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SEROTONERGIC ERGOLINE DERIVATIVES.

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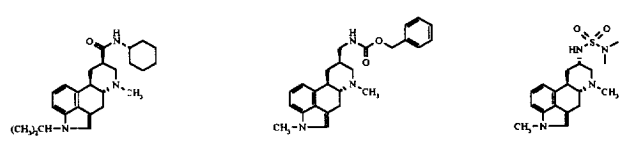
Abstract. Novel classes of 13- and 14-tertbutyl-ergoline derivatives were prepared, and characterised *in vitro* for their affinity for adrenergic, dopaminergic and serotonergic binding sites. This study particularly examines the importance of the presence and the position of the tert-butyl group in conferring either significant 5-HT_{1A} or 5-HT_2 affinity and selectivity respectively.

Serotonin (5-HT, 5-hydroxytryptamine) is an important neurotransmitter mediating many central and peripheral physiological functions including food intake, sleep, sexual behaviour, memory and blood pressure.[1] 5-HT attains such a variety of functions by acting on distinct receptor types. The most recent classification of 5-HT receptor subtypes is based upon the amino acid sequence, gene structure, coupling to second messenger and pharmacological profile. Of these, 5-HT_{1A} and 5-HT_2 receptor sites have been the most studied as it is generally accepted that they are involved in psychiatric disorders such as depression, schizophrenia and anxiety.[2] Following the proposal of Humphrey, Hartig and Hoyer, the 5-HT_2 receptor has been subdivided into three subtypes, namely 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor sites.[3] Several classes of agents such as arylpiperazines, phenylalkylamines, indolylalkylamines and ergoline derivatives bind at these receptor sites.[4] The latter display a wide array of pharmacological effects in the peripheral and in central nervous systems by interacting with adrenergic, dopaminergic and serotonergic receptor types.[5] Notwithstanding the receptorial non-selectivity of most ergolines, compounds such as the 5-HT_{2A} antagonist amesergide, or the 5-HT_{1A} agonist/5-$HT_{2A/2C}$ antagonist metergoline, or the 5-$HT_{2A/2C}$ antagonist mesulergine do show relative serotonergic selectivity. Thus, the ergolines may be considered a valuable template in the design of serotonergic agents.



Amesergide **Metergoline** **Mesulergine**

A synthetic program started with the objective of identifying novel ergolines having high and selective affinity for either 5-HT_{1A} or 5-HT_2 receptor.

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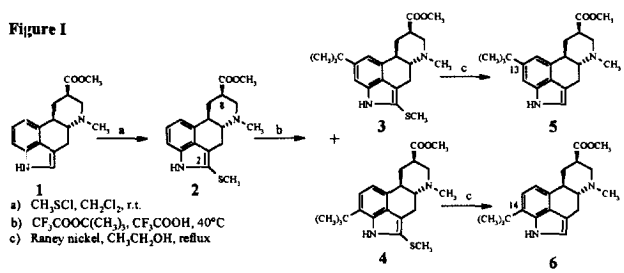
Consideration of the general SAR requirements for DA receptor binding, based on the hypothetical model of DA receptor developed by Mc Dermed et al., suggested that the interaction of ergoline derivatives with DA receptors might be prevented by a bulky group on the phenyl ring of the ergoline skeleton.[6] [7] Following this assumption, the introduction of the tert-butyl group on the phenyl ring formed part of our strategy to enhance the selectivity of the serotonergic component by precluding DA interactions. Preliminary results with a set of 6-methyl-8β-acylaminomethyl-ergoline derivatives indicated that the 5-HT_{1A} selectivity was achieved when a tert-butyl group was present in position 13.[8] Subsequently, we undertook the synthesis of various classes of 13- and 14-tertbutyl-ergoline derivatives, the purpose of which was to gain more insight into the role of the tert-butyl group with respect to 5-HT_{1A} and 5-HT_2 affinity and selectivity.

