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Synthesis and in vitro and in vivo evaluation of dopaminergic ergoline derivatives

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Abstract

A series of ergoline-amides was synthesised in the discovery of new dopaminomimetic agents. Several compounds exhibited in vivo high prolactin lowering activity (indirectly measured by the nidation test) in rats. For the most active, the potential anti-Parkinson activity was evaluated by observation of the contralateral turning behaviour in 6-OH-DA lesioned rats. The acute toxicity by oral route in mice was also studied.

Keywords: Ergolines; Nidation inhibitory activity; Dopaminergic activity; D_2 receptor affinity; Anti-Parkinson activity

1. Introduction

Parkinson's disease (PD) is a prototype neurodegenerative disorder in which the pathogenic process affects only a small portion of neurons within the mammalian CNS with a very high degree of selectivity. Parkinson's disease is characterised behaviourally by movement disorders such as akinesia, rigidity, tremors and flexed postures, and most of the motor symptoms of Parkinsonian patients can be ascribed to forebrain dopamine (DA) depletion [1]. These motor impairments can be corrected by supplying exogenously the immediate DA precursor L-DOPA. A major limitation of the DA replenishment approach with L-DOPA, however, is its relatively short-lived efficacy. After 3–7 years of treatment a significant diminution of efficacy is often accompanied by the appearance of late motor complications consisting of alternation of blocks and relative fluency of the motor performance [2]. The latter is often accompanied by involuntary movements or dyskinesias. In recent years, a number of ergot derivatives have been described as dopamine agonists [3] with selectivity for D_2 receptors and some of them, e.g. bromocriptine [4] and lisuride [5] (Fig. 1), have been evaluated as replacement for levodopa therapy in the treatment of PD and for prolactin-related disorders and acromegaly [6].

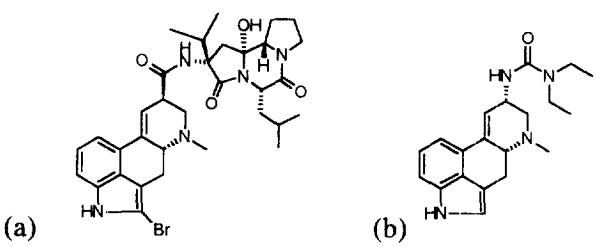

Fig. 1. (a) Bromocriptine; (b) lisuride.

These ergot-related compounds, however, show undesirable side effects such as nausea and vomit and short-lasting activity that limit their clinical application. Therefore, a great deal of work has been done in the search of agents more suitable for human therapy. It is well known that the complex pharmacological profile of the ergolines can be substantially modified by introduction of a suitable pharmacogenic group in the 8-position [7]. Against this background, a screening program aimed at the discovery of potent, safe and long-lasting dopaminergic ergoline derivatives with limited side-effects was initiated in our laboratories. We had found previously that the amide **1** (Fig. 2) is endowed with a nidation inhibitory activity comparable to that of bromocriptine [8].

In the present study, the synthesis and biological examination of related compounds to **1** bearing the arylaminocarbonyl moiety linked to the ergoline skeleton, either by a rigid methylidene or ethylidene unit or by the more flexible meth-

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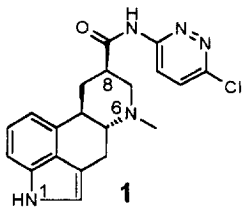
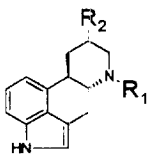
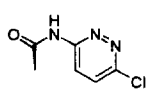
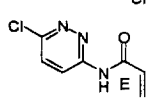
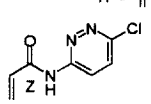
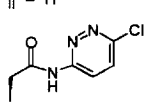
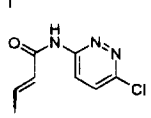
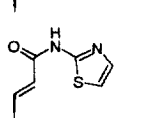
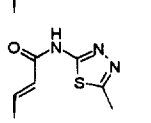
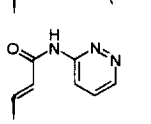


Fig. 2.

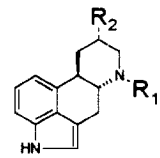
Table 1
Analytical data of ergoline derivatives **1**, **6–8**, **13–16**

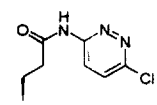
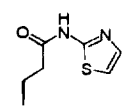
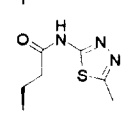
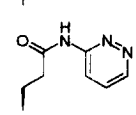
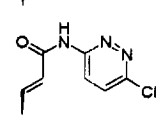
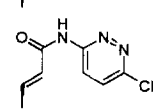
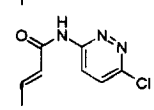


Compound	R_1	R_2	Formula	M.p. (°C)
1	CH_3		$C_{20}H_{20}ClN_5O$	201–203
6	CH_3		$C_{21}H_{20}ClN_5O$	251–253
7	CH_3		$C_{21}H_{20}ClN_5O$	239–242
8	CH_3		$C_{21}H_{22}ClN_5O$	285–288
13	CH_3		$C_{22}H_{22}ClN_5O$	273–275
14	CH_3		$C_{21}H_{22}N_4OS$	250–251
15	CH_3		$C_{21}H_{23}N_5OS$	275–277
16	CH_3		$C_{22}H_{23}N_5O$	260–263

ylene or ethylene chain is reported (see Tables 1 and 2). The effect of different heterocyclic amines and alkyl groups at position 6 were also explored with regard to the nidation inhibitory activity and toxicity. Some of these compounds markedly inhibit the nidation activity and show limited toxicity. Structure–activity relationship within this class is discussed.

