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Original paper

Synthesis and nidation inhibitory activity of a new class of ergoline derivatives

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Summary — The synthesis and the nidation inhibitory activity (indirect evidence of prolactin activity) of a new class of ergolinyl-acylureas is described. Some structure–activity relationship considerations are also reported. *N*-[3-(Dimethyl-amino)propyl]-*N*-[(ethylamino)carbonyl]-6-(2-propenyl)ergoline-8β-carboxamide (laboratory code FCE 21336; international non-proprietary name cabergoline) was the most interesting compound of the series and is now under extensive clinical evaluation in treatment of hyperprolactinemic disorders.

Résumé — **Synthèse et activité inhibitrice sur la nidation d'une nouvelle classe de dérivés de l'ergoline.** *Les auteurs ont décrit la synthèse et l'activité inhibitrice de la nidation d'une nouvelle classe d'acylurées d'ergolinyle. En outre, quelques considérations concernant la corrélation entre structure et activité ont été rapportées. Le composé le plus intéressant de la série était l'*N*-[(diméthylamino)-3 propyl]-*N*-[(éthylamino)carbonyl](propényl-2)-6 ergoline-carboxamide-8β (code de laboratoire: FCE 21336, dénomination internationale commune: cabergoline) qui est soumis maintenant à une évaluation clinique approfondie.*

ergoline-acylureas / nidation inhibitory activity / FCE 21336 / cabergoline

Introduction

Natural ergot alkaloids and synthetic ergoline derivatives display a remarkable variety of pharmacological activities depending mainly upon the nature and the configuration of substituents at position 8 [1, 2]. Various ergot derivatives, *e.g.*, bromocriptine, lisuride and pergolide, have shown potent dopamine agonist properties and have been useful as anti-Parkinson drugs and as prolactin inhibitors [3, 4]. In fact, by decreasing pituitary prolactin secretion, these compounds can be used for inhibition or suppression of puerperal lactation and in prolactin-dependent diseases, such as amenorrhea–galactorrhea. Dopamine receptors are located not only on the lactotrophic cells in the anterior pituitary, but also in the central nervous system and, they have now been found in many peripheral sites including autonomic nerve endings in the cardiovascular system [5]. The anti-hypertensive properties of dopaminergic ergoline derivatives are now under extensive pharmacological evaluation [6].

As a part of a project related to the synthesis of novel semisynthetic ergolines having a broad spectrum of pharmacological activity [7], we planned to prepare a series of amides derived from 9,10-dihydrolysergic acid **I** [8]. Among the various synthetic methods for the acid-to-amide transformation, the activation of the carboxylic group by dicyclohexylcarbodiimide and the subsequent reaction of the acyl isourea with an amine is a well-known procedure commonly employed [9]. One of the major difficulties associated with this method when applied to dihydrolysergic acid, is the tedious separation of the dicyclohexylurea and, sometimes, when the amine is only weakly nucleophilic, the formation of the acylurea, namely *N*-cyclohexyl-*N'*-[(cyclohexylamino)-carbonyl]-6-methyl-ergoline-8β-carboxamide **II**.

The observation that **II** was endowed with some anti-hypertensive activity in spontaneous hypertensive rats stimulated us to carry out further research on this class of compounds with the aim of seeking agents having increased anti-hypertensive activity.

While the anti-hypertensive activity of the first terms of the new class of compounds did not show an increase in potency, some of these compounds appeared to exhibit prolactin secretion inhibitory activity, indirectly shown by the nidation inhibitory activity in rats. Our research activity was therefore redirected to obtain, through modification of the acylurea side chain, a compound displaying a potent anti-nidation activity and possibly devoid of anti-hypertensive activity and untowards side effects (*e.g.*, emesis).

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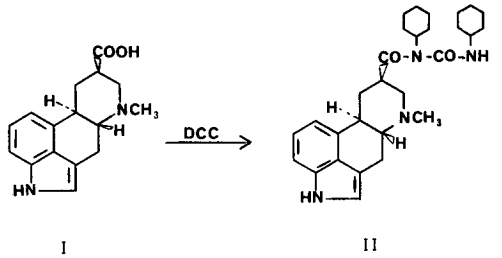
Chemistry and Pharmacology

The reaction of an organic acid with a carbodiimide generally occurs rapidly and the acyl isourea, thus formed, in absence of a nucleophile leads by internal rearrangement to *N*-acylureas (Scheme 1).

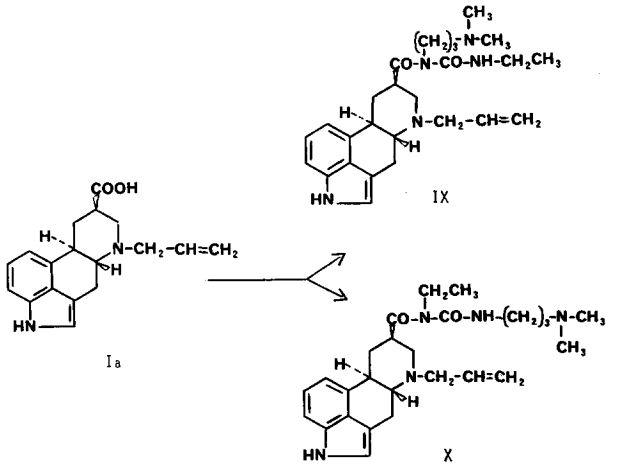


I II

Scheme 1.

In the first instance, the syntheses of **II**–**V** (listed in Table I) were performed by reaction of 9,10-dihydrolysergic acid with commercially available carbodiimides, such as *N*,*N*′-dicyclohexyl, *N*,*N*′-diisopropyl, *N*,*N*′-diterbutyl, *N*-(3-dimethylaminopropyl)-*N*′-ethyl or freshly prepared ones [10], in tetrahydrofuran or dimethylformamide as solvent with the reaction time depending upon the carbodiimide.