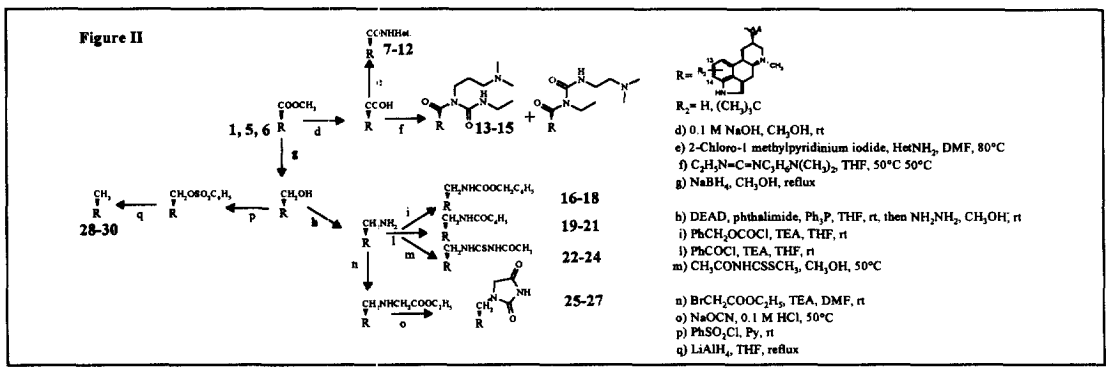
Chemistry The syntheses of the key intermediates, 13-tertbutyl- and 14-tertbutyl-dihydrolysergic acid methyl ester were carried out as outlined in **Figure I**

Figure I

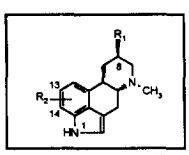


The key step involved the introduction of a tert-butyl group on the phenyl ring. Removable protection of the more electrophilic position 2 seemed a way of directing tert-butylation to the phenyl ring. The thiomethyl group was expected to fulfil this requirement. It can be readily removed by action of Raney nickel or nickelborohydride and furthermore, it has been shown that the 2-thiomethyl group can facilitate electrophilic aromatic substitution on the phenyl ring of the ergolines.[9] The 2-methylthiodihydrolysergic acid methyl ester **2** was readily obtained by direct thiomethylation of **1** with methylsulphenyl chloride, prepared by action of sulphuryl chloride on dimethyldisulphide at low temperature.[10] On exposure to tert-butylacetate/trifluoroacetic acid, **2** underwent a smooth aromatic tert-butylation to provide, after chromatography and crystallisation, **3** (65% yield) and **4** (15% yield). Removal of the thiomethyl group with Raney nickel afforded the 13- and 14-tertbutyl-ergolines **5** and **6**.[11] The ester moiety of **1**, **5** and **6** was then converted into different pharmacophoric groups observed to impart central or peripheral biological activity in the case of the unsubstituted analogues, following the depicted pathways of **Figure II**. Amides **7-12** were prepared by condensing the heteroarylamines with the dihydrolysergic acids employing 2-chloro-1-methylpyridinium iodide as condensing agent.[12] Condensation of the dihydrolysergic acids with N-ethyl-N′-(3-dimethylamino)propylcarbodiimide gave a mixture of two regioisomer acylureas, with the desired **13-15** as the major isomer.[13] Purification by chromatography provided pure **13-15**. Sodium borohydride reduction of **1**, **5**, **6** afforded the corresponding alcohols that were converted into the amine analogues using the Mitsunobu procedure.[14] Carbamates **16-18**,