Table 2
Analytical data of ergoline derivatives **17–20**, **23–25**



Compound	R_1	R_2	Formula	M.p. (°C)
17	CH_3		$C_{22}H_{24}ClN_5O$	281–283
18	CH_3		$C_{21}H_{24}N_4OS$	244–246
19	CH_3		$C_{21}H_{25}N_5OS$	253–255
20	CH_3		$C_{23}H_{25}N_5O$	273–275
23	C_3H_5		$C_{24}H_{24}ClN_5O$	220–222
24	C_3H_7		$C_{24}H_{26}ClN_5O$	203–206
25	C_2H_5		$C_{23}H_{24}ClN_5O$	227–229

2. Chemistry

The unsaturated amides **6**, **7**, **13–16** were synthesised by condensing the acids **5** (*E* and *Z*) and **12** (*E*) (Fig. 3) with the corresponding heterocyclic amine [9] (Scheme 1).

The condensation was conveniently carried out by reaction of the triethylammonium salt of the acid with trifluoroacetic anhydride at low temperature, and subsequent reaction of the resulting labile mixed anhydride with the heteroarylamine in pyridine [10]. The unsaturated acids **5** and **12** stemmed from the saponification of the corresponding ethyl esters **4** and **11**,

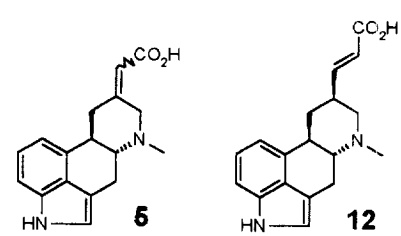
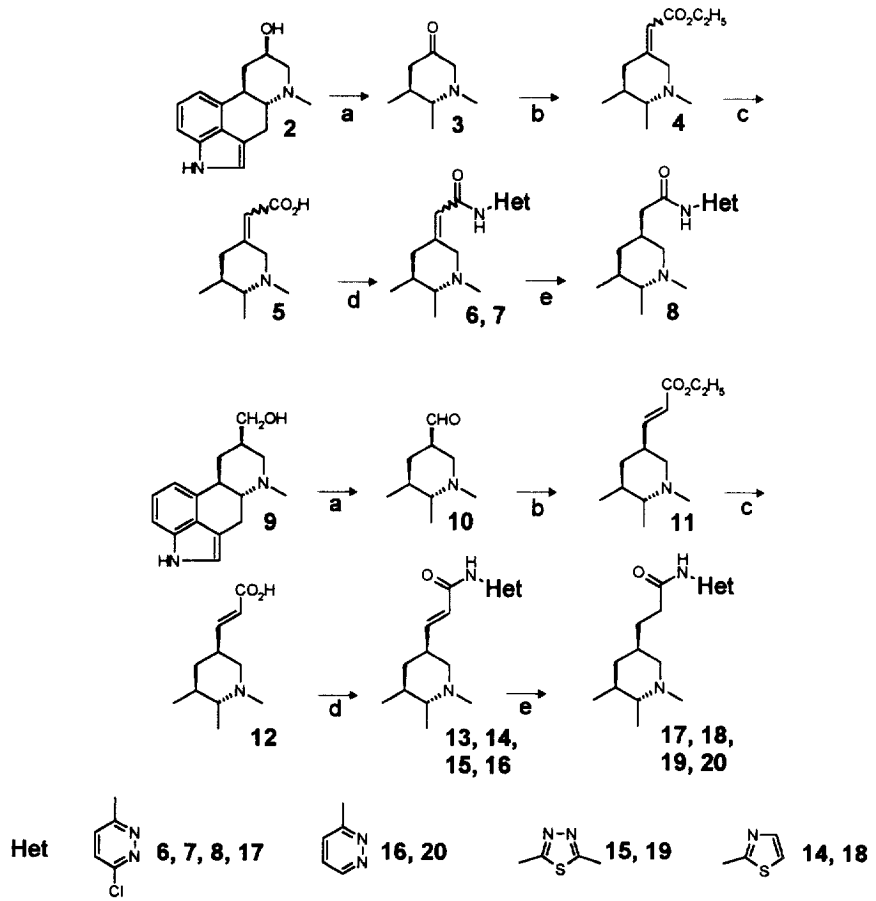
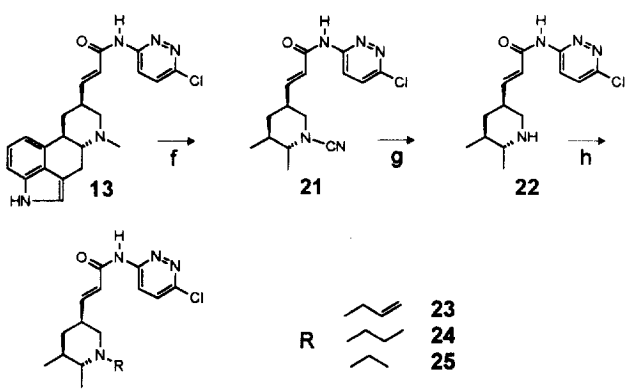


Fig. 3.



Scheme 1. (a) TEA·SO_3, DMSO, TEA, r.t. (b) $(C_2H_5O)_2POCH_2COOC_2H_5$, NaH, THF, low temperature. (c) KOH, C_2H_5OH. (d) TFAA, THF, TEA, low temperature. (e) H_2 1 atm., Pd/C 10%, AcOH.



Scheme 2. (f) BrCN, DMF, r.t. (g) Zn, AcOH, r.t. (h) RBr, DMF, Hunig's base.

prepared respectively by Horner–Emmons condensation [11] of 6-methyl-8-oxo-ergoline (**3**) [12] and 6-methyl-8β-formyl-ergoline [12] (**10**) with triethylphosphonoacetate sodium salt in tetrahydrofuran at low temperature. In the first case, the two diastereomers *E* and *Z* were equally formed. Conversely, the *E* isomer was formed exclusively when the reaction occurred on 6-methyl-8β-formyl-ergoline. Controlled hydrogenation in acidic medium of the unsaturated amides **6**, **7**, **13–16** provided the saturated analogues **8**, **17–20**, as shown in Scheme 1.