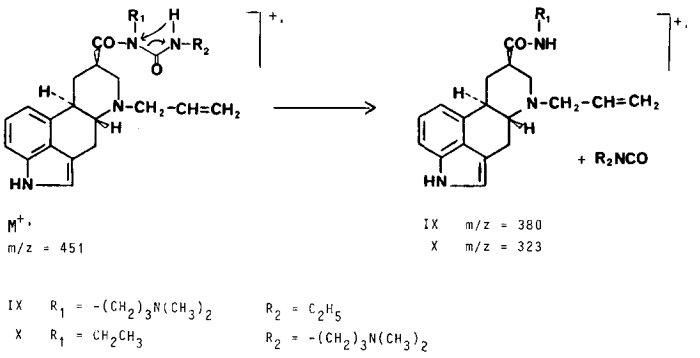
Among these acylureas, the mixture of acylureas, obtained by treatment of dihydrolysergic acid with *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide showed an appreciable activity in the nidation test, indicative of an anti-prolactin activity. Since it is known that the substitution of the 6-methyl group with a higher linear homolog increases the anti-prolactin activity in ergolines [11, 12], the introduction of a 6-propyl and 6-(2-propenyl) substituent was considered. The required *N*-alkyl dihydrolysergic acids were prepared by alkylation of 6-nor-dihydrolysergic acid methyl ester using the procedure described in the literature [13]. The *N*-alkyl esters were subsequently saponified to give the required acid. In fact, the mixture of regioisomers obtained by reaction of 6-(2-propenyl)-dihydrolysergic acid **1a** with the above-mentioned asymmetric carbodiimide (Scheme 2) showed a greater anti-prolactin activity with an approximate ED_{50} of 0.04 mg/kg evaluated in the nidation inhibition test. At this point, it was deemed appropriate to isolate the two isomers by means of preparative high-pressure liquid chromatography (HPLC). Of the two compounds thus isolated, **IX** showed a very high activity (ED_{50} = 0.025 mg/kg) whereas that of **X** was one order of activity lower.



Ia IX X

Scheme 2.

Regiochemical assignments were based on analysis of mass spectral data which for **IX** and **X** provided two different fragmentations that could as well be linked to a thermic degradation reaction. The main fragments were originated by loss of ethyl isocyanate for **IX** (*m*/*z* 380) and 3-dimethylaminopropyl isocyanate for **X** (*m*/*z* 323), respectively, probably *via* a 4-membered ring mechanism, as indicated in Scheme 3.



Scheme 3.

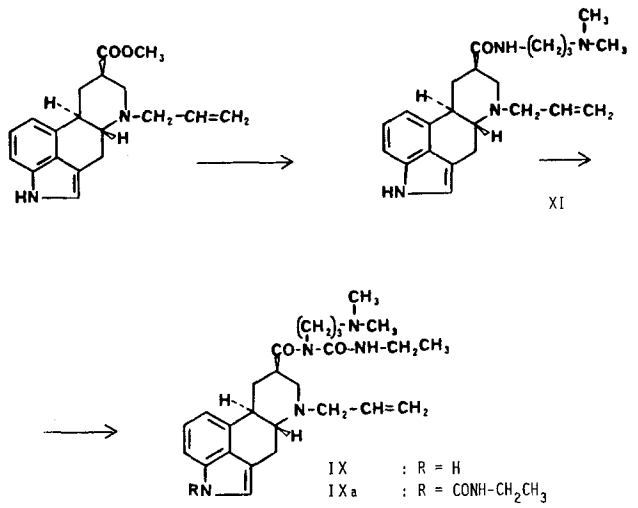
The activity displayed by **IX** was considered outstanding and the subsequent need for a consistent amount of this compound, for further biological studies, required the development of a different synthetic process.

The phenomena observed on mass spectroscopy (loss of isocyanate) suggested that, on the contrary, the addition of isocyanate to an amide could be a possible efficient process for the target compound and analogues.

The first step of the planned synthesis involved the amidation of 6-(2-propenyl)-9,10-dihydrolysergic acid methyl ester by 3-dimethylamino-1-propylamine in the presence of one equivalent of the amine salt to give the amide **XI** in good yield [14]. The subsequent reaction of amide **XI** with ethyl isocyanate was investigated in detail in regard to the concentration of reagents, solvent and temperature. The best results were obtained by using a very large excess of ethyl isocyanate in refluxing toluene.

In addition to the main product **IX**, the addition of ethyl isocyanate to the nitrogen of indole yielded a small amount **IXa** (Scheme 4).

Using this new procedure, the analogs of **IX** bearing various substituents on position 6 and on the side chain were synthesized and the results are summarized in Table I, together with the biological data (nidation inhibition and acute toxicity). The required amides **XII**, **XIII**, **XIV** were synthesized similarly to compound **XI**.

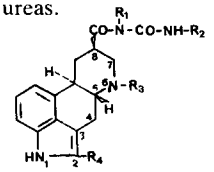


Scheme 4.

Discussion

The results of our study showed that 6-substituted ergolinyl-8β-acylureas are endowed with a strong anti-prolactin activity in rats, as revealed in the nidation inhibition test. Among them, **IX**, namely *N*-[3-(dimethylamino)propyl]-*N*-[(ethylamino)carbonyl]-6-(2-propenyl)ergoline-8β-carboxamide, was the most interesting, having a very high potency in the anti-nidation test (ED_{50} 0.025 mg / kg *p.o.*) and a low toxicity ($LD_{50} \approx 400$ mg / kg *p.o.*). On the basis of these considerations, **IX** was preferred over **XV** for further development, since the latter had similar activity but considerably higher toxicity. Bromocriptine, the reference compound, was about 200 times less potent than **IX** in the anti-nidation test, its ED_{50} being 5.7 mg / kg *p.o.* As seen from the data reported in Table I, replacement in the side chain of the ethyl residue with methyl **XVIII** resulted in a reduced potency and the introduction of a phenyl **XIX** practically abolished it. Replacement of the 6-methyl with

Table I. Chemical and pharmacological data of ergolinyl-acyl ureas.



