d_5): δ 2.13 (dd, *J* = 11.8, 11.8 Hz, 1 H, H-7ax), 2.47 (s, 3 H, CH_3N), 2.49 (m, 2 H, CH_2-8), 2.77 (dd, *J* = 11.0, 14.6 Hz, 1 H, H-4ax), 3.0–3.2 (m, 3 H, H-5ax, H7e, H-8ax), 3.62 (m, 4 H, $N(CH_2CO)_2$), 3.63 (dd, *J* = 5.6, 14.6 Hz, 1 H, H-4e), 6.55 (bs, 1 H, H-9), 73–7.4 (m, 3 H, H-12, H-13, H-14), 12.80 (bs, 1 H, NH-1), 13.18 (bs, 1 H, CONHCO).

*6.1.7. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-2-methylthio-9,10-didehydro-6-methylergoline **7***

A solution of sulphurylchloride (2.2 g, 15 mmol)) in dichloromethane (40 mL) was slowly added dropwise to a stirred solution of dimethyldisulphide (0.8 g, 22 mmol) in dichloromethane (50 mL) at –20 °C. The yellow solution was set aside at room temperature for 1 h, and was then added dropwise to a stirred solution of **1** (1.75 g, 4.76 mmol) in dichloromethane (50 mL) at 0 °C. After being kept for 1 h at this temperature, the solution was slowly warmed to room temperature and partitioned with 0.1 M ammonium hydroxyde. The organic phase was washed with brine and then dried. After removal of the solvent, the residue was crystallised twice from ethylacetate to give 0.9 g of **7** (45% yield). MS *m/z*: 396 ($C_{21}H_{24}N_4O_2S$, 56, $[M]^{+\cdot}$), 381 (23), 269 (100), 267 (93), 253 (51), 239 (36), 238 (32), 192 (19), 191 (22), 180 (11), 127 (16), 47 (26), 42 (19). ^{1}H-NMR (200 MHz, Py-d_5): δ 2.14 (dd, *J* = 11.6, 11.6 Hz, 1 H, H-7ax), 2.44 (s, 3H, CH_3S), 2.46 (m, 2 H, CH_2-8), 2.48 (s, 3 H, CH_3N), 2.90 (dd, *J* = 11.2, 14.7 Hz, 1 H, H-4ax), 3.0–3.2 (m, 2 H, H-7e, H-8ax), 3.22 (m, 1 H, H-5ax), 3.62 (m, 4 H, $N(CH_2CO)_2$), 3.83 (dd, *J* = 5.3, 14.7 Hz, 1 H, H-4e), 6.57 (m, 1 H, H-9), 7.3–7.4 (m, 3 H, H-12, H-13, H-14), 12.23 (bs, 1 H, NH-1), 13.18 (bs, 1 H, CONHCO).

*6.1.8. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-2,3β-dihydro-9,10-didehydro-6-methylergoline **8***

Sodium borohydride (0.5 g, 12 mmol) was slowly added portionwise to a stirred solution of **1** (3.5 g, 10 mmol) in trifluoroacetic acid (30 mL) under nitrogen at –10 °C. After stirring for 30 min, the solution was diluted with ethylacetate and basified with 0.1 M ammonium hydroxyde. The organic phase was dried over sodium sulphate and the solvent removed off. The residue was chromatographed on silica gel eluting with acetone/cyclohexane 1:1.5 to provide, after crystallisation from diethyl ether 1.3 g of **8** (37% yield). MS *m/z*: 352 ($C_{20}H_{24}N_4O_2$, 83, $[M]^{+\cdot}$), 237 (19), 225 (66), 223 (100), 194 (23), 168 (31), 167 (23), 154 (16), 127 (64), 71 (19), 42 (31). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.39 (ddd, *J* = 11.5, 11.5, 11.5 Hz, 1 H, H-4ax), 2.12 (dd, *J* = 10.8, 10.8 Hz, 1 H, H-7ax), 2.4-2.6 (m, 3 H, H-4e, CH_2-8), 2.49 (s, 3 H, CH_3N, 2.85 (m, 1 H, H-8ax), 2.93 (m, 1 H, H-5ax), 3.06 (dd, *J* = 48, 10.8 Hz, 1 H, H-7e), 3.1–3.3 (m, 2 H, H-2α, H-3β), 3.42 (s, 4 H, $N(CH_2CO)_2$), 3.70 (dd, *J* = 6.7, 6.7 Hz, 1 H, H-2β), 6.22 (bs, 1 H, H-9), 6.50 (d, *J* = 7.2 Hz, 1 H, H-14), 6.9–7.1 (m, 2 H, H-12, H-13), 8.01 (bs, 1 H, CONHCO).

*6.1.9. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-9,10-didehydro-ergoline **9***

Cyanogen bromide (3.3 g, 31 mmol) was added to a stirred solution of **1** (9 g, 26 mmol) in dimethylformamide (75 mL) After stirring overnight, the solvent was removed in vacuo and the residue was twice crystallised from acetone to provide 7.3 g of 8β-(3,5-dioxopiperazin-1-ylmethyl)-9,10-didehydro-6-cyanoergoline, m.p. 234–237 °C MS-FD (EHC = 25 mA) *m/z*: 353. A suspension of 8β-(3,5-dioxopiperazin-1-ylmethyl)-9,10-didehydro-6-cyanoergoline (7 g, 19 mmol) and Zn dust (3.5 g, 54 mmol) in acetic acid (150 mL) was stirred for 5 h at 45 °C. After filtration, the solvent was removed in vacuo, and the residue was taken up in dichlorometane and washed with 0.1 M of ammonium hydroxyde. The organic phase was dried over sodium sulphate and evaporated. The residue was filtered on a small pad of silica gel eluting with acetone/cyclohexane 1:1.5, to furnish after crystallisation from ethylacetate 4.7 g of **9**