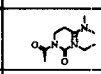
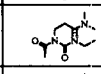
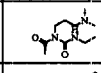
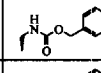
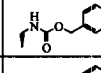
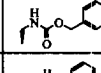
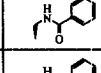
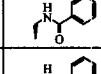
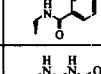
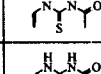
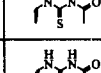
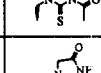
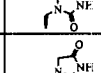
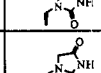
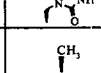
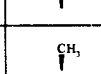
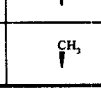
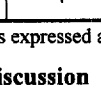
benzamides **19-21** and acetylthioureas **22-24** were prepared in a straightforward way by reaction of the amines with benzyl chloroformate, benzoyl chloride and methyl N-acetyldithiocarbamate respectively, whilst alkylation with ethyl bromoacetate to the glycine derivatives and subsequent cyclization with hydrocyanic acid gave the hydantoins **25-27**. Conversion of the alcohols into benzensulphonate esters followed by lithium aluminium hydride reduction allowed the preparation of **28-30**.



Pharmacology The compounds described in this study were evaluated for their α_1, α_2, D_1, D_2, 5-HT_{1A} and 5-HT_2 receptor binding affinities (see **Table I** and **II**), assessed by measuring the displacement of [^{3}H]-prazosin binding in rat frontal cortex, [^{3}H]-yohimbine binding in rat frontal cortex, [^{3}H]-SCH-23390 binding in rat striatum, [^{3}H]-spiroperidol binding in rat striatum, [^{3}H]-8-OH-DPAT binding in rat hippocampus and [^{3}H]-ketanserin binding in rat pre-frontal cortex, respectively. Considering the ligand ([^{3}H]-ketanserin) and the homogenate (rat pre-frontal cortex) used for the assessment of the 5-HT_2 affinity, the values reported essentially mirror the 5-HT_{2A} component.



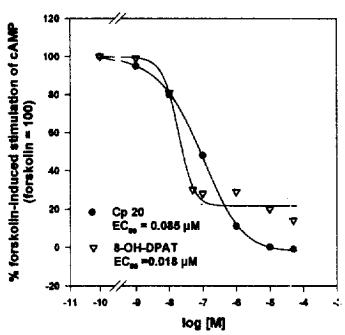
Cp	R_1	R_2	α_1	α_2	D_1	D_2	5-HT_{1A}	5-HT_2
7		H	0.73	0.41	0.19	0.17	0.82	0.26
8		13-$(CH_3)_3C$	5.23	1.82	>10	1.12	0.12	0.43
9		14-$(CH_3)_3C$	1.11	0.81	>10	1.26	0.58	0.002
10		H	0.35	0.51	0.57	0.22	0.69	0.52
11		13-$(CH_3)_3C$	6.57	0.83	3.81	2.62	0.34	1.22
12		14-$(CH_3)_3C$	1.55	0.87	7.22	0.96	0.57	0.003

Cp	R_1	R_2	α_1	α_2	D_1	D_2	5-HT_{1A}	5-HT_2
13		H	2.55	1.27	0.85	0.05	0.31	0.28
14		13-$(CH_3)_3C$	2.95	0.007	>10	1.06	0.09	0.54
15		14-$(CH_3)_3C$	2.58	0.087	>10	0.57	0.21	0.032
16		H	3.33	0.39	1.42	0.15	0.06	0.25
17		13-$(CH_3)_3C$	7.41	2.17	>10	1.25	0.015	0.52
18		14-$(CH_3)_3C$	8.62	0.67	>10	0.83	0.027	0.054
19		H	1.32	0.48	4.15	0.075	0.014	0.43
20		13-$(CH_3)_3C$	6.53	>10	3.43	5.17	0.025	6.73
21		14-$(CH_3)_3C$	>10	0.71	>10	2.07	0.031	0.048
22		H	>10	0.08	1.23	0.73	0.24	0.31
23		13-$(CH_3)_3C$	>10	3.57	>10	5.47	0.18	0.61
24		14-$(CH_3)_3C$	0.78	0.45	4.58	1.41	0.93	0.003
25		H	0.13	0.17	2.21	1.12	0.73	0.27
26		13-$(CH_3)_3C$	2.20	0.26	7.39	7.63	0.078	0.32
27		14-$(CH_3)_3C$	3.27	0.14	3.54	0.94	0.21	0.019
28		H	2.79	0.18	4.34	0.25	0.26	1.67
29		13-$(CH_3)_3C$	8.95	1.76	>10	9.13	0.23	4.94
30		14-$(CH_3)_3C$	9.43	1.23	7.53	3.74	0.28	0.12