Compd.	R_1	R_2	R_3	R_4		mp °C	Nidation inhibition ED_{50} mg / kg	Acute toxicity DL_{50} mg / kg
	Brocriptine						5.7	>800
Ia	6-Allyl-dihidrolysergic acid						>8	>800
II	$-CH-(CH_2)_4-CH_2$	$-CH-(CH_2)_4-CH_2$	$-CH_3$	H	$C_{29}H_{40}N_4O_2$	205–207	>8	>800
III	$-CH-(CH_3)_2$	$-CH-(CH_3)_2$	$-CH_3$	H	$C_{23}H_{32}N_4O_2$	202–204	>8	50–100
IV	$-C-(CH_3)_3$	$-C-(CH_3)_3$	$-CH_3$	H	$C_{25}H_{36}N_4O_2$	194–196	>8	>800
V	$-CH_3$	$-CH_3$	$-CH_3$	H	$C_{19}H_{24}N_4O_2$	215–217	3.2	50–100
VI	$-CH-(CH_2)_4-CH_2$	$-CH-(CH_2)_4-CH_2$	$-CH_2-CH=CH_2$	H	$C_{31}H_{42}N_4O_2$	150–152	3.2	>800
VII	$-CH-(CH_3)_2$	$-CH-(CH_3)_2$	$-CH_2-CH_2-CH_3$	H	$C_{25}H_{36}N_4O_2$	143–145	0.02	100–200
VIII	$-CH_3$	$-CH_3$	$-CH_2-CH=CH_2$	H	$C_{21}H_{26}N_4O_2$	106–108	0.5	100–200
IX	$-(CH_2)_3N(CH_3)_2$	$-CH_2-CH_3$	$-CH_2-CH=CH_2$	H	$C_{26}H_{37}N_5O_2$	102–104	0.025	>400
X	$-CH_2-CH_3$	$-(CH_2)_3N(CH_3)_2$	$-CH_2-CH=CH_2$	H	$C_{26}H_{43}N_5O_{10}P_2$*	150–153	0.27	400–800
XV	$-(CH_2)_3N(CH_3)_2$	$-CH_2-CH_3$	$-CH_2-CH_2-CH_3$	H	$C_{26}H_{39}N_5O_2$	64– 68	0.015	=100
XVI	$-(CH_2)_3N(CH_3)_2$	$-CH_2-CH_3$	$-CH_2-CH_2-CH_3$	Br	$C_{26}H_{38}N_5O_2Br$	foam	0.05	200–400
XVII	$-(CH_2)_3N(CH_3)_2$	$-CH_2-CH_3$	$-CH-(CH_3)_2$	H	$C_{26}H_{39}N_5O_2$	foam	>8	>800
XVIII	$-(CH_2)_3N(CH_3)_2$	$-CH_3$	$-CH_2-CH=CH_2$	H	$C_{25}H_{35}N_5O_2$	126–128	0.1	200–400
XIX	$-(CH_2)_3N(CH_3)_2$	$-C_6H_5$	$-CH_2-CH=CH_2$	H	$C_{30}H_{37}N_5O_2$	82– 85	2	200–400
XX	$-H$	$-CH_2-CH_3$	$-CH_2-CH=CH_2$	H	$C_{21}H_{26}N_4O_2$	210–212	1	50–100

Diphosphate salt (doses as free base).

a 6-isopropyl group **XVII** yielded, as expected, an almost inactive compound confirming the importance of the steric factor for dopaminergic activity, as previously observed for other dopaminomimetic compounds.

Compound **IX** (laboratory code: FCE 21336; international non-proprietary name: cabergoline) was found to be a long lasting prolactin lowering agent in rats [15, 16] and was selected for further pharmaco-toxicological studies. The compound is now under extensive clinical evaluation and its potent long lasting prolactin lowering effect in conjugation with the absence of side effects in healthy male volunteers and hyperprolactinemic patients [17] should make cabergoline a welcome advance in the medical treatment of hyperprolactinemia [4].

Experimental protocols

Chemistry

Melting points were determined on a Büchi melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 125 spectrophotometer. ^{1}H NMR spectra were recorded on Varian XL-200 spectrometer, except for compounds **II**, **III**, **IV**, **X**, **XII**, **XVII** and **XVIII** (chemical shifts are given in ppm (δ) downfield from tetramethyl silane (TMS). E.I. Mass spectra were recorded at 70 eV on a Varian MAT 311 A mass spectrometer. All compounds had IR, NMR and mass spectra that were fully consistent with their structure. The results of elemental analysis (C,H,N) were within ± 0.4% of the theoretical value.

HPLC separation was performed with Prep LC/System 500 apparatus (Waters) using a Prep-Pak 500/Silica column (Waters).

Method A

N-*Cyclohexyl*-N-*[(cyclohexylamino)-carbonyl]-6-methylergoline-8β-carboxamide* ***II***

A mixture of 5 g (0.018 mol) of dihydrolysergic acid, 3.8 g (0.018 mol) of *N, N'*-dicyclohexylcarbodiimide in tetrahydrofuran (500 ml) was refluxed overnight. The solution was evaporated to give a solid residue. The residue was taken up in 10% $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated. The solid residue was crystallized from methanol to give 5.4 g (61%) of pure **II**. mp: 205–207°C. Anal. ($C_{29}H_{40}N_4O_2$) C,H,N. IR (KBr): 3500–2700 cm^{-1} (ν N–H, ν C–H); 1660 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) (60 MHz) δ: 2.48 (s, 3H, NCH_3); 6.8–7.3 (5H, aromatic H's, CO–*NH*); 9.17 (bs, 1H, *NH*-1). MS (FD) *m/z*: 476(100, M$^+$), 351(74).