The replacement of the 6-methyl with different low linear alkyl groups, a common strategy used to enhance the dopaminomimetic action [13], was accomplished via a Von Braun reaction [14] by exposure of **13** to the action of cyanogen bromide in DMF at 35°C to give **21** in high yield. Compound **21** was reduced with activated zinc in acetic acid [15] to afford the nor derivative **22**. Alkylation of **22** with different alkyl halides in DMF in the presence of ethyldiisopropylamine (Hünig's base) at 30–45°C yielded **23–25**, as depicted in Scheme 2.

3. Results and discussion

Compounds **1**, **6–8**, **13–20**, **23–25** were evaluated for their receptor binding profile (α_1, α_2, D_1, D_2, 5-HT, 5-HT_2), nidation inhibition activity and toxicity. With respect to **1**, four main modifications are represented in the compounds under investigation. These are: the methylidene derivatives **6**, **7** and their saturated analogue **8**, the vinylidene derivatives **13–16** and their saturated analogues **17–20**. From the binding data listed in Table 3, some general trends can be easily identified. The methylidene derivatives **6**, **7** are almost devoid of affinity towards the different receptors. Otherwise the majority of the remaining compounds exhibit their highest potency on the

Table 3
In vitro and in vivo evaluation of compounds **1**, **6–8**, **13–20**, **23–25**

Compound	α_1	α_2	D_1	D_2	5-HT	5-HT_2	ED_{50}	LD_{50}
1	350	500	520	210	530	500	8–12	400–800
6	660	$>10^4$	$>10^4$	1800	$>10^4$	130	NT	NT
7	4200	$>10^4$	$>10^4$	2800	$>10^4$	160	NT	NT
8	800	$>10^4$	$>10^4$	710	1100	1000	>32	>800
13	122	470	400	17	170	40	0.1–0.2	400–800
14	1500	280	240	30	400	30	3	200–400
15	3000	150	70	30	$>10^4$	60	2–4	>800
16	100	70	1100	20	700	40	4	100–200
17	380	340	1100	180	260	100	>32	>800
18	100	440	1400	350	700	350	>32	>800
19	280	$>10^4$	350	290	320	250	>8	>800
20	800	550	340	310	700	340	8	>800
23	900	190	700	10	240	110	0.1–0.2	100–200
24	$>10^4$	300	750	8	170	50	0.05	25–50
25	2000	200	1800	20	110	100	0.1–0.2	100–200
Bromocriptine	130	350	2000	6	360	280	5.7	>800

IC_{50} expressed in nM, standard errors were ± 10% of the mean reported values.
Nidation inhibitory activity ED_{50} and toxicity LD_{50} are reported in mg/kg p.o.

D_2 and 5-HT_2 receptor sites. This fact is particularly associated in the case of the vinylidene derivatives **13–16**, **23–25**. Only a few compounds bind to the other receptors significantly, e.g. **15** to the D_1, **16** to the α_2 and **18** to the α_1 receptor sites.

Concerning the potency to inhibit nidation, this is related to the nature of the substituent in position 8 and correlates with the D_2 component as revealed by comparing the results reported in Table 3. In fact, the highest activity is present in the vinylidene derivatives **13–16** whereas it is sharply decreased in the saturated analogues **17–20**. This tendency is notably represented by **13** whose nidation inhibiting activity is hundreds-fold greater than that of its saturated analogue **17**. This compound, notwithstanding its being endowed with lower D_2 affinity, was fifty times more potent than bromocriptine.

Within this series, the replacement of the 6-chloro-pyridazin-3-yl residue with the pyridazin-3-yl (**16**), thiazol-2-yl (**14**) and 1,3,4-thiadiazol-2-yl (**15**) moiety resulted in less active and more toxic compounds. Furthermore, replacement of the 6-methyl group at position 6 with 2-propenyl, propyl and ethyl groups provided **23–25** with a higher D_2 component and nidation inhibitory activity albeit accompanied by an increased toxicity.

The high nidation inhibitory activity associated with low toxicity represented by compound **13** prompted further investigation. In addition to the inhibitory implantation activity, the dopaminergic component was evaluated by observation of the contralateral rotational behaviour after subcutaneous administration in rats with unilateral 6-hydroxydopamine-induced lesions of the substantia nigra. The results shown in Table 4 indicate that **13** induced turning behaviour contralateral to the side of the lesion in 6-OHDA lesioned rats in a dose-related manner. This effect was more potent than bromocriptine, and suggests therapeutic potential in the treatment of Parkinsonism.

Table 4
Contralateral turning behaviour induced by **13** in 6-OHDA lesioned rats

Compound	No. rats	Dose (mg/kg ip.)	Turns/6 h	Percentage of rats turning
Bromocriptine	8	1	814 ± 280	50
13	7	0.25	1285 ± 459	57
13	7	0.5	1382 ± 427	86
13	8	1	1985 ± 622	88

In conclusion, the results presented in Tables 3 and 4 indicate that potent and quite selective dopaminergic activity can be successfully achieved by chemical variation of ergot structures.

4. Experimental

4.1. Chemistry

Melting points were determined on a Büchi melting point apparatus and were not corrected.

IR spectra were recorded on a Perkin-Elmer 125 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian XL-200 spectrometer in $CDCl_3$ or DMSO-d_6 solutions. Chemical shifts are expressed in δ (ppm from zero TMS). Assignments were supported by suitable decoupling experiments. EI were recorded at 70 eV on a Varian MAT 311-A spectrometer. All compounds had IR, ^{1}H NMR and mass spectra that were fully consistent with their structure.

The results of the elemental analysis (C, H, N) were within ± 0.4% of the theoretical value.

The examples presented below are representative of all the synthetic procedures.