(affinity is expressed as IC_{50} in μM, standard errors are ± 10% of the mean reported value)

Result and discussion As shown in **Table I** and **II**, the unsubstituted lead compounds **7**, **10**, **13**, **22**, **25** and **28** had little and unselective affinity on 5-HT_{1A} and 5-HT_2 activity. In contrast, compounds **16** and **19** showed a significant, albeit unselective, 5-HT_{1A} component. Introduction of a tert-butyl group in position 13 did not affect 5-HT_{1A} affinity substantially. On the other hand, α_1, α_2, D_1 and D_2 affinities were remarkably decreased. This fact led to an enhanced 5-HT_{1A} selectivity as shown for **8**, **11**, **14**, **17**, **20**, **23**, **26** and **29**. For instance, **17** and **20** are endowed with a quite high 5-HT_{1A} affinity and selectivity, the latter being more hundred-fold in respect to the D_1 and D_2 component. A consistent increase in α_2 and less extension in 5-HT_{1A} affinity was observed for **14**. Similar to what was observed with 13-tert-butyl analogues, introduction of a tert-butyl group in position 14

not only decreased adrenergic and dopaminergic affinity substantially, but diminished the 5-HT_{1A} affinity significantly. 5-HT_2 affinity increased in all series considered, independently from the pharmacophoric group in position 8. Among the compounds considered in this study, amides **9** and **12** demonstrated more than a hundred-fold increase with respect to the parents **7** and **10** in terms of 5-HT_2 affinity and selectivity. An hundred-fold enhancement in 5-HT_2 affinity was also seen for **24** in comparison with the parent **22**. However, the increase in selectivity was less pronounced than in the cases previously mentioned. Compound **20** displaying a relatively high 5-HT_{1A} selectivity for an ergoline derivative, without being a particulary high affinity agent, was chosen to test its effect on receptor function. For this purpose, the compound was evaluated on 5-HT_{1A} receptor, negatively coupled to adenylyl cyclase. HeLa cells transfected with 5-HT_{1A} were incubated with forskolin (0.5 μM), which stimulates cAMP production by acting on the catalytic site of adenylyl cyclase, and different concentrations (from 10^{-9} to $5 \cdot 10^{-4}$ M) of the tested compounds. After cell lysis by HCl, the cAMP released was measured with a cAMP-binding protein method.[15]



The results of the adenylyl cyclase assay, presented in figure, showed that the 5-HT_{1A} agonist 8-OH-DPAT and similarly **20** inhibit forskolin-stimulated adenylyl cyclase in dose-dependent manner. 8-OH-DPAT exhibited a EC_{50} = 0.018 μM, without restoring the basal level of adenylyl cyclase, in fact about 20 % of forskolin activity was retained even at the highest concentrations. Compound **20** presented an EC_{50} = 0.085 μM and was able to bring back at about $5 \cdot 10^{-5}$ M the cAMP production at the basal level, indicating, in this assay, an agonistic activity on the receptor function.