(74% yield). MS *m/z*: 336 ($C_{19}H_{20}N_4O_2$, 44, $[M]^{+\cdot}$), 221 (10), 209 (100), 207 (84), 192 (61), 180 (16), 167 (18), 154 (19), 127 (49), 71 (29), 42 (75). ^{1}H-NMR (200 MHz, Py-d_5): δ 2.45 (m, 2 H, CH_2-8), 2.68 (dd, *J* = 9.7, 11.9 Hz, 1 H, H-7ax), 2.94 (m, 1 H, H-8ax), 3.05 (ddd, *J* = 1.7, 11.7, 14.6 Hz, 1 H, H-4ax), 3.3–3.5 (m, 2 H, H-4e, H-7e), 3.58 (s, 4 H, $N(CH_2CO)_2$), 4.06 (m, 1 H, H-5ax), 6.61 (bs, 1 H, H-9), 7.2–7.5 (m, 4 H, H-2, H-12, H-13, H-14), 11.66 (bs, 1 H, NH-1), 13.14 (bs, 1 H, CONHCO).

*6.1.10. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-9,10-didehydro-6-propylergoline **10***

A stirred solution of **9** (4.6 g, 14 mmol), triethylamine (1.8 g, 17 mmol) and propyliodide (2.9 g, 17 mmol) in dimethylformamide (50 mL) was heated at 50 °C for 2 h. The solvent was removed in vacuo and the residue taken up in ethylacetate was washed with 0.1 M of ammonium hydroxyde, then dried over sodium sulphate. After removal of the solvent, the residue was chromatographed on silica gel eluting with ethylacetate to afford after crystallisation from acetone 3.2 g of **10** (65% yield). FD-MS (EHC = 22 mA) *m/z*: 378 ($C_{22}H_{26}N_4O_2$, 100, $[M]^{+\cdot}$). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 0.86 (t, *J* = 7.1, 3 H, $CH_3CH_2CH_2N$), 1.46 (m, 2 H, $CH_3CH_2CH_2N$), 2.11 (dd, *J* = 9.4, 11.0 Hz, 1 H, H-7ax), 24–2.5 (m, 3 H, H-4ax, CH_2-8), 2.6–2.7 (m, 3 H, H-8ax, $CH_3CH_2CH_2N$), 2.95 (dd, *J* = 4.2, 11.0 Hz, H-7e), 3.23 (m, 1 H, H-5ax), 3.36(s, 4 H, $N(CH_2CO)_2$), 3.39 (dd, *J* = 5.4, 14.5 Hz, 1 H, H-4e), 6.21 (bs, 1 H, H-9), 6.9–7.2 (m, 4 H, H-2, H-12, H-13, H-14), 10.65 (bd, *J* = 1.9 Hz, 1 H, NH-1), 11.16 (bs, 1 H, CONHCO).

*6.1.11. 8α-(3,5-Dioxopiperazin-1-ylmethyl)-9,10-didehydro-6-methylergoline **11***

Compound **11** was synthesised from **28** following the procedure reported for **1**.

MS *m/z*: 350 ($C_{20}H_{22}N_4O_2$, 100, $[M]^{+\cdot}$), 235 (11), 223 (66), 221 (11), 207 (20), 193 (45), 192 (67), 180 (25), 167 (23), 154 (21), 127 (12), 42 (14). ^{1}H-NMR (200 MHz, Py-d_5): δ 2.44 (s, 3 H, CH_3N), 2.5–3.0 (m, 6 H, H-4ax, CH_2-7, H-8e, CH_2-8), 3.21 (m, 1 H, H-5ax), 3.60 (s, 4 H, $N(CH_2CO)_2$), 3.62 (dd, *J* = 5.5, 14.3 Hz, 1 H, H-4e), 6.59 (dd, *J* = 1.3, 4.8Hz, 1 H, H-9), 7.2–7.5 (m, 4 H, H-2, H-12, H-13, H-14), 11.70 (bs, 1 H, NH-1), 13.14 (bs, 1 H, CONHCO).

*6.1.12. 8β-(3,5-Dioxopiperazin-1-yl)-9,10-didehydro-6-methylergoline **12***

Compound **12** was synthesised from **32** following the procedure reported for **1**.

MS *m/z*: 336 ($C_{19}H_{20}N_4O_2$, 12, $[M]^{+\cdot}$), 293 (100), 292 (45), 221 (21), 206 (36), 205 (26), 192 (14), 180 (70), 167 (29), 154 (25), 42 (30). ^{1}H-NMR (200 MHz, Py-d_5): δ 2.45 (s, 3 H, CH_3N), 2.50 (dd, *J* = 9.5, 10.6 Hz, 1 H, H-7ax), 2.81 (ddd, *J* = 1.6, 11.2, 14.5 Hz, 1 H, H-4ax), 3.01 (dd, *J* = 5.0, 10.6 Hz, 1 H, H-7e), 3.22 (m, 1 H, H-5ax), 3.56 (dd, *J* = 5.7, 14.5 Hz, 1 H, H-4e), 3.80 (s, 4 H, $N(CH_2CO)_2$), 3.94 (m, 1 H, H-8ax), 6.65 (bs, 1 H, H-9), 7.2–7.5 (m, 4 H, H-2, H-12, H-13, H-14), 11.74 (bs, 1 H, NH-1), 13.10 (bs, 1 H, CONHCO).