N-*Isopropyl*-N-*[(isopropylamino)-carbonyl]-6-methyl-ergoline-8β-carboxamide* ***III***

Compound **III** was prepared starting from dihydrolysergic acid by reaction with *N, N'*-diisopropylcarbodiimide, to give the title compound in 65% yield. mp: 202–204°C. Anal. ($C_{23}H_{32}N_4O_2$) C,H,N. IR (KBr): 3500–2700 cm^{-1} (ν N–H, ν C–H); 1650 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) (60 MHz) δ: 1.20 (d, J=6.5 Hz, 6H, NCH(CH_3)$_2$; 1.43 (d, J=6.5 Hz, 6H, NHCH(CH_3)$_2$; 2.48 (s, 3H, NCH_3); 4.03 (dq, J=6.5 Hz, 1H, NH*CH*); 4.52 (q, J=6.5 Hz, 1H, NC*H*); 6.8–7.3 (m, 44, *H-2, H-13, H-14*); 7.30 (bd, J=7.0 Hz, 1H NHCH); 8.39 (bs, 1H, N*H*1). MS (EI) *m/z*: 398 (84, M$^+$); 311(100); 270(50); 255(48); 223(48); 167(39); 154(62); 144(42); 127(14); 114(27).

N-t-*Butyl-[(t-butylamino)-carbonyl]-6-methylergoline-8β-carboxamide* ***IV***

Compound **IV** was prepared starting from dihydrolysergic acid by reaction with *N,N'*-di-*t*-butylcarbodiimide, to give the title compound in 75% yield. mp: 194–196°C. Anal. ($C_{25}H_{36}N_4O_2$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, ν C–H); 1660 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$, 60 MHz): δ 1.42, 1.53 (two s, 18H, NC(CH_3)$_3$, NHC(CH_3)$_3$); 2.47 (s, 3H, NCH_3); 5.40 (bs, 1H, N*H*C$_3$)$_3$); 6.8–7.3 (m, 4H, *H-2, H-12, H-13, H-14*); 8.23 (bs, 1H, *NH*-1). MS (EI) *m/z*: 424(37, M$^+$); 325(39); 249(24); 270(100); 225(31); 223(34); 167(42); 154(49); 144(34); 57(46).

N-*Methyl*-N-*[(methylamino)-carbonyl]-6-methylergoline-8β-carboxamide* ***V***

Compound **V** was prepared from dihydrolysergic acid by reaction with *N,N'*-dimethylcarbodiimide, to give the title compound in 62% yield. mp: 215–217°C. Anal. ($C_{19}H_{24}N_4O_2$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, ν C–H); 1660 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) δ: 2.50 (s, 3H, NCH_3); 2.89 (d, J=5.0 Hz, 3H, N*H*CH$_3$); 3.48 (s, 3H, CONC*H*$_3$); 6.8–7.3 (m, 4H, *H-2, H-12, H-13, H-14*); 8.18 (bq, J=5.0 Hz, 1H, N*H*CH$_3$); 9.46 (bs, 1H, *NH*-1). MS (EI) *m/z*: 340(58, M$^+$); 283(89); 225(43); 223(61); 167(79); 154(98); 144(100); 127(43); 116(78); 86(67).

N-*Cyclohexyl*-N-*[(cyclohexylamino)-carbonyl]-6-(2-propenyl)-ergoline-8β-carboxamide* ***VI***

Compound **VI** was prepared starting from 6-(2-propenyl)-dihydrolysergic acid by reaction with *N, N'*-dicyclohexylcarbodiimide, to give the title compound in 66% yield. mp: 150–152°C. Anal. ($C_{31}H_{42}N_4O_2$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, ν C–H); 1660 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) δ: 1.0–2.0 (m, 21H, *H-9ax*, CH_2's of cyclohexyls); 2.5–2.3 (m, 4H, *H-4ax, H-5ax, H-7ax, H-8ax*); 2.9–3.2 (m, 2H, *H-7eq, H-10ax*); 3.34 (dd, J=7.5 & 14.2 Hz, 1H, NC*H*(H)CH=CH$_2$); 3.44 (dd, J=3.8 & 14.0 Hz, 1H, *H-4e*); 3.57 (dd, J=5.8 & 14.2 Hz, 1H, NCH(*H*)CH=CH$_2$); 3.70 (m, 1H, NHC*H*); 4.03 (m, 1H, NC*H*); 5.22 (m, 2H, CH_2=); 5.97 (m, 1H, C*H*=); 6.8–7.2 (m, 4H, *H-2, H-12, H-13, H-14*); 6.80 (bm, 1H, N*H*CH); 7.92 (bs, 1H, *NH*-1). MS (EI) *m/z*: 502(2, M$^+$); 377(42); 336(19); 306(20); 278(13); 251(25); 167(41); 154(100); 144(33); 125(70).

N-*Isopropyl*-N-*[(isopropylamino)-carbonyl]-6-*n*-propyl-ergoline-8β-carboxamide* ***VII***

Compound *VII* was prepared starting from 6-*n*-propyldihydrolysergic acid by reaction with *N',N'*-diisopropylcarbodiimide, to give the title compound in 60% yield, mp: 143–145°C. Anal. ($C_{25}H_{36}N_4O_2$) C,H,N. IR (KBr): 3550–2700 cm^{-1}(ν N–H, ν C–H); 1650 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=6.0 Hz, 3H, $NCH_2CH_2CH_3$); 1.15, 1.22 (two d, J=6.5 Hz, 6H, NHCH(CH_3)$_2$, NCH(CH_3)$_2$); 3.87 (m, 1H, NHC*H*(CH_3)$_2$O; 4.38 (m, 1H, NC*H*(CH_3)$_2$); 6.7–7.2 (m, 4H, *H-2, H-12, H-13, H-14*); 8.32 (bd, J=8.0 Hz, 1H, N*H*CH); 10.49 (bs, 1H, N*H*-1). MS (EI) *m/z*: 424(46, M$^+$); 239(100); 310(49); 298(29); 253(55); 251(40); 167(61); 154(90); 144(85); 70(78).