4.1.1. 6-Methyl-8-oxo-ergoline (3)

A solution of 22.8 g (126 mmol) of triethylamine–sulfur trioxide complex in 30 ml of DMSO was added to a stirred solution of 15.2 g (63 mmol) of 6-methyl-8β-hydroxy-ergoline (**2**) in 75 ml of DMSO and 25 ml of TEA at room temperature. After stirring for 10 min, the solution was diluted with 50 ml of glacial acetic acid and 500 ml of ice-water and 500 ml of ethyl acetate were added and the mixture was then slowly basified with concentrated ammonium hydroxide solution. The organic layer was washed with brine and dried. After removal of the solvent, the residue was rinsed with acetone to furnish 9.3 g of **3** (61% yield); m.p. 210–213°C.

Anal. ($C_{15}H_{16}N_2O$): C, H, N. IR (KBr, cm^{-1}): 1700–1710 (νCO); 1330 (νCH_3N). ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.34 (s, 3H, NCH_3); 2.54 (dd, J = 16.0, 16.0 Hz, 1H, H-9ax); 2.59 (m, 1H, H-5); 2.78 (ddd, J = 1.5, 11.0, 14.0 Hz, 1H, H-4ax); 3.00 (d, J = 15.0 Hz, 1H, H-7ax); 3.38 (d, J = 15.0 Hz, 1H, H-7e); 3.3–4.7 (m, 3H, H-4e, H-9e, H-10); 6.8–7.4 (m, 4H, H-2, H-12, H-13, H-14). MS (EI) m/z: 240 (100, $C_{15}H_{16}N_2O$, $[M]^+$); 212 (46, $[M-CO]^+$); 211 (41, $[M-CHO]^+$); 181 (54); 168 (76), 167 (53); 154 (99); 144 (26); 127 (49); 115 (39).

4.1.2. (E, Z)-(6-Methyl-ergolin-8-ylidene)-acetic acid ethyl ester (4)

A solution of 10 g (45 mmol) of triethylphosphonoacetate in 25 ml of THF was added to a stirred suspension of 1.7 g (36 mmol) of 50% NaH in 200 ml of THF at −15°C. After 30 min, a solution of 7.3 g (29 mmol) of **3** in 150 ml of THF was added dropwise and the stirring was continued for 5 h at room temperature. The solvent was removed and the residue taken up in ethyl acetate was washed with brine and dried. The organic phase was evaporated and the residue chromatographed on silica gel eluting with ethyl acetate/cyclohexane 2/3 afforded 5.8 g (64% yield) of (*E*, *Z*) **4** as a 1/1 mixture. By preparative TLC, the two diastereomers were separated and characterised.

(E)-(6-Methyl-ergolin-8-ylidene)-acetic acid ethyl ester. *Anal.* ($C_{19}H_{22}N_2O_2$): C, H, N. IR (KBr, cm^{-1}): 1720 (νCOO); 1600–1610 (νCH=CH). ^1H NMR (DMSO-d_6, 200 MHz) δ: 1.22 (t, 3H, $COOCH_2CH_3$); 1.96 (m, 1H, H-9ax); 2.34 (s, 3H, NCH_3); 2.3–3.4 (m, 6H, CH_2-4, H-5, CH_2-7, H-10); 4.13 (q, 2H, $COOCH_2CH_3$); 4.59 (dd, 1H, H-9e); 5.82 (s, 1H, $HCCOOCH_2CH_3$); 6.7–7.2 (m, 4H, H-2, H-12, H-13, H-14); 10.67 (d, 1H, NH-1). MS (EI) m/z: 310 (27, $C_{19}H_{22}N_2O_2$, $[M]^{+\cdot}$); 281 (100, $[M-C_2H_5]^+$); 265 (11, $[M-C_2H_5O]^+$); 263 (54); 235 (10); 192 (11); 191 (10); 167 (20); 154 (58); 127 (28).

(Z)-(6-Methyl-ergolin-8-ylidene)-acetic acid ethyl ester. *Anal.* ($C_{19}H_{22}N_2O_2$): C, H, N. IR (KBr, cm^{-1}): 1725 (νCOO); 1600–1610 (νCH=CH). ^1H NMR (DMSO-d_6, 200 MHz) δ: 1.22 (t, 3H, $COOCH_2CH_3$); 2.26 (s, 3H, NCH_3); 2.2–3.3 (m, 7H, CH_2-4, H-5, H-7ax, CH_2-9, H-10); 4.09 (q, 2H, $COOCH_2CH_3$); 4.73 (d, 1H, Hh-7e); 5.90 (s, 1H, $HCCOOCH_2CH_3$); 6.78 (d, 1H, H12); 7.01 (dd, 1H, H-13); 7.12 (d, 1H, H-14); 10.66 (d, 1H, NH-1). MS (EI) m/z: 310 (32, $C_{19}H_{22}N_2O_2$, $[M]^{+\cdot}$); 281 (100, $[M-C_2H_5]^+$); 265 (15, $[M-C_2H_5O]^+$); 263 (60); 235 (9); 192 (14); 191 (10); 167 (24); 154 (61); 127 (30).

4.1.3. (E)-N-(6-Chloro-pyridazin-3-yl)-2-(6-methyl-ergolin-8-ylidene)-acetamide (6); (Z)-N-(6-chloro-pyridazin-3-yl)-2-(6-methyl-ergolin-8-ylidene)-acetamide (7)

A solution of 8 g (26 mmol) of **4** and 52 ml of 1 M potassium hydroxide in 250 ml of ethanol was heated at 65°C for 3 h. The solvent was removed and the residue dissolved in 200 ml of water. This solution was then treated with 53 ml of 1 M hydrochloric acid. The precipitate was filtered, washed with acetone and dried, to furnish 5.8 g (79% yield) of (*E*, *Z*) **5**. A suspension of 3.5 g (12 mmol) of (*E*, *Z*) **5** in 75 ml of pyridine and 15 ml of TEA was treated with a solution of 2 g (10 mmol) of trifluoroacetic anhydride in 10 ml of THF at −10°C. After stirring for 5 min, 1.9 g (15 mmol) of 3-amino-6-chloro-pyridazine dissolved in 25 ml of pyridine were added to that clear solution. After stirring for 3 h, the solvent was removed and the residue taken up in dichloromethane was washed with a saturated $KHCO_3$ solution then dried. After removal of the solvent, the residue was chromatographed on silica gel eluting with chloroform/cyclohexane 2/5, to provide after crystallisation from methanol 1.3 g of **6**.