*6.1.13. 8α-(3,5-Dioxopiperazin-1-yl)-9,10-didehydro-6-methylergoline **13***

Compound **13** was synthesised from **33** following the procedure reported for **1**.

MS *m/z*: 336 ($C_{19}H_{20}N_4O_2$, 10, $[M]^{+\cdot}$), 293 (100), 221 (35), 207 (38), 206 (44), 205 (38), 192 (19), 180 (64), 167 (30), 154 (25), 43 (13), 42 (14). ^{1}H-NMR (200 MHz, Py-d_5): δ 2.32 (s, 3 H, CH_3N), 2.45 (dd, *J* = 4.1, 12.5 Hz, 1 H, H-7ax), 2.79 (ddd, *J* = 1.7, 11.2, 14.3 Hz, 1 H, H-ax), 3.0–3.1 (m, 2 H, H-5ax, H-7e), 3.45 (m, 1 H, H-8e), 3.52 (dd, *J* = 5.9, 14.3 Hz, 1 H, H-4e), 3.91 (s, 4 H, $N(CH_2CO)_2$), 6.58 (bd, *J* = 5.7 Hz, 1 H, H-9), 7.2–7.5 (m, 4 H, H-2, H-12, H-13, H-14), 11.77 (bs, 1 H, NH-1), 12.91 (bs, 1 H, CONHCO).

*6.1.14. 8β-(3,5-Dioxopiperazin-1-ylethyl)-9,10-didehydro-6-methylergoline **14***

Compound **14** was synthesised from **34** following the procedure reported for **1**. MS *m/z*: 364 ($C_{21}H_{24}N_4O_2$, 96, $[M]^{+\cdot}$), 237 (30), 223 (46), 221 (71), 206 (45), 194 (88), 192 (48), 167 (44), 154 (79), 127 (96), 71 (25), 44 (100), 42 (90). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.47 (m, 2 H, CH_2CH_2N 8β-chain), 2.04 (dd, *J* = 10.5, 10.5 Hz, 1 H, H-7ax), 2.3–2.6 (m, 4 H, H-4ax, H-8ax, H-CH_2CH_2N 8β-chain), 2.40 (s, 3 H, CH_3N), 2.8–3.0 (m, 2 h, H-5ax, H-7e), 3.33 (s, 4 H, $N(CH_2CO)_2$), 342 (dd, *J* = 5.4, 14.8 Hz, 1 H, H-4e), 6.29 (bs, 1 H, H-9), 7.0–7.2 (m, 4 H, H-2, H-12, H-13, H-14), 10.65 (bs, 1 H, NH-1), 11.12 (bs, 1 H, CONHCO).

*6.1.15. [8β-(3,5-dioxo-4-methylpiperazin-1-yl)-methyl]-9,10-didehydro-6-methylergoline **15***

Diethylazodicarboxylate (1.8 g, 0.11 mmol) was slowly added dropwise to a stirred solution of **1** (3 g, 8.6 mmol), triphenylphosphine (2.7 g, 10 mmol) and methanol (0.8 g, 26 mmol) in tetrahydrofuran (50 mL) at room temperature. After stirring for 3 h, the solvent was evaporated and the residue was chromatographed on silica gel eluting with acetone/cyclohexane 1:2. After crystallisation from ethylacetate, 2.1 g of **15** (65% yield) were obtained. MS *m/z*: 364 ($C_{21}H_{24}N_4O_2$, 67, $[M]^{+\cdot}$), 236 (16), 235 (17), 223 (100), 221 (97), 192 (76), 180 (31), 167 (37), 154 (38), 141 (43), 113 (69), 42 (98). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.18 (dd, *J* = 9.8, 10.8 Hz, 1 H, H-7ax), 2.47 8m, 2 H, CH_2-8), 2.56 (s, 3 H,

CH_3N), 2.67 (ddd, *J* = 1.8, 11.3, 14.4 Hz, H-4ax), 2.91 (m, 1 H, H-8ax), 3.0–3.2 (m, 2 H, H-5ax, H-7e), 3.18 (s, 3 H, $CON(CH_3)CO$), 3.47 (s, 4 H, $N(CH_2CO)_2$), 3.52 (dd, *J* = 5.3, 14.4 Hz, 1 H, H-4e), 6.29 (bd, 1 H, H-9), 6.90 (t, *J* = 1.8 Hz, 1 H, H-2), 7.1–7.2 (m, 3 H, H-12, H-13, H-14), 7.91 (bs, 1 H, NH-1).

6.1.16. [8β-(3,5-dioxo-4-phenylpiperazin-1-yl)-methyl]-9,10-didehydro-6-methylergoline ***16***