N-*Methyl*-N-*(methylamino)-carbonyl]-6-(2-propenyl)-ergoline-8β-carboxamide* ***VIII***

Compound **VIII** was prepared starting from 6-(2-propenyl)dihydrolysergic acid by reaction with freshly prepared *N,N'*-dimethylcarbodiimide to give the title compound in 68% yield. mp: 106–108°C. Anal. ($C_{21}H_{26}N_4O_2$) C,H,N. IR (KBr): 3500–2700 cm^{-1} (ν N–H, ν C–H); 1650 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) δ: 2.87 (d, J=6.0 Hz, 3H, NHCH_3); 3.50 (s, 3H, NCH_3); 5.0–6.3 (m, H, N–CH_2–C*H*=CH_2); 6.6–7.3 (m, 4H, *H-2, H-12, H-13, H-14*); 8.08 (bq, J=6.0 Hz, 1H, N*H*CH$_3$); 9.23 (bs, 1H, N*H*-1). MS (EI) *m/z*: 366(39, M$^+$); 309(57); 268(24); 251(28); 209(54); 167(66); 154(100); 144(56); 127(45); 57(70).

N-*[3-(Dimethylamino)propyl]*-N-*[(ethylamino)-carbonyl]-6-(2-propenyl)-ergoline-8β-carboxamide* ***IX*** *and* N-*[[[3-(dimethylamino)propyl]amino]carbonyl]*-N-*ethyl-6-(2-propenyl)-ergoline-8β-carboxamide* ***X***

A mixture of 5 g (0.017 mol) of 6-(2-propenyl)-9,10-dihydrolysergic acid, 5.95 g (0.031 mol) of *N*-ethyl-*N'*-(3-dimethylamino)propyl-carbodiimide hydrochloride and 4.34 ml (0.031 mol) of triethylamine in dimethylformamide (100 ml) was stirred at room temperature overnight. The reaction mixture was concentrated and ice and 1 N NaOH were added to adjust to pH 9. The basic mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated. Thin layer chromatography (TLC) of the residue on a silica gel plate indicated that the residue consisted of two components. HPLC separation was carried out using as eluent a mixture of ethyl acetate/*n*-butanol/pyridine/dimethylformamide 30/20/7/20. After crystallization from diethyl ether, 3.5 g (46%) of pure **IX** were obtained as a white solid, mp: 102–104°C. Anal. ($C_{26}H_{37}H_5O_2$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν

N–H, ν C–H); 1690 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) δ: 9.43 (bt, *J*=5.0 Hz, 1H, CO–*NH*–CH_2); 7.99 (bs, 1H, *NH*-1); 5.96 (m, 1H, *CH*=CH_2); 5.21 (m, 2H, CH_2=CH–); 3.84 (m, 2H, CO–N–CH_2–CH_2); 3.30 (dq, *J*=7.5 & 5.0 Hz, 1H, NH–CH_2–CH_3); 2.56 (dd, *J*=11.5 & 11.5 Hz, 1H, *H-7ax*); 2.34 (t, *J*=6.5 Hz, 2H, CH_2–$N(CH_3)_2$); 2.23 (s, 6H, $N(CH_3)_2$); 1.77 (ddd, *J*=12.0, 12.0 & 12.0 Hz, 1H, *H-9ax*); 1.18 (t, *J*=7.5 Hz, 3H, CH_3–CH_2–N). MS (EI) *m/z*: 451(18, M$^{+\cdot}$); 380(22); 339(12); 251(29); 154(53); 58(100).

Continuing elution with the same eluent, 2.7 g (35%) of pure *N*-[[[3-(dimethylamino)propyl]amino]-carbonyl]-*N*-ethyl-6-(2-propenyl)-ergoline 8β-carboxamide were obtained as a white foam. The compound was characterized as diphosphate salt **X**. Anal. ($C_{26}H_{43}N_5O_{10}P_2$) C,H,N. IR (KBr) free base: 3600–2200 cm^{-1} (ν N–H, ν C–H), 1700 cm^{-1} (ν C=O). ^{1}H NMR (DMSO–d_6) (60 MHz) free base: δ 10.5 (s, 1H, *NH*-1); 8.50 (bs, 1H, *NH*–CH_2–); 6.6–7.2 (m, 4H, *H-12*, *H-13*, *H-14*, *H-2*); 5.6–6.3 (m, 1H, –*CH*=CH_2); 5.0–5.6 (m, 2H, CH_2=CH–); 2.60 (s, 6H, $N(CH_3)_2$); 1.13 (t, 3H, CH_3–CH_2–N). MS (EI) *m/z*: 451(8, M$^{+\cdot}$); 323(49); 283(18); 251(18); 154(38); 58(100).

N-*[3-(Dimethylamino)propyl]-6-(2-propenyl)-ergoline-8β-carboxamide* ***XI***

A solution of 25 g (0.08 mol) of 6-(2-propenyl)-9,10-dihydrolysergic acid methyl ester in 3-dimethylamino-1-propylamine (150 ml) and glacial acetic acid (5 ml) was refluxed overnight and the 3-dimethylamino-1-propylamine in excess was evaporated. The brown oily residue was taken up in a 10% $NaHCO_3$ solution and extracted with ethyl acetate. The organic extracts were washed with brine, dried (Na_2SO_4) and the solvent evaporated. The residue was crystallized from acetone to give 26.2 g (85%) of pure amide. mp: 200–202°C. Anal. ($C_{23}H_{32}N_4O$) C,H,N. IR (KBr): 3380 and 3000 cm^{-1} (ν N–H); 1625 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) δ: 7.97 (bs, 1H, *NH*-1); 7.61 (bt, *J*=5.0 Hz, 1H, *NH*–CO–8); 5.97 (m, 1H, –*CH*=CH_2); 5.25 (m, 2H, CH_2=CH–); 2.46 (dd, *J*=11.5 & 11.5 Hz, 1H, *H-7ax*); 2.42 (t, *J*=6.0 Hz, 2H, CH_2–$N(CH_3)_2$); 2.24 (s, 6H, –$N(CH_3)_2$); 1.67 (dt, *J*=6.0 & 6.0 Hz, 2H, CH_2–CH_2–$N(CH_3)_2$); 1.63 (ddd, *J*=12.0, 12.0 & 12.0 Hz, 1H, *H-9ax*). MS (EI) *m/z*: 380(8, M$^{+\cdot}$); 339(5); 251(8); 154(33); 58(100).