Anal. ($C_{21}H_{20}ClN_5O$): C, H, N. IR (KBr, cm^{-1}): 1670–1680 (νCONHHet); 1630–1640 (νCONHHet); 1335 (νCH_3N). ^1H NMR (DMSO-d_6, 200 MHz) δ: 1.97 (dd, 1H, H-9ax); 2.37 (s, 3H, NCH_3); 2.0–3.4 (m, 6H, CH_2-4, H-5, CH_2-7, H-10); 4.78 (dd, 1H, H-9e); 6.21 (s, 1H, $HCCO$-NHHet); 6.76 (d, 1H, H-12); 6.98 (s, 1H, H-2); 7.01 (d, 1H, H-14); 7.12 (dd, 1H, H-13); 7.86 (d, 1H, (H-5)pyridazine); 8.53 d, 1H, (H-4)pyridazine); 10.66 (d, 1H, NH-1); 11.41 (s, 1H, CONHHet). MS (EI) m/z: 393 (1, $C_{21}H_{20}ClN_5O$, $[M]^{+\cdot}$); 264 (43, $[M-C_4H_4N_3Cl]^{+\cdot}$); 263 (23); 237 (13); 168 (23); 167 (100); 155 (44); 154 (45); 129(16); 127(18).

Continuing the elution with chloroform/cyclohexane 3/5, 0.9 g of **7** was obtained after crystallisation from acetone.

Anal. ($C_{21}H_{20}ClN_5O$): C, H, N. IR (KBr, cm^{-1}): 1660–1680 (νCONHHet); 1630–1645 (νCONHHet); 1340 (νCH_3N). ^1H NMR (DMSO-d_6, 200 MHz) δ: 2.38 (s, 3H, NCH_3); 2.73 (d, 1H, H-7ax); 2.2–3.2 (m, 6H, CH_2-4, H-5, CH_2-9, H-10); 4.97 (d, 1H, H-7e); 6.28 (s, 1H, $HCCO$-NHHet); 6.82 (d, 1H, H-12); 6.99 (s, 1H, H-2); 7.03 (dd, 1H, H-13); 7.86 (d, 1H, (H-5)pyridazine); 8.47 d, 1H, (H-4)pyridazine); 10.67 (d, 1H, NH-1); 11.51 (s, 1H, CONHHet). MS (EI) m/z: 393 (1, $C_{21}H_{20}ClN_5O$, $[M]^{+\cdot}$); 264 (49, $[M-C_4H_4N_3Cl]^{+\cdot}$); 263 (29); 237 (13); 168 (23); 167 (100); 155 (394); 154 (48); 129(19); 127(15).

*4.1.4. 6-Methyl-8β-formyl-ergoline (**10**)*

A solution of 27.9 g (154 mmol) of triethylamine–sulfur trioxide complex in 30 ml of DMSO was added dropwise to a stirred solution of 15.8 g (62 mmol) 6-methyl-8β-hydroxymethyl-ergoline (**9**) in 50 ml of DMSO and 35 ml of TEA at room temperature. After 15 min, 100 ml of glacial acetic acid in 1000 ml of ice-water were added and the stirring continued for 30 min. The solution was slowly basified with 5 M sodium hydroxide until pH 9, then partitioned with ethyl acetate. The organic phase was thoroughly washed with brine and dried. The solvent was removed and the residue crystallised from isopropanol provided 12.7 g of **10** (82% yield); m.p. 169–172°C.

Anal. ($C_{16}H_{18}N_2O$): C, H, N. IR (KBr, cm^{-1}): 2830 (ν*CH*O); 1720 (νCO). ^1H NMR (DMSO-d_6, 60 MHz) δ: 1.3 (ddd, 1H, H-9ax); 2.4 (s, 3H, NCH_3); 6.8–7.2 (m, 4H, H-2, H-12, H-13, H-14); 9.7 (s, 1H, CHO); 10.1 (bs, 1H, NH-1). MS (EI) *m/z*: 254 (52, $C_{16}H_{18}N_2O$, $[M]^{+\cdot}$); 226 (28, $[M-CO]^{+\cdot}$); 225 (16, $[M-CHO]^+$); 211 (22); 195 (25); 168 (39); 176 (48); 154 (100); 144 (40), 127 (48), 115 (22).

*4.1.5. (E) 3-(6-Methyl-ergolin-8β-yl)-acrylic acid ethyl ester (**11**)*

A solution of 30 g (143 mmol) of triethylphosphonoacetate in 50 ml of THF was slowly added dropwise to a stirred suspension of 6.1 g (128 mmol) of 50% NaH at −10°C. After stirring for 45 min, a solution of 25.5 g (100 mmol) of **10** in 300 ml of THF was added dropwise and set aside at room temperature for 2 h. After removal of the solvent, the residue was taken up in ethyl acetate, washed with brine and dried. The solvent was evaporated off and the residue crystallised twice from ethanol to provide 27.4 g of **11** (84% yield); m.p. 203–205°C.

Anal. ($C_{20}H_{24}N_2O_2$): C, H, N. IR (KBr, cm^{-1}): 1715 (νCOO); 1610 (νCH=CH). ^1H NMR ($CDCl_3$, 200 MHz) δ: 1.30 (t, 3H, $COOCH_2CH_3$); 1.35 (ddd, 1H, H-9ax); 2.12 (m, 1H, H-5); 2.13 (dd, 1H, H-7ax); 2.48 (s, 3H, NCH_3); 2.6–2.9 (m, 3H, H-4ax, H-8, H-9e); 2.9–3.1 (m, 2H, H-7e, H-10); 3.40 (dd, 1H, H-4e); 4.20 (q, 2H, $COOCH_2CH_3$); 5.94 (dd, 1H, CH=*CH*$COOCH_2CH_3$); 6.8–7.2 (H-2, H-13, H-14, *CH*=$CHCOOCH_2CH_3$); 7.99 (bs, 1H, NH-1). MS (EI) *m/z*: 324 (100, $C_{20}H_{24}N_2O_2$, $[M]^{+\cdot}$); 295 (11, $[M-C_2H_5]^+$); 279 (9, $[M-C_2H_5O]^+$); 251 (6, $[M-COOC_2H_5]^+$); 197 (19); 167 (14); 155 (58); 154 (72); 144 (9); 127 (20).