1 M Potassium hydroxyde (60 mL, 60 mmol) was added dropwise to a solution of N-[(9,10-didehydro-6-methylergoline-8-yl)methyl]-iminodiacetic acid diethyl ester (25 g, 59 mmol) in ethanol (100 mL). After stirring for 3 h, the precipitate was filtered, dissolved in boiling water and treated with 1 M hydrochloric acid (62 mL, 62 mmol). The precipitate was washed with a small volume of water, then crystallised from ethanol/water 5:1 to provide 18.3 g of N-[(9,10-didehydro-6-methylergoline-8-yl)methyl]-iminodiacetic acid monoethyl ester (79% yield), m.p. 248–251 °C. MS-FD (EHC = 25 mA) *m/z*: 397 ($C_{21}H_{28}N_4O_4$, 100, $[M]^{+\cdot}$). 1,1'-Carbonyldiimidazole (1.6 g, 10 mmol) was added to a stirred suspension of N-(9,10-didehydro-8β-ylmethyl-6-methylergoline)-iminodiacetic acid monoethyl ester (3.5 g, 8.8 mmol) in dioxane (75 mL), then heated at reflux for 15 min. After cooling, aniline (2 g, 24 mmol) was added and the solution was refluxed for 3 h. The solvent was removed and the residue was chromatographed on silica gel eluting with ethyl-acetate/cyclohexane 1:1 to give 2.4 g of N-[(9,10-didehydro-6-methylergoline-8β-yl)methyl]-N-(phenyl-carbamoylmethyl)-glycine ethyl ester (60% yield), m.p. 196–198 °C. MS-FD (EHC = 30 nA) *m/z*: 472 ($C_{28}H_{30}N_4O_3$, 100, $[M]^{+\cdot}$). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.28 (t, *J* = 7.2 Hz, 3 H, $COOCH_2CH_3$), 2.25 (dd, *J* = 9.7, 11.4 Hz, 1 H, H-7-ax), 2.56 (s, 3 H, CH_3N), 2.6–2.9 (m, 3 H, H-4ax, CH_2-8), 2.90 (m, 1, H-8ax), 3.12 (m, 1 H, H-5ax), 3.16 (dd, *J* = 5.4, 11.4 Hz, 1 H, H-4e), 3.43 (s, 2 H, NCH_2CON), 3.51 (dd, *J* = 5.4, 11.4 Hz, 1 H, H-4e), 3.52 (s, 2 H, $NCH_2COOCH_2CH_3$), 4.30 (q, *J* = 7.2 Hz, 2 H, $COOCH_2CH_3$), 6.39 (bs, 1 H, H-9), 6.89 (t, *J* = 1.9 Hz, 1 H, H-2), 7.0–7.4 (m, 6 H, H-12, H-13, H-14, N-phenyl hydrogen H-3', H-4', H-5'), 7.68 (d, *J* = 8.0, Hz, N-phenyl hydrogen H-2', H-6'), 7.93 (bs, 1 H, NH-1), 9.79 (bs, 1 H, CONH).

A solution of N-[(9,10-didehydro-6-methylergoline-8β-yl)methyl]-N-(phenylcarbamoylmethyl)-glycine ethyl ester (4 g, 8.6 mmol) and 1 M potassium hydroxyde (10 mL, 10 mmol) in ethanol (50 mL) was heated at 50 °C for 3 h, then treated dropwise with 1 M of hydrochloric acid (11 mL, 11 mmol). The precipitate was filtered off, washed with water, then with acetone to provide 2.9 g of N-[(9,10-didehydro-6-methylergoline-8β-yl)methyl]-N-(phenylcarbamoylmethyl)-glycine (77% yield), m.p. 252–255 °C. MS *m/z*: 444 ($C_{26}H_{28}N_4O_3$, 4, $[M]^{+\cdot}$), 351 (3), 337 (4), 236 (13), 235 (14), 223 (11), 221 (16), 192 (17), 151 (15), 106 (16), 93 (100). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.63 (s, 3 H, CH_3N), 3.39 (s, 4 H, $N(CH_2COOH)CH_2CONHC_6H_5$), 6.51 (bs, 1 H, H-9), 6.9–7.6 (m, 9 H, H-2, H-12, H-13, H-14, N-phenyl hydrogen H-2', H-3', H-4', H-5', H-6'), 10.22 (bs, 1 H, CONH), 10.64 (d, *J* = 2.1 Hz, 1 H, NH-1).

1,1'-Carbonyldiimidazole (5.6 g, 34 mmol) was added to a stirred suspension of N-[(9,10-didehydro-6-methyl-ergoline-8β-yl)methyl]-N-(phenylcarbamoylmethyl)-glycine (5 g, 11.4 mmol) in dioxane (75 mL), then refluxed for 3 h. The solvent was removed and the residue was chromatographed on silica gel eluting with acetone/cyclohexane 3:2. After crystallisation from acetone, 3.7 g of **16** (77% yield) were obtained. MS *m/z*: 426 ($C_{26}H_{26}N_4O_2$, 49, $[M]^{+\cdot}$), 295 (14), 223 (67), 221 (100), 207 (28), 193 (51), 192 (63), 167 (39), 154 (35), 106 (36). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.24 (dd, *J* = 10.6, 10.6 Hz, 1 H, H-7ax), 2.59 (m, 2 H, CH_2-8), 2.59 (s, 3 H, CH_3N), 2.70 (ddd, *J* = 1.8, 11.4, 14.5 Hz, 1 H, H-4ax), 2.98 (m, 1 H, H-8ax), 3.1–3.2 (m, 2 H, H-5ax, H-7e), 3.54 (dd, *J* = 5.2, 14.5 Hz, 1 H, H-4e), 3.65 (s, 4 H, $N(CH_2CO)_2$), 6.34 (bs, 1 H, H-9), 6.92 (t, *J* = 1.8 Hz, H-2), 7.2–7.5 (m, 9 H, H-2, H-12, H-13, H-14, N-phenyl hydrogen H-2', H-3', H-4', H-5', H-6'), 7.91 (bs, 1 H, NH-1).

