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Effective cerebral antihypoxic activity of new aminocyclopentanones

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Summary — Effective antihypoxic activity of new aminocyclopentanones which was higher than that of the reference compounds has been demonstrated by the SCR hypoxia test.

antihypoxic activity / aminoindanones / aminocyclopentathiophenones / SCR – hypoxia test / skin conductance reaction

Introduction

In continuation of our work on screening of new compounds with potential antihypoxic effect and in relation to recent studies which have demonstrated the CNS stimulating, antihypoxic or cerebroprotective properties of some indanamine derivatives [1, 2] we report herein the effective cerebral antihypoxic activity of new 3-aminoindanones and 4-aminocyclopentathiophenones.

The study of antihypoxic activity was conducted with the SCR (skin conductance reaction) hypoxia test, the adaptation under hypoxic conditions of the previously described SCR-test [3–5]. This activity was measured by 2 parameters: minimal recovery dose (MRD) and recovery percentage (RP). Determination of these 2 parameters permitted a global approach to improvement in cerebral activity depressed through hypoxia.

Although the chemistry of all compounds whose antihypoxic activity was evaluated has been the subject of several publications [6–12], in this paper we have summarized their synthesis and analytical data.

Chemistry

3-Trifluoroacetylaminoindan-1-ones **1a–g** and 4-trifluoroacetylaminocyclopenta [*b*] thiophen-6-one **1h** were synthesized by intramolecular cyclization of 3-amino-3-arylpropionic acids **2a–h** following 2 previously described pathways [6–9] (scheme 1).

The β-amino acids **2a–h** were obtained by treatment of the aromatic carboxaldehydes **3a–h** with ammonium acetate and malonic acid in refluxing ethanol.

The intramolecular cyclization could be conducted in all cases by the Friedel–Crafts method after protection of the amino group with trifluoroacetic anhydride leading to the trifluoroacetamido derivatives **4a–h**. The acid chlorides **5a–h** were then obtained by refluxing in thionyl chloride. Cyclization was achieved by refluxing in dichloromethane with aluminium chloride [7].

3-Amino-3-(3-thienyl)propionic acid **2h** and 3-amino-3-(3,4,5-trimethoxyphenyl) propionic acid **2b** could be also cyclized in one step. Thus, **2h** and **2b** were dissolved at room temperature in trifluoroacetic acid and the solution was then added with an equivalent amount of trifluoroacetic anhydride. The reaction mixture was refluxed for 3 h and evaporated to dryness to afford the expected cyclized compounds **1h** and **1b** [7–9].

Chlorination of the cyclopentanones **1a** and **1h** was carried out at room temperature in chloroform with chlorine flow. It afforded monochloro derivatives **6a** and **6h**, whose *cis* structure was assigned by ^{1}H-NMR spectrum analysis (**6a**: $J_{H\text{-}2H\text{-}3}$ = 7.3 Hz; **6h**: $J_{H4H\text{-}5}$ = 6.4 Hz). At refluxing temperature, chlorination led to the dichloro compounds **7a** and **7h**. The reaction mechanism has been studied previously [10, 11].

Trifluoroacetamido groups were hydrolyzed in acidic medium to yield the ammonium chlorides **8b–h**, **9a**, **10a** and **10h**. In this reaction, *cis* monochloro derivatives **6a** and **6h** were converted into *trans* compounds **9a** ($J_{H\text{-}2H\text{-}3}$ = 3 Hz) and **9h** ($J_{H\text{-}4H\text{-}5}$ = 2.4 H) [11, 12].

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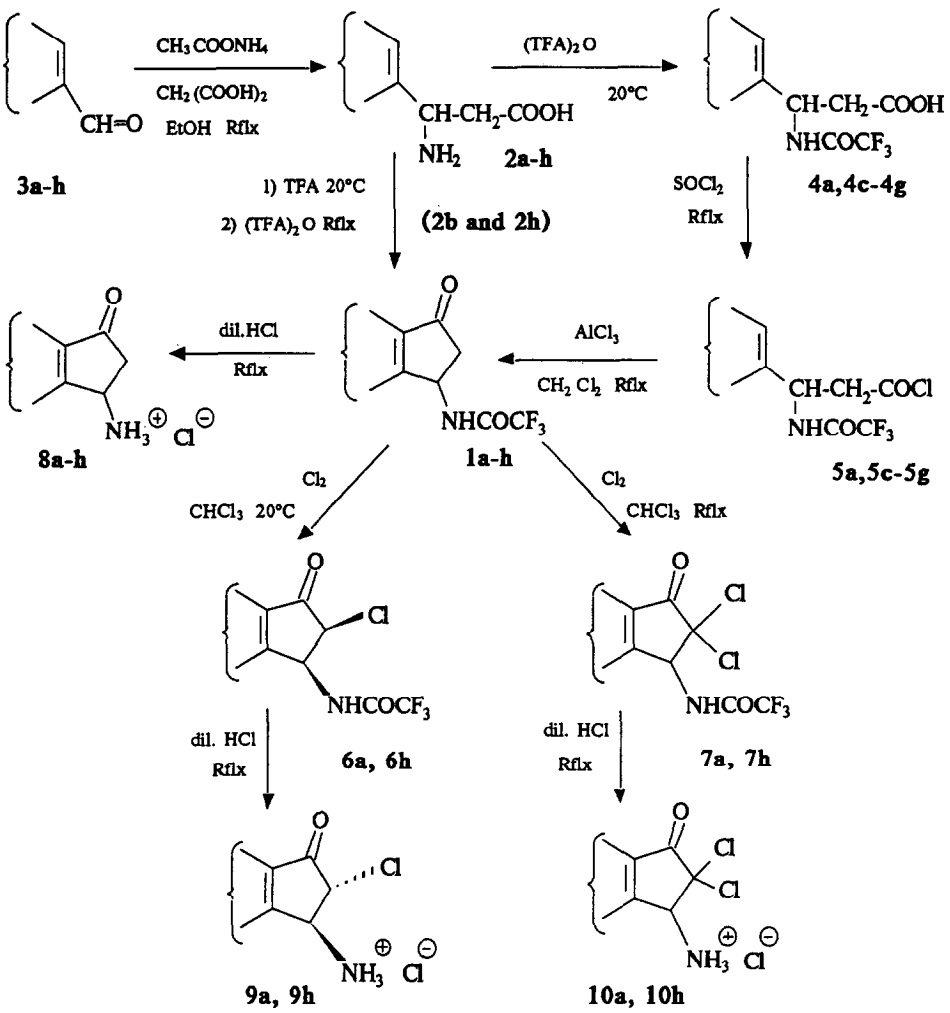
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20°C
CH-CH2-COOH
NHCOCF3
4a,4c-4g
1) TFA 20°C
2) (TFA)2O Rflx
(2b and 2h)
SOCl2
Rflx
dil.HCl
Rflx
AlCl3
CH2Cl2 Rflx
NHCOCF3
1a-h
CH-CH2-COCl
NHCOCF3
5a,5c-5g
NH3 Cl
8a-h
Cl2
CHCl3 20°C
Cl2
CHCl3 Rflx
Cl
NHCOCF3
6a, 6h
Cl
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