*4.1.6. (E) 3-(6-Methyl-ergolin-8β-yl)-acrylic acid (**12**)*

A stirred solution of 45 g (139 mmol) of **11** and 208 ml (208 mmol) of 1 M sodium hydroxide in 900 ml of ethanol was heated at 50°C for 2 h. The solvent was removed and the residue dissolved in 300 ml of water was then charcoalised. The resulting clear solution was slowly acidified with 209 ml (209 mmol) of 1 M hydrochloric acid. The precipitate was filtered and washed with water and thoroughly with acetone and dried, furnishing 32 g of **12** (77% yield); m.p. 288–280°C.

Anal. ($C_{18}H_{22}N_2O_2$): C, H, N. IR (KBr, cm^{-1}): 3300–2700 (νOH stretch); 1715 (νCO). ^1H NMR (DMSO-d_6, 200 MHz) δ: 1.18 (ddd, 1H, H-9ax); 2.0–2.1 (m, 2H, H-5, H-7e); 2.36 (s, 3H, NCH_3); 2.5–3.0 (m, 5H, H-4ax, H-7e, H-8, H-9e, H-10); 3.29 (dd, 1H, H-4e); 5.87 (dd, $J=1.1$, 16.7 Hz, 1H, CH=*CH*COOH); 6.7–7.0 (m, 4H, H-2, H-12, H-13, *CH*=CHCOOH); 7.11 (d, 1H, H-14); 10.61 (d, 1H, NH-1). MS (EI) *m/z*: 296 (82, $C_{18}H_{22}N_2O_2$, $[M]^{+\cdot}$); 251 (8, $[M-COOH]^+$); 237 (10); 223 (9); 197 (30); 167 (38); 155 (54); 154 (100); 144 815); 127 (43).

*4.1.7. (E) N-(6-Chloro-pyridazin-3-yl)-3-(6-methyl-ergolin-8β-yl)-acrylamide (**13**)*

A solution of 4.5 g (11.5 mmol) of trifluoroacetic anhydride in 25 ml of THF was added dropwise to a stirred suspension of 2.7 g (9.2 mmol) of **12** in 50 ml of pyridine and 5 ml of TEA at −25°C and, after 15 min, a solution of 1.8 g (13.8 mmol) of 3-amino-6-chloro-pyridazine in 50 ml of pyridine was added dropwise to that clear solution. After stirring for 20 min at −15°C, the solution was set aside at room temperature for 1 h. The solvent was removed and the residue dissolved in dichloromethane was washed with a saturated $NaHCO_3$ solution and dried. The crude product was then filtered on a small pad of silica gel eluting with chloroform. After crystallisation from methanol, 2.4 g of **13** (64% yield) were recovered.

Anal. ($C_{22}H_{22}ClN_5O$): C, H, N. IR (KBr, cm^{-1}): 1670–1680 (νCONHHet); 1630–1655 (νCONHHet); 1350 (νCH$_3$N). ^1H NMR (DMSO-d_6, 200 MHz) δ: 1.23 (ddd, 1H, H-9ax); 2.0–2.1 (m, 2H, H-5, H-7ax); 2.37 (s, 3H, NCH_3); 2.5–3.1 (m, 5H, H-4ax, H-7e, H-8, H-9e, H-10); 3.30 (dd, 1H, H-4e); 6.47 (d, $J=15.8$ Hz, 1H, CH=*CH*CONHHet); 6.8–7.1 (m, 5H, H-2, H-12, H-13, H-14, *CH*=CHCONHHet); 7.82 (d, 1H, (H-5)pyridazine); 8.46 d, 1H, (H-4)pyridazine); 10.54 (d, 1H, NH-1). MS (EI) *m/z*: 407 (3, $C_{22}H_{22}ClN_5O$, $[M]^{+\cdot}$); 372 (11, $[M-Cl]^+$); 278 (15, $[M-C_4H_4N_3Cl]^+$); 249 (8); 237 (10); 223 (16); 167 (58); 155 (68); 154 (100); 127 (27).

*4.1.8. (E) N-(6-Chloro-pyridazin-3-yl)-3-(6-methyl-ergolin-8β-yl)-propionamide (**17**)*

A solution of 2 g (5 mmol) of **13** in 50 ml of acetic acid was hydrogenated at atmospheric pressure over 0.5 g of 10% Pd/C. The calculated amount of H_2 was taken up in 1 h. The catalyst was removed by filtration and the solvent was evaporated off. The residue dissolved in chloroform was washed with dilute ammonium hydroxide solution, then the organic phase was dried and evaporated. The crude product crystallised from methanol gave 1.6 g (80% yield) of **17**.

Anal. ($C_{22}H_{24}ClN_5O$): C, H, N. IR (KBr, cm^{-1}): 1705 (νCONHHet). ^1H NMR (DMSO-d_6, 60 MHz) δ: 2.3 (s, 3H, NCH_3); 6.8–7.2 (m, 4H, H-2, H-12, H-13, H-14); 7.9 (d, 1H, (H-5)pyridazine); 8.5 (d, 1H, (H-4)pyridazine); 10.6 (bs, 1H, NH-1); 11.38 (s, 1H, CONHHet). MS (EI) *m/z*: 409 (37, $C_{22}H_{24}ClN_5O$, $[M]^{+\cdot}$); 374 (21, $[M-Cl]^+$);

280 (100, $[M-C_4H_4ClN_3]^{+\cdot}$); 252 (20), 237 (56); 224 (66); 225 (65); 167 (41); 154 (76); 144 (29); 129 (17); 127 (28).