6.1.17. [8β-(3,5-dioxo-4-(2,6-dimethylphenyl)piperazin-1-yl)-methyl]-9,10-didehydro-6-methylergoline ***17***

Compound **17** was synthesised from N-(9,10-didehydro-8β-ylmethyl-6-methylergoline)-iminodiacetic acid monoethyl ester and 2,6-dimethylaniline following the procedure reported for **16**. MS *m/z*: 454 ($C_{28}H_{30}N_4O_2$, 100, $[M]^{+\cdot}$), 236 (18), 231 (37), 223 (50), 221 (64), 193 (28), 192 (32), 167 (9), 154 (14), 42 (21). ^{1}H-NMR (200 MHz, Py-d_5): δ 2.20 (s, 6 H, N-xylyl methyl CH_3-2', CH_3-6'), 2.24 (dd, *J* = 11.2, 11.2 Hz, 1 H, H-7ax), 2.50 (s, 3 H, CH_3N), 2.65 (m, 2 H, CH_2-8), 2.91 (ddd, *J* = 1.7, 11.2, 14.3 Hz, 1 H, H-4ax), 3.1–3.3 (m, 3 H, H-5ax, H-7e, H-8ax), 3.66 (dd, *J* = 5.1, 14.3 Hz, H-4e), 3.95 (s, 4 H, $N(CH_2CO)_2$), 6.62 (bs, 1 H, H-9), 7.1–7.4 (m, 7 H, H-2, H-12, H-13, H-14, N-xylyl hydrogen H-3', H-4', H-5'), 11.68 (bs, 1 H, NH-1).

6.1.18. [8β-(3,5-dioxo-4-cyclohexylpiperazin-1-yl)-methyl]-9,10-didehydro-6-methylergoline ***18***

Compound **18** was synthesised from **1** and cyclohexanol following the procedure reported for **15**. MS *m/z*: 432 ($C_{26}H_{32}N_4O_2$, 94, $[M]^{+\cdot}$), 236 (27), 223 (100), 221

(96), 209 (28), 192 (38), 181 (16), 167 (17), 154 (15), 127 (27), 71 (32), 43 (41), 42 (41). ^{1}H-NMR (200 Mhz, Py-d_5): δ 1.0–1.7 (m, 10 H, N-cyclohexyl hydrogen H-2'ax, H-6'ax, CH_2-3', CH_2-4', CH_2-5'), 2.14 (d, *J* = 11.9, 11.9 Hz, 1 H, H-7ax), 2.3–2.6 (m, 4 H, CH_2-8, N-cyclohexyl hydrogen H-2'e, H-6'e), 2.46 (s, 3 H, CH_3N), 2.89 (ddd, *J* = 1.5, 11.2, 14.2 Hz, 1 H, H-4ax), 3.0–3.2 (m, 2 H, H-7e, H-8ax), 3.23 (m, 1 H, H-5ax), 3.63 (dd, *J* = 5.3, 14.2 Hz, 1 H, H-4e), 3.65 (s, 4 H, $N(CH_2CO)_2$), 4.72 (m, 1 H, N-cyclohexyl H-1'ax), 6.55 (bs, 1 H, H-9), 7.2–7.5 (m, 4 H, H-2, H-12, H-13, H-14), 11.69 (bs, 1 H, NH-1).

*6.1.19. [8β-(3,5-dioxo-4-aminopiperazin-1-yl)-methyl]-9,10-didehydro-6-methylergoline **19***

Compound **19** was synthesised from N-(9,10-didehydro-8β-ylmethyl-6-methylergoline)-iminodiacetic acid diethyl ester and hydrazine hydrate following the procedure reported for **1**. MS *m/z*: 365 ($C_{20}H_{23}N_5O_2$, 76, $[M]^{+\cdot}$), 236 (17), 223 (95), 221 (100), 207 (26), 193 (70), 180 (23), 167 (26), 154 (27), 42 (38). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.08 (dd, *J* = 9.8, 10.7 Hz, 1 H, H-7ax), 2.42 (s, 3 H, CH_3N), 2.84 (m, 1 H, H-8ax), 3.43 (dd, *J* = 5.5, 14.6 Hz, 1 H, H-4e), 3.55 (s, 4 H, $N(CH_2CO)_2$), 5.12 (bs, 2 H, N–NH_2), 6.22 (bs, 1 H, H-9), 6.9–7.2 (m, 4 H, H-2, H-12, H-13, H-14).

*6.1.20. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-6-methyl-ergoline **20***

A solution of **1** (7 g, 20 mmol) in acetic acid (170 mL) was hydrogenated at 3 atmosphere pressure over 2.5 g of 10% Pd/C. The calculated amount of H_2 was taken up in 3 h. The catalyst was removed by filtration and the solvent was evaporated off. The residue dissolved in chloroform, was washed with dilute ammonium hydroxyde solution, then the organic phase was dried and evaporated. The residue was crystallised from acetone to afford 5.3 g of **20** (75% yield). MS *m/z*: 352 ($C_{20}H_{24}N_4O_2$, 93, $[M]^{+\cdot}$), 237 (40), 225 (46), 223 (39), 207 (24), 194 (39), 167 (69), 154 (100), 144 (61), 127 (60), 44 (46), 42 (68). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 0.96 (ddd, *J* = 12.3, 12.3, 12.3 Hz, 1 H, H9-ax), 1.79 (dd, *J* = 11.4, 11.4 Hz, 1 H, H-7ax), 1.93 (dd, *J* = 4.3, 9.2 Hz, 1 H, H-8ax), 2.23 (ddd, *J* = 4.3, 9.5, 11.5 Hz, 1 H, H-5ax), 2.10 (m, 1H, H-9ax), 2.32 (s, 3 H, CH_3N), 2.3–2.36 (m, 2 H, H-4ax, H-9e), 2.59 (m, CH_2-8), 2.75 (ddd, *J* = 3.5, 9.5, 12.3 Hz, 1 H, H-10ax), 2.88 (bd, *J* = 11.4, 1 H, H-7e), 3.28 (dd, *J* = 4.3, 14.6 Hz, 1 H, H-4e), 3.38 (s, 4 H, $N(CH_2CO)_2$), 6.77 (d, *J* = 8.0 Hz, 1 H, H-12), 6.99 (t, *J* = 8.0 Hz, 1 H, H-13), 7.10 (d, *J* = 8.0 Hz, 1 H, H-14), 10.59 (bs, 1H, NH-1), 11.20 (bs, 1 H, CONHCO).