N-*[3-(Dimethylamino)propyl]-6-propyl-ergoline 8β-carboxamide* ***XII***

Compound **XII** was prepared according to the procedure for compound **XI**, starting from 6-*n*-propyl-dihydrolysergic acid methyl ester, to give the title compound in 78% yield. mp: 202–204°C. Anal. ($C_{23}H_{34}N_4O$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, ν C–H); 1660 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$, 80 MHz) δ: 0.90 (t, *J*=7.0 Hz, 3H, CH_2CH_3); 2.25 (s, 6H, $N(CH_3)_2$); 6.8–7.2 (m, 4H, *H-2*, *H-12*, *H-13*, *H-14*); 7.40–7.85 (two bs, 2H, N*H*–1, CON*H*). MS (EI) *m/z*: 382(39, M$^{+\cdot}$); 253(20); 251(24); 167(21); 154(30); 144(28); 127(11); 84(46), 58(100); 43(22).

N-*[3-(Dimethylamino)propyl]-6-isopropyl-ergoline-8β-carboxamide* ***XIII***

Compound **XIII** was prepared according to the procedure described for compound **XI**, starting from 6-isopropyl-lysergic acid methyl ester, to give the title compound in 65% yield as a white foam. Anal. ($C_{23}H_{34}N_4O$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, ν C–H); 1660 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.92, 1.23 (two d, *J*=6.5 Hz, $CH(CH_3)_2$); 1.65 (ddd, *J*=12.4, 12.4 & 12.4 Hz, 1H, *H-9ax*); 1.71 (m, 2H, $CH_2CH_2N(CH_3)_2$); 2.26 (s, 6H, $N(CH_3)_2$); 2.28 (dd, *J*=11.0 & 11.0 Hz, 1H, *H-7ax*); 2.44 (m, 2H, $CH_2N(CH_3)_2$); 2.5–2.7 (m, 3H, *H-4ax*, *H-5az*, *H-8ax*); 2.83 (m, 1H, *H-9eq*); 3.00 (m, 1H, *H-10ax*); 3.26 (m, 1H, *H-7eq*); 3.40 (m, 3H, CONHC*H*$_2$, *H-4eq*); 3.63 (qq, *J*=6.5 Hz, 1H, NC*H*$(CH_3)_2$); 6.9–7.2 (m, 4H, *H-2*, *H-12*, *H-13*, *H-14*); 7.58 (bm, 1H, N*H*CH_2); 7.94 (bs, 1H, N*H*–1). MS (EI) *m/z*: 382(18, M$^{+\cdot}$); 339(4); 253(10); 167(11); 154(20); 144(19); 84(34); 72(9); 58(100).

6-(2-Propenyl)-ergoline-8β-carboxamide ***XVI***

A solution of 10 g (0.032 mol) of 6-(2-propenyl)-dihydrolysergic acid methyl ester and 3.4 g (0.064 mol) of ammonium chloride in methanol (500 ml) was saturated with gaseous ammonia at –20°C. The resulting solution was heated overnight at 120°C in a stainless steel vessel. The solvent was removed and the residue was taken up in 10% Na_2CO_3 solution and extracted with ethyl acetate. The organic extracts were washed with brine, dried (Na_2SO_4) and the solvent evaporated. The crude amide was crystallized from acetone to afford 6.5 g of pure **XIV**. mp: 197–199°C. Anal. $C_{18}H_{21}N_3O$) C,H,N. IR (KBr): 3500–2700 cm^{-1} (ν N–H, ν C–H); 1660 cm^{-1} (ν C=O). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.37 (ddd, *J*=12.0, 12.0 & 12.0 Hz, 1H, *H-9ax*); 2.28 (dd, *J*=11.0 & 11.0 Hz, 1H, *H-7ax*); 2.30 (m, 1H, *H-5ax*); 2.5–2.8 (m, 4H, *H-4ax*, *H-8ax*, *H-9eq*, H-10 *ax*); 3.01 (m, 1H, *H-7eq*); 3.34 (dd, *J*=4.0 & 14.5 Hz, 1H, *H-4eq*); 3.36 (m, 2H, NCH_2); 5.20 (m, 2H, =CH_2); 5.93 (m, 1H, C*H*=); 6.60 (bs, 2H, CONH_2); 6.8–7.2 (m, 4H, *H-2*, *H-12*, *H-13*, *H-14*); 10.61 (bs, 1H, N*H*-1). MS (EI) *m/z*: 295(92, M$^{+\cdot}$); 254(28); 215(21); 249(18); 209(80); 167(75); 154(100); 144(65); 127(55); 41(39).

N-*[3-(dimethylamino)propyl]*-N-*[(ethylamino)carbonyl]-6-(2-propenyl)-ergoline-8β-carboxamide* ***IX*** *and* N-*[3-(dimethylamino)propyl]*-N-*[(ethylamino)carbonyl]-1-ethylaminocarbonyl-6-(2-propenyl)-ergoline-8β-carboxamide* ***IXa***