*4.1.9. (E) N-(6-Chloro-pyridazin-3-yl)-3-(6-cyano-ergolin-8β-yl)-acrylamide (**21**)*

A solution of 5 g (12.2 mmol) of **13** and 1.55 g (14.6 mmol) of cyanogen bromide in 50 ml of DMF was stirred overnight at room temperature. After removal of the solvent, the residue was taken up in ethyl acetate and washed with a dilute solution of ammonium hydroxide then with brine and dried. The solvent was evaporated off and the crude product was crystallised twice from methanol to afford 4.2 g (80% yield) of **21**; m.p. 277–280°C.

Anal. ($C_{22}H_{19}ClN_6O$): C, H, N. IR (KBr, cm^{-1}): 2210 (νNCN); 1670–1650 (νCONHHet); 1640–1655 (νCONHHet); 1340 (νCH_3N). ^1H NMR (DMSO-d_6, 200 MHz) δ: 1.41 (ddd, 1H, H-9ax); 2.7–3.3 (m, 7H, H-4ax, H-5, CH_2-7, H-8, H-9e, H-10); 3.63 (dd, 1H, H-4e); 6.47 (d, $J = 15.8$ Hz, 1H, CH=*CH*CONHHet); 6.8–7.2 (m, 5H, H-2, H-12, H-13, H-14, *CH*=CHCONHHet); 7.87 (d, 1H, (H-5)pyridazine); 8.47 d, 1H, (H-4)pyridazine); 10.77 (d, 1H, NH-1); 11.43 (s, 1H, CONHHet). MS (EI) *m/z*: 418 (38, $C_{22}H_{19}ClN_6O$, $[M]^{+\cdot}$); 383 (26, $[M-Cl]^+$); 289 (100, $[M-C_4H_4ClN_3]^{+\cdot}$); 155 (56); 154 (100), 127 (29).

*4.1.10. (E) N-(6-Chloro-pyridazin-3-yl)-3-(6-nor-ergolin-8β-yl)-acrylamide (**22**)*

A stirred solution of 8 g (19 mmol) of **21** in 100 ml of acetic acid and 210 ml of water was treated with 16 g of Zn at 70°C. After stirring for 1 h, the suspension was filtered and the solvent removed. The residue was taken up in chloroform and washed with 0.05 M NaOH then with brine and dried. The solvent was removed and the residue was filtered on a small pad of silica gel eluting with acetone/cyclohexane 1/1. After crystallisation from ethanol, 4.8 g (64% yield) of **22** were obtained; m.p. 225–228°C.

Anal. ($C_{21}H_{20}ClN_5O$): C, H, N. IR (KBr, cm^{-1}): 1670–1650 (νCONHHet); 1640–1655 (νCONHHet); 1340 (νCH_3N). ^1H NMR (DMSO-d_6, 200 MHz) δ: 1.28 (ddd, 1H, H-9ax); 1.9–2.2 (m, 2H, H-5, H-7ax); 2.5–3.3 (m, 5H, H-4ax, H-7e, H-8, H-9e, H-10); 3.40 (dd, 1H, H-4e); 6.5 (d, $J = 15.8$ Hz, 1H, CH=*CH*CONHHet); 6.9–7.1 (m, 5H, H-2, H-12, H-13, H-14, *CH*=CHCONHHet); 7.95 (d, 1H, (H-5)pyridazine); 8.66 (d, 1H, (H-4)pyridazine); 10.3 (d, 1H, NH-1). MS (EI) *m/z*: 393 (12, $C_{21}H_{20}ClN_5O$, $[M]^{+\cdot}$); 358 (9, $[M-Cl]^+$); 264 (17, $[M-C_4H_4ClN_3]^{+\cdot}$); 155 (73); 154 (100), 127 (31).

*4.1.11. (E) N-(6-Chloro-pyridazin-3-yl)-3-[6-(2-propenyl)-ergolin-8β-yl]-acrylamide (**23**)*

A stirred solution of 5.4 g (13.8 mmol) of **22** in 75 ml of DMF and 6 ml of diisopropylethylamine was treated with 2.8 g (16.5 mmol) of propyl iodide at r.t. After stirring for 1 h, the solution was diluted with ethyl acetate and washed with brine and dried. Removal of the solvent and crystallisation from ethanol provided 4.3 g (72% yield) of **23**.

Anal. ($C_{24}H_{24}ClN_5O$): C, H, N. IR (KBr, cm^{-1}): 1680–1660 (νCONHHet); 1640–1660 (νCONHHet). ^1H NMR (Py-d_5, 200 MHz) δ: 1.39 (ddd, 1H, H-9ax); 2.16 (dd, 1H, H-7ax); 2.4–2.6 (m, 1H, H-5); 2.6–2.9 (m, 3H, H-4ax, H-8, H-9e); 3.00 (m, 1H, H-10); 3.13 (m, 1H, H-7e); 3.33 (m, 1H, NC*H*HCH=CH_2); (3.42 (dd, 1H, H-4e); 3.55 (m, 1H, NC*H*HCH=CH_2); 5.2 (m, 2H, $NCH_2CH=CH_2$); 5.95 (m, 1H, $NCH_2CH=CH_2$); 6.4–7.6 (m, 5H, H-2, H-12, H-13, H-14, *CH*=CHCONHHet); 7.75 (d, 1H, (H-5)pyridazine); 8.98 (d, 1H, (H-4)pyridazine); 12.51 (d, 1H, NH-1); 12.65 (s, 1H, CONHHet). MS (EI) *m/z*: 433 (5, $C_{24}H_{24}ClN_5O$, $[M]^{+\cdot}$); 398 (17, $[M-Cl]^+$); 304 (11, $[M-C_4H_4N_3Cl]^+$); 237 (12); 223 (8); 167 (46); 155 (68); 154 (100); 127 (33).