*6.1.21. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-1,6-dimethyl-ergoline **21***

Compound **21** was synthesised from **2** following the procedure reported for **20**. MS *m/z*: 366 ($C_{21}H_{26}N_4O_2$, 43, $[M]^{+\cdot}$), 251 (13), 239 (15), 237 (14), 194 (9), 181 (25), 168 (48), 158 (26), 127 (18), 44 (37), 42 (100). ^{1}H-NMR (200 MHz, Py-d_5): δ 0.93 (ddd, *J* = 12.2, 12.2, 12.2 Hz, 1 H, H9-ax), 1.81 (dd, *J* = 11.3, 11.3 Hz, 1 H, H-7ax), 1.95 (dd, *J* = 4.3, 9.2 Hz, 1 H, H-8ax), 2.24 (ddd, *J* = 4.5, 9.5, 11.6 Hz, 1 H, H-5ax), 2.14 (m, 1H, H-9ax), 2.34 (s, 3 H, CH_3N), 2.3–2.4 (m, 2 H, H-4ax, H-9e), 2.61 (m, CH_2-8), 2.77 (ddd, *J* = 3.5, 9.5, 12.3 Hz, 1 H, H-10ax), 2.89 (bd, *J* = 11.3, 1 H, H-7e), 3.31 (dd, *J* = 4.3, 14.6 Hz, 1 H, H-4e), 3.35 (s, 4 H, $N(CH_2CO)_2$) 3.74 (s, 3 H, CH_3-1), 6.87 (d, *J* = 7.8 Hz, 1 H, H-12), 6.96 (t, *J* = 7.8 Hz, 1 H, H-13), 7.15 (d, *J* = 7.8 Hz, 1 H, H-14), 11.25 (bs, 1 H, CONHCO).

*6.1.22. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-6-propyl-ergoline **22***

Compound **22** was synthesised from **10** following the procedure reported for **20**. MS *m/z*: 380 ($C_{22}H_{28}N_4O_2$, 29, $[M]^{+\cdot}$), 265 (9), 253 (14), 251 (9), 223 (22), 167 (30), 154 (59), 153 (41), 144 (30), 127 (58), 71 (37), 44 (41), 43 (74), 42 (100). ^{1}H-NMR (200 MHz, Py-d_5): δ 0.85 (t, *J* = 7.3. Hz, 3 H, $CH_3CH_2CH_2N$), 1.18 (ddd, *J* = 12.2, 12.2, 12.2 Hz, 1 H, H-9ax), 1.42 (m, 2 H, $CH_3CH_2CH_2N$), 2.02 (dd, *J* = 11.2, 11.2 Hz, 1 H, H-7ax), 2.20 (m, 2 H, H-5ax, H-8ax), 2.27 (ddd, *J* = 1.7, 11.4, 14.8 Hz, 1 H, H-4ax), 2.45 (m, 2 H, $CH_3CH_2CH_2N$), 2.50 (m, 2 H, CH_2-8), 2.60 (m, 1 H, H-9e), 2.95 (m, 2 H, H-7e, H-10ax), 3.35 (s, 4 H, $N(CH_2CO)_2$), 6.71 (d, *J* = 8.0 Hz, 1 H, H-12), 6.93 (t, *J* = 8.0 Hz, 1 H, H-13), 7.15 (d, *J* = 8.0 Hz, 1 H, H-14), 10.63 (bs, 1H, NH-1), 11.27 (bs, 1 H, CONHCO).

*6.1.23. [8β-(3,5-dioxo-4-methylpiperazin-1-yl)-methyl]-6-methylergoline **23***

Compound **23** was synthesised from **15** following the procedure reported for **20**.

MS *m/z*: 366 ($C_{21}H_{26}N_4O_2$, 100, $[M]^{+\cdot}$), 237 (31), 225 (31), 223 (22), 167 (37), 154 (37), 144 (20), 141 (13), 127 (13), 44 (19), 42 (26). δ ^{1}H-NMR (200 MHz, Py-d_5): δ 0.98 (ddd, *J* = 12.2, 12.2, 12.2 Hz, 1 H, H9-ax), 1.76 (dd, *J* = 11.4, 11.4 Hz, 1 H, H-7ax), 1.94 (dd, *J* = 4.2, 9.2 Hz, 1 H, H-8ax), 2.24 (ddd, *J* = 4.2, 9.5, 11.5 Hz, 1 H, H-5ax), 2.12 (m, 1H, H-9ax), 2.32 (s, 3 H, CH_3N), 2.3–2.4 (m, 2 H, H-4ax, H-9e), 2.58 (m, CH_2-8), 2.77 (ddd, *J* = 3.5, 9.5, 12.2 Hz, 1 H, H-10ax), 2.88 (bd, *J* = 11.4, 1 H, H-7e), 3.15 (s, 3 H, $CON(CH_3)CO$), 3.28 (dd, *J* = 4.2, 14.6 Hz, 1 H, H-4e), 3.38 (s, 4 H, $N(CH_2CO)_2$), 6.77 (d, *J* = 8.0

Hz, 1 H, H-12), 6.96 (t, *J* = 8.1 Hz, 1 H, H-13), 7.15 (d, *J* = 8.1 Hz, 1 H, H-14), 10.59 (bs, 1H, NH-1).