A mixture of 10 g (0.026 mol) of *N*-[3-(dimethylamino)propyl]-6-(2-propenyl)-ergoline-8β-carboxamide in toluene (200 ml) and ethyl isocyanate (40 ml) was refluxed overnight. The reaction mixture was evaporated to give an oil which was dissolved in chloroform, washed several times with brine and dried (Na_2SO_4). The solvent was removed and the resulting crude compound was chromatographed on silica gel using acetone as eluent. The first fractions gave, after evaporation of the eluent and crystallization from diisopropyl ether, 0.57 g of pure **IXa**, mp: 125–127°C. Anal. ($C_{29}H_{42}N_6O_3$) C,H,N. IR (KBr): 3300 and 3000 cm^{-1} (ν N–H); 1660 cm^{-1} (ν C=O). ^{1}H NMR ($CDCl_3$) δ: 9.46 (t, *J*=5.0 Hz, 1H, *NH*-CH_2–CH_3); 7.68 (d, *J*=7.5 Hz, 1H, *H-14*); 7.26 (t, *J*=7.5 Hz, 1H, *H-13*); 7.02 (d, *J*=7.5 Hz, 1H, *H-12*); 5.94 (m, 1H, –*CH*=CH_2); 5.49 (t, *J*=5.5 Hz, 1H, N(1)–CO–N*H*); 5.20 (m, 2H, CH_2=CH–); 3.50 (dq, *J*=7.0 & 5.5 Hz, 2H, N(1)–CONH–CH_3); 3.29 (dq, *J*=7.5 & 5.0 Hz, 2H, NH–CH_2–CH_3); 2.54 (dd, *J*=11.5 & 11.5 Hz, 1H, *H-7ax*); 2.23 (s, 6H, –$N(CH_3)_2$); 1.72 (ddd, *J*=12.0, 12.0 & 12.0 Hz, 1H, *H-9ax*); 1.29 (t, *J*=7.0 Hz, 3H, CH_3–CH_2–NH–CO–N(1); 1.17 (t, *J*=7.5 Hz, 3H, CH_3–CH_2–NH). MS (EI) *m/z*: 522(0.11, M$^{+\cdot}$); 451(0.3); 380(2); 339(0.8); 71(100).

Continuing the elution with acetone, the desired product **IX** was obtained and crystallized from diethyl ether to give 9.7 g (81%), mp: 102–104°C.

Method B

N-*[3-(Dimethylamino)propyl]*-N-*[(ethylamino)carbonyl]-6-*n*-propyl-ergoline-8β-carboxamide* ***XV***

Compound **XV** was prepared starting from compound **XII** by reaction with ethylisocyanate, to give the title compound in 87% yield. mp: 64–68°C. Anal. ($C_{26}H_{39}N_5O_2$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, C–H); 1670 cm^{-1} (ν C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.89 (t, *J*=7.3 Hz, 3H, NCH_2CH_3); 1.18 (t, *J*=7.4 Hz, 3H, NH–CH_2CH_3); 1.55 (m, 2H, $NCH_2CH_2CH_3$); 1.82 (ddd, *J*=12.5, 12.5 & 12.5 Hz, 1H, *H-9ax*); 1.85 (m, 2H, $CH_2CH_2N(CH_3)_2$); 2.24 (s, 6H, $N(CH_3)_2$); 2.26 (m, 1H, *H-7ax*); 2.36 (m, 2H, $CH_2N(CH_3)_2$); 2.6–2.8 (m, 6H, H-4ax, *H-5ax*, *H-8ax*, *H-9eq*, $NCH_2CH_2CH_3$); 3.03 (m, 1H, *H-10ax*); 3.18 (m, 1H, *H-7eq*); 3.2–3.4 (m, 3H, *H-4eq*, NHCH_2CH_3); 3.86 (m, 2H, $CH_2CH_2CH_2NCH_3$); 6.8–7.2 (m, 4H, *H-2*, *H-12*, *H-13*, *H-14*); 8.06 (bs, N*H*-1); 9.43 (bm, 1H, N*H*CH_2CH_3). MS (EI) *m/z*: 453(2, M$^{+\cdot}$); 382(23); 251(31); 173(23); 167(28); 154(36); 84(39); 71(36); 58(100); 56(65).

N-*[3-(Dimethylamino)propyl]*-N-*[(ethylamino)carbonyl]-2-bromo-6-*n*-propyl-ergoline-8β-carboxamide* ***XVI***

To a stirred solution containing 1 g (0.0022 mol) of compound **XV** in dioxane (50 ml) was added portionwise, *N*-bromosuccinimide (0.43 g, 0.0024 mol) at room temperature. After 3 h, the resulting dark solution was diluted with ethyl acetate, and washed first with a $NaHSO_3$ aqueous solution, then with $NaHCO_3$ solution. After drying (Na_2SO_4) the solvent was removed *in vacuo* and the residue was chromatographed on silica gel eluting with acetone. The combined fractions afforded, after evaporation of the solvent, 0.66 g (56%) of **XVI** as a foam. Anal. ($C_{26}H_{38}N_5O_2Br$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, ν C–H); 1670 cm^{-1} (ν C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.89 (t, *J*=7.3 Hz, 3H, NCH_2CH_3); 1.18 (t, *J*=7.4 Hz, 3H, NH–CH_2CH_3); 1.55 (m, 2H, $NCH_2CH_2CH_3$); 1.82 (ddd, *J*=12.5, 12.5 & 12.5 Hz, 1H, *H-9ax*); 1.85 (m, 2H, $CH_2CH_2N(CH_3)_2$); 2.24 (s, 6H, $N(CH_3)_2$); 2.26 (m, 1H, *H-7ax*); 2.36 (m, 2H, $CH_2N(CH_3)_2$); 2.6–2.8 (m, 6H, H-4ax, *H-5ax*, *H-8ax*, *H-9eq*, $NCH_2CH_2CH_3$); 3.03 (m, 1H, *H-10ax*); 3.18 (m, 1H, *H-7eq*); 3.2–3.4 (m, 3H, *H-4eq*, NHCH_2CH_3); 3.86 (m, 2H, $CH_2CH_2CH_2NCH_3$); 6.9–7.3 (m, 3H, *H-12*, *H-13*, *H-14*); 8.06 (bs, N*H*-1); 9.43 (bm, 1H, N*H*CH_2CH_3). MS (FD) *m/z*: 533(100, M$^{+\cdot}$); 531(98, M$^{+\cdot}$).