*4.1.12. (E) N-(6-Chloro-pyridazin-3-yl)-3-(6-propyl-ergolin-8β-yl)-acrylamide **24***

A stirred solution of 1.8 g (4.6 mmol) of **22** in 20 ml of DMF and 2 ml of diisopropylethylamine was treated with 0.9 g (5.5 mmol) of propyl iodide at 50°C. After stirring for 2 h, the solution was diluted with ethyl acetate and washed with brine and dried. Removal of the solvent and crystallisation from acetone provided 1.1 g (51% yield) of **24**.

Anal. ($C_{24}H_{26}ClN_5O$): C, H, N. IR (KBr, cm^{-1}): 1670–1650 (νCONHHet); 1640–1655 (νCONHHet). ^1H NMR (Py-d_5, 200 MHz) δ: 0.87 (t, 3H, $NCH_2CH_2CH_3$); 1.38 (ddd, 1H, H-9ax); 1.4–1.6 (m, 2H, $NCH_2CH_2CH_3$); 2.15 (dd, 1H, H-7ax); 2.4–2.6 (m, 1H, H-5); 2.6–2.9 (m, 5H, H-4ax, H-8, $NCH_2CH_2CH_3$, H-9e); 3.00 (m, 1H, H-10); 3.13 (m, 1H, H-7e); 3.42 (dd, 1H, H-4e); 6.7–7.5 (m, 5H, H-2, H-12, H-13, H-14, *CH*=CHCONHHet); 7.65 (d, 1H, (H-5)pyridazine); 8.93 (d, 1H, (H-4)pyridazine); 12.49 (d, 1H, NH-1); 12.61 (s, 1H, CONHHet). MS (EI) *m/z*: 435 (3, $C_{24}H_{26}ClN_5O$, $[M]^{+\cdot}$); 400 (11, $[M-Cl]^+$); 306 (15, $[M-C_4H_4N_3Cl]^+$); 237 (14); 223 (13); 167 (59); 155 (65); 154 (100); 127 (23).

*4.1.13. (E) N-(6-Chloro-pyridazin-3-yl)-3-(6-ethyl-ergolin-8β-yl)-acrylamide (**25**)*

A stirred solution of 3.6 (9.2 mmol) of **22** in 50 ml of DMF and 4 ml of diisopropylethylamine was treated with 1.7 g (11 mmol) of ethyl iodide at 60°C. After stirring for 3 h, the solution was diluted with ethyl acetate and washed with brine and dried. Removal of the solvent and crystallisation from isopropanol provided 2.7 g (71% yield) of **25**.

Anal. ($C_{23}H_{24}ClN_5O$): C, H, N. IR (KBr, cm^{-1}): 1680–1650 (νCONHHet); 1630–1650 (νCONHHet). ^1H NMR (Py-d_5, 200 MHz) δ: 1.38 (ddd, 1H, H-9ax); 1.4–1.6 (t, 3H, NCH_3); 2.18 (dd, 1H, H-7ax); 2.4–2.6 (m, 1H, H-5); 2.7–2.9 (m, 1H, H-4ax, H-8, NCH_2CH_3, H-9e); 3.15 (m, 1H, H-10); 3.2 (m, 1H, H-7e); 3.42 (dd, 1H, H-4e); 6.8–7.6 (m, 5H, H-2, H-12, H-13, H-14, *CH*=CHCONHHet); 7.85 (d, 1H, (H-5)pyridazine); 8.98 (d, 1H, (H-4)pyridazine); 12.50 (d, 1H, NH-1); 12.61 (s, 1H, CONHHet). MS (EI)

m/z: 421 (7, $C_{23}H_{24}ClN_5O$, $[M]^{+\cdot}$); 386 (11, $[M-Cl]^+$); 292 (11, $[M-C_4H_4N_3Cl]^+$); 237 (16); 223 (19); 167 (63); 155 (75); 154 (100); 127 (21).

4.2. Pharmacology

4.2.1. Receptor binding profile

The α_1, α_2, D_1, D_2, 5-HT and 5-HT_2 receptor binding affinities of the compounds synthesised were determined by measurement of displacement of [^{3}H] prazosin binding in rat frontal cortex [16], [^{3}H] yohimbine binding in rat frontal cortex [17], [^{3}H] SCH-23390 binding in rat striatum [18], [^{3}H] spiroperidol binding in rat striatum [19], [^{3}H] 5-HT binding in rat hippocampus [20] and [^{3}H] ketanserin binding in rat pre-frontal cortex [21], respectively (see Table 3).

4.2.2. Prolactin inhibitory activity

The prolactin secretion inhibitory activity was evaluated indirectly using the nidation inhibition test in rats [22]. Adult Sprague–Dawley female rats (Charles River, Italy) were caged with fertile male rats in the evening of the day of proestrus. Only rats with spermatozoa in vaginal smears on the following day (1 day of pregnancy) were included in the protocol. They were treated on the morning of day 5 of pregnancy with a suspension of the test compounds in methocel 0.5% wt./vol. orally. On day 14, the animals were anaesthetised and the uteri were examined for the presence of implantation sites. Compounds were tested at different doses (6–8 animals per group) and the dose inhibiting nidation in 50% of the animals (ED_{50}) was determined.

4.2.3. Acute toxicity

The acute oral toxicity was evaluated in male Swiss mice (Charles River, Italy). Each compound was suspended in methocel (0.5% wt./vol.) and administered at various dose levels (3 animals per dose). The dose causing the death of 50% of the animals (LD_{50}) within the 7th day following treatment was determined.

4.2.4. Contralateral turning behaviour in 6-OH-lesioned rats

After lesioning in the substantia nigra with 6-hydroxydopamine [23], rats were selected for further testing on the basis of completing at least 200 contralateral turns in response to an injection of 1–2 mg/kg i.p. of apomorphine. Rats were tested more than once at intervals of at least 1 week. After administration of the test compounds, the percentage of rats treated that turn and the number of contralateral of turns within 6 h were recorded.

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