*6.1.24. [8β-(3,5-dioxo-4-phenylpiperazin-1-yl)-methyl]-6-methylergoline **24***

Compound **24** was synthesised from **16** following the procedure reported for **20**. MS *m/z*: 428 ($C_{26}H_{28}N_4O_2$, 34, $[M]^{+\cdot}$), 237 (22), 225 (27), 223 (21), 167 (44), 154 (47), 144 (32), 127 (14), 106 (18), 42 (100). ^{1}H-NMR (200 MHz, Py-d_5): δ 0.92 (ddd, *J* = 12.2, 12.2, 12.2 Hz, 1 H, H9-ax), 1.75 (dd, *J* = 11.2, 11.2 Hz, 1 H, H-7ax), 1.94 (dd, *J* = 4.2, 9.1 Hz, 1 H, H-8ax), 2.23 (ddd, *J* = 4.2, 9.5, 11.6 Hz, 1 H, H-5ax), 2.12 (m, 1H, H-9ax), 2.31 (s, 3 H, CH_3N), 2.3–2.5 (m, 2 H, H-4ax, H-9e), 2.53 (m, CH_2-8), 2.69 (ddd, *J* = 3.4, 9.5, 12.2 Hz, 1 H, H-10ax), 2.88 (bd, *J* = 11.4, 1 H, H-7e), 3.28 (dd, *J* = 4.2, 14.6 Hz, 1 H, H-4e), 3.38 (s, 4 H, $N(CH_2CO)_2$), 7.1–7.5 (m, 9 H, H-2, H-12, H-13, H-14, N-phenyl hydrogen H-2', H-3', H-4', H-5', H-6'), 10.89 (bs, 1H, NH-1).

*6.1.25. [8β-(3,5-dioxo-4-aminopiperazin-1-yl)-methyl]-6-methylergoline **25***

Compound **25** was synthesised from **19** following the procedure reported for **20**.

MS *m/z*: 367 ($C_{20}H_{25}N_5O_2$, 71, $[M]^{+\cdot}$), 237 (49), 225 (51), 223 (68), 194 (24), 181 (25), 167 (74), 154 (88), 144 (52), 127 (43), 115 (28), 44 (96), 42 (100). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 0.93 (ddd, *J* = 12.3, 12.3, 12.3 Hz, 1 H, H9-ax), 1.86 (dd, *J* = 11.2, 11.2 Hz, 1 H, H-7ax), 1.94 (dd, *J* = 4.2, 9.2 Hz, 1 H, H-8ax), 2.24 (ddd, *J* = 4.2, 9.5, 11.5 Hz, 1 H, H-5ax), 2.13 (m, 1H, H-9ax), 2.37 (s, 3 H, CH_3N), 2.3–2.4 (m, 2 H, H-4ax, H-9e), 2.61 (m, CH_2-8), 2.69 (ddd, *J* = 3.5, 9.5, 12.2 Hz, 1 H, H-10ax), 2.88 (bd, *J* = 11.2, 1 H, H-7e), 3.28 (dd, *J* = 4.2, 14.6 Hz, 1 H, H-4e), 3.41 (s, 4 H, $N(CH_2CO)_2$), 5.23 (bs, 2 H, N-NH_2), 6.64 (d, *J* = 8.2 Hz, 1 H, H-12), 6.93 (t, *J* = 8.2 Hz, 1 H, H-13), 7.18 (d, *J* = 8.2Hz, 1 H, H-14), 10.71 (bs, 1H, NH-1).

*6.1.26. 8β-(3,5-Dioxopiperazin-1-ylmethyl)-9α-hydroxy-6-methylergoline **26***

Compound **26** was synthesised from **35** following the procedure reported for **1**.

MS *m/z*: 368 ($C_{20}H_{24}N_4O_3$, 36, $[M]^{+\cdot}$), 254 (40), 253 (93), 241 (34), 239 (37), 223 (62), 214 (83), 193 (38), 183 (27), 154 (80), 127 (56), 71 (27), 44 (60), 42 (100). ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.92 (m, 2 H, H-8ax, H-7ax), 2.01 (ddd, *J* = 4.0, 10.0, 11.0 Hz, 1 H, H-5), 2.29 (s, 3 H, CH_3N), 2.54 (ddd, *J* = 1.0, 11.0, 14.0 Hz, 1 H, H-4ax), 2.80 (dd, *J* = 10.0, 10.0 Hz, 1 H, H-10), 2.92 (m, 1 H, H-7eq), 3.24 (dd, *J* = 4.0, 14.0 Hz, 1H, H-4eq), 3.39 (s, 4 H, $N(CH_2CO)_2$), 3.51 (m, 1 H, H-9), 4.82 (d, *J* = 7.7 Hz, 1 H, OH-9), 6.91 (m, 1 H, H-2), 6.94 (m, 1 H, H-13), 7.08 (d, *J* = 8.0 Hz, 1 H, H-12), 7.54 (d, *J* = 7.0 Hz, 1 H, H-14), 10.53 (bs, 1 H, NH-1), 11.20 (bs, 1 H, CONHCO).