N-*[3-(Dimethylamino)propyl]*-N-*[(ethylamino)carbonyl]*-6-isopropyl-ergoline-β-carboxamide ***XVII***

Compound **XVII** was prepared starting from compound **XIII** by reaction with ethyl isocyanate, to give the title compound as a foam in 78% yield. Anal. ($C_{26}H_{39}N_5O_2$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, ν C–H); 1670 cm^{-1} (ν C=O). ^1H NMR (60 MHz, DMSO–d_6) δ: 0.83, 1.13 (two d, J=6.5 Hz, 6H, $NCH(CH_3)_2$); 1.10 (t, J=7.0 Hz, 3H, $NHCH_2CH_3$); 2.15 (s, 6H, $N(CH_3)_2$); 6.8–7.2 (m, 4H, *H-2*, *H-12*, *H-13*, *H-14*); 8.89 (bm, 1H, $NHCH_2$); 10.40 (bs, 1H, *NH*-1). MS (EI) *m/z*: 453(2, $M^{+\cdot}$); 382(5); 253(4); 201(5); 173(5); 154(7); 144(5); 71(83); 58(30); 56(100).

N-*[3-(Dimethylamino)propyl]*-N-*[(methylamino)carbonyl]*-6-(2-propenyl)-ergoline-β-carboxamide ***XVIII***

Compound **XVIII** was prepared starting from compound **XI** by reaction with methyl isocyanate, to give the title compound in 80% yield. mp: 126–128°C. Anal. ($C_{25}H_{35}N_5O_2$) C,H,N. IR (KBr): 3600–2700 cm^{-1} (ν N–H, ν C–H); 1670 cm^{-1} (ν C=O). ^1H NMR (60 MHz, $CDCl_3$) δ: 2.23 (s, 6H, $N(CH_3)_2$); 2.85 (d, J=4.4 Hz, 3H, $NHCH_3$); 5.30 (m, 2H, $=CH_2$); 5.90 (m, 1H, $CH=$); 6.8–7.2 (m, 4H, *H-2*, *H-12*, *H-13*, *H-14*); 8.20 (bs, 1H, *NH*-1); 9.40 (bq, J=4.4 Hz, 1H, $NHCH_3$). MS (EI) *m/z*: 437(15, $M^{+\cdot}$); 380(35); 251(19); 159(17); 154(28); 144(14); 84(24); 58(84); 57(100); 56(29).

N-*[3-(Dimethylamino)propyl]*-N-*[(phenylamino)carbonyl]*-6-(2-propenyl)-ergoline-β-carboxamide ***XIX***

Compound **XIX** was prepared starting from compound **XI** by reaction with phenyl isocyanate, to give the title compound in 74% yield. mp: 82–85°C. Anal. ($C_{30}H_{37}N_5O_2$) C,H,N. IR (KBr): 3370 and 3000 cm^{-1} (ν N–H); 1700 and 1620–1600 cm^{-1} (ν C=O). ^1H NMR ($CDCl_3$) δ: 11.74 (s, 1H, CO-*NH*-Ph); 7.97 (s, 1H, *NH*-1); 6.8–7.6 (m, 9H, *H-12*, *H-13*, *H-14*, *H-2*, *-Ph*); 5.97 (m, 1H, $-CH=CG_2$); 5.21 (m, 2H, $-CH=CH_2$); 2.60 (dd, J=11.5 & 11.5 Hz, 1H, *H-7ax*); 2.42 (t, J=6.5 Hz, 2H, $CH_2-CH_2-N(CH_3)_2$); 2.32, 2.30 (two s, 6H, $N(CH_3)_2$); 1.94 (t, J=6.5 Hz, 2H, $CH_2-CH_2-CH_2-N(CH_3)_2$); 1.80 (ddd, J=12.5, 12.5 & 12.5 Hz, 1H, *H-9ax*). MS (EI) *m/z*: 380(0.12); 339(0.06); 119(100).

N-*[(Ethylamino)carbonyl]*-6-(2-propenyl)-ergoline-8 β-carboxamide ***XX***

Compound **XX** was prepared starting from compound **XIV** by reaction with ethyl isocyanate, to give the title compound in 90% yield. mp: 210–212°C. Anal. ($C_{21}H_{26}N_4O_2$) C,H,N. IR (KBr): 3400–3000 cm^{-1} (ν N–H); 1650 cm^{-1} (ν C=O). ^1H NMR (60 MHz, $CDCl_3$) δ: 1.20 (t, J= 7.0 Hz, 3H, $NHCH_2CH_3$); 5.30 (m, 2H, $=CH_2$); 5.90 (m, 1H, $CH=$); 6.8–7.2 (m, 4H, *H-2*, *H-12*, *H-13*, *H-14*); 8.18 (bs, 1H, *NH*-1); 8.45 (bm, 1H, $NHCH_2$); 9.80 (bs, 1H, CONHCO). MS (EI) *m/z*: 366(45, $M^{+\cdot}$); 321(95); 295(54); 251(21); 209(69); 167(82); 154(100); 144(47); 127(43); 115(18).

Pharmacology

Anti-nidation activity

The prolactin secretion inhibitory activity was indirectly evaluated utilizing the nidation inhibition test in rats. In fact, the dose inhibiting nidation in 50% of the animals is related to the dose inhibiting basal prolactin secretion by 50% for at least 24 h in male rats [18]. Adult female Sprague–Dawley rats (180–230 g), supplied by Charles River, were housed with fertile male rats during the night of proestrus. The following morn-ing, vaginal smears were checked for the presence of spermatozoa (first day of pregnancy). Animals with positive vaginal smears were treated in the morning of the 5th gestational day (6–8 animals per group) with test compounds, suspended in 5% gum arabic, at the screening dose of 8 mg/kg *p.o.* On the 14th day of pregnancy, laparotomy was performed under light ether anesthesia and the uterine horns were checked for the presence or absence of implantation sites. The percentage of animals without implantations was calculated. The active compounds were tested at lower doses for ED_{50} evaluation. Bromocriptine, well known for both its anti-nidation and anti-prolactin activities [19], was used as a reference standard.

Acute toxicity

The acute oral toxicity of the compounds was evaluated in male Swiss mice, supplied by Charles River. Each compound, suspended in 5% gum arabic, was administered at various doses (5–6 animals per dose) and the dose causing the death of 50% of the animals (LD_{50}) within the 7th day following treatment was approximately evaluated.

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