Conclusion In conclusion, the introduction of a tert-butyl group either in position 13 or 14 of the ergoline skeleton has resulted a valid strategy for the identification of reasonably potent and selective 5-HT_{1A} or 5-HT_2 ligands. From the binding data listed in **Table I** and **II**, some general trends can be easily identified. Selectivity for 5-HT_{1A} *versus* α_1, α_2, D_1, D_2 and 5-HT_2 receptor sites appears to be determined by the presence of a tert-butyl group in position 13. The most favorable affinity-selectivity profile is exemplified by **20**, that furthermore exhibits a 5-HT_{1A} agonist profile as suggested by the results of the adenylyl cyclase assay. In contrast, selectivity for 5-HT_2 versus α_1, α_2, D_1, D_2 and 5-HT_{1A} receptor sites is originated by the presence of a tert-butyl group in position 14. Some compounds of this class, for example **9**, **12** and **24**, display nM affinity for the 5-HT_2 receptor accompanied by at least a hundred-fold separation in selectivity over the other receptors. Within this set of ergoline derivatives, the adrenergic and dopaminergic binding sites seem to be very sensitive to increases in steric demand in the area of the phenyl ring. The changes in steric demands caused by the aromatic tert-butylation have a noteworthy effect on either 5-HT_{1A} selectivity or 5-HT_2 affinity and selectivity. These results, eventhough 5-HT_{1A} or 5-HT_{2A} high affinity and selectivity were not completely achieved, provide

further evidence of the delicate balance between structure and biological activity for ergot derivatives and furthermore attest the pharmacological potential of this class.

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9. Mantegani, S., personal comunication (Different percentage and distribution of 13- and 14-tertbutyl derivatives were observed when 2-bromo- and 2-chlorodihydrolysergic acid methyl ester were submitted to the reaction's condition herein reported)
10. SIMES Società Italiana Medicinali e Sintetici 1977 Swiss Patent 8255/77.
11. Spectral data for **5** ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.36 (s, 9 H, $(CH_3)_3C$); 1.5-1.7 (m, 1 H, H-9ax); 2.18 (ddd, J=4.3, 9.2, 11.1 Hz, 1 H, H-5); 2.35 (dd, J=11.4, 11.4 Hz, 1 H, H-7ax); 2.48 (s, 3 H, CH_3-N); 2.64 (ddd, J=1.7, 11.1, 14.7 Hz, 1 H, H-4ax); 2.8-3.1 (m, 3 H, H-8, H-9eq, H-10); 3.2-3.3 (m, 1 H, H-7eq); 3.32 (dd, J=4.3, 14.7 Hz, 1 H, H-4eq); 3.75 (s, 3 H, $COOCH_3$); 6.82 (dd, J=1.7, 1.7 Hz, 1 H, H-2); 7.02 (s, 1 H, H-14); 7.20 (s, 1 H, H-12); 7.82 (bs, 1 H, NH-1). MS: *m/z* 340 (100, [M]+·); 325 (8, $[M\text{-}CH_3]^+$); 309 (5, $[M\text{-}CH_3O]^+$); 284 ($[M\text{-}C_4H_3]^+$); 281 (4, $[M\text{-}COOCH_3]^+$); 223 (8); 210 (6); 200 (8); 154 (8); 94 (5); 57 (5). mp 175-177 °C. Spectral data for **6** ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.48 (s, 9 H, $(CH_3)_3C$); 1.50 (m, 1 H, H-9ax); 2.17 (m, 1 H, H-5); 2.34 (dd, J=11.4, 11.4 Hz, 1 H, H-7ax); 2.49 (s, 3 H, CH_3-N); 2.67 (ddd, J=1.7, 11.1, 14.6 Hz, 1 H, H-4ax); 2.94 (m, 3 H, H-8, H-9eq, H-10); 3.25 (ddd, J=1.9, 4.7, 11.4 Hz, 1 H, H-7eq); 3.41 (dd, J=4.4, 14.6 Hz, 1 H, H-4eq); 3.74 (s, 3 H, $COOCH_3$); 6.91 (m, 1 H, H-12); 7.09 (J=7.6 Hz, 1 H, H-13); 8.10 (bs, 1 H, NH-1). MS: *m/z* 340 (100, $[M]^+$); 325 (78, $[M\text{-}CH_3]^+$); 309 (7, $[M\text{-}CH_3O]^+$); 281 (3, $[M\text{-}COOCH_3]^+$); 180 (10); 176 (10); 154 (8); 57 (13). mp 191-193 °C.
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