6.2. Pharmacology

All binding studies were performed in rat brain areas. Male Wistar rats weighing 175–200 g, were killed by decapitation under light anesthesia and the various brain regions dissected out very quickly on an ice-cold plate. Depending on the receptor to be studied the different areas were used following the methods reported.

6.2.1. α_1 Adrenergic binding assay

Brain cortex was homogenized in 30 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25 °C) using a Polytron PT10 (setting 5 for 20 sec). Homogenates were centrifuged twice for 10 min at 50000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in ice-cold 50 mM Tris-HCl (pH 7.4 at 25 °C). Each assay tube contained 50 mL of drug solution, 50 mL of [^{3}H]-prazosin to achieve a final concentration of 0.4 nM, and 900 mL of resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was termined by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25 °C). The radioactivity bound to the filters was measured by liquid scintillation counter. Specific [^{3}H]-prazosin binding was defined as the difference between binding in the absence or presence of 1 μM phentolamine.

6.2.2. α_2 Adrenergic binding assay

Brain cortex was homogenized in 30 volumes (w/v) of ice-cold 5 mM Tris-HCl, 5 mM EDTA buffer (pH 7.3 at 25 °C) using a Polytron PT10 (setting 5 for 20 sec). Homogenates were centrifuged three times for 10 min at 50000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in ice-cold 50 mM Tris-HCl, 0.5 mM EDTA (pH 7.5 at 25 °C). Each assay tube contained 50 mL of drug solution, 50 mL of [^{3}H]-yohimbine to achieve a final concentration of 1 nM, and 900 mL of resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was termined by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl, 0.5 mM EDTA buffer (pH 7.5 at 25 °C). The radioactivity bound to the filters was measured by liquid scintillation counter. Specific [^{3}H]-yohimbine binding was defined as the difference between binding in the absence or presence of 10 μM phentolamine.

6.2.3. D_1 Dopaminergic binding assay

Striata were homogenized in 30 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 sec). Homogenates were centrifuged twice for 10 min at 50000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in ice-cold Tris-HCl 50 mM containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 mL of drug solution, 50 mL of [^{3}H]-SCH 23390 to achieve a final concentration of 0.4 nM, and 900 mL of resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37 °C and the incubation was termined by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The radioactivity bound to the filters was measured by liquid scintillation counter. Specific [^{3}H]-SCH 23390 binding was defined as the difference between binding in the absence or presence of 0.1 μM piflutixol.

6.2.4. D_2 Dopaminergic binding assay

Striata were homogenized in 30 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 sec). Homogenates were centrifuged twice for 10 min at 50000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in ice-cold Tris-HCl 50 mM containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 mL of drug solution, 50 mL of [^{3}H]-spiroperidol to achieve a final concentration of 0.4 nM, and 900 mL of resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37 °C and the incubation was termined by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The radioactivity bound to the filters was measured by liquid scintillation counter. Specific [^{3}H]-spiroperidol binding was defined as the difference between binding in the absence or presence of 1 μM (+)-butaclamol.

6.2.5. 5-HT_{1A} Serotonergic binding assay

Hippocampus was homogenized in 30 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25 °C) using a Polytron PT10 (setting 5 for 20 sec). Homogenates were centrifuged twice for 10 min at 50000 *g* with resuspension of the pellet in fresh buffer. After the second centrifugation the pellet was resuspended in homogenization buffer and the suspension incubated 10 min at 37 °C. After other two centrifugations and washing the final pellet was resuspended in ice-cold Tris-HCl 50 mM containing 4 mM $CaCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.4 at 25 °C). Each assay tube contained 50 mL of drug solution, 50 mL of [^{3}H]-8-OH-DPAT to achieve a final concentration of 0.8 nM, and 900 mL of resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was termined by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25 °C). The radioactivity bound to the filters was measured by liquid scintillation counter. Specific [^{3}H]-8-OH-DPAT binding was defined as the difference between binding in the absence or presence of 10 μM 5-HT.

6.2.6. 5-HT_2 Serotonergic binding assay

Prefrontal cortex was homogenized in 30 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25 °C) using a Polytron PT10 (setting 5 for 20 sec). Homogenates were centrifuged three times for 10 min at 50000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in ice-cold 50 mM Tris-HCl (pH 7.4 at 37 °C). Each assay tube contained 50 mL of drug solution, 50 mL of [^{3}H]-ketanserin to achieve a final concentration of 0.8 nM, and 900 mL of resuspended membranes (5 mg fresh tissue). The tubes were incubated for 15 min at 37 °C and the incubation was termined by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25 °C). The radioactivity bound to the filters was measured by liquid scintillation counter. Specific [^{3}H]-ketanserin binding was defined as the difference between binding in the absence or presence of 1 μM methysergide. The binding profile is reported in *table III*.

6.2.7. Antagonism of apomorphine-induced climbing behaviour in rats

60 and 30 min after s.c. administration of the test compounds, rats (175–200 g) were injected s.c. with 1.5 mg/kg of apomorphine (APO), and put into individual cylindrical cages with walls of smooth metal bars. The animals were observed 15 min after apomorphine administration according to the method of Protais et al. [29].

6.2.8. Contralateral turning behaviour in 6-OHDA-lesioned rats

After 6-OHDA nigral lesion, rats were selected for further testing on the basis of completing at least 250 contralateral turns after injection of apomorphine

(0.5 mg/kg s.c.). Rats were tested more than once at intervals of at least 1 week. After administration of the test compounds, turning behaviour was recorded for at least 6 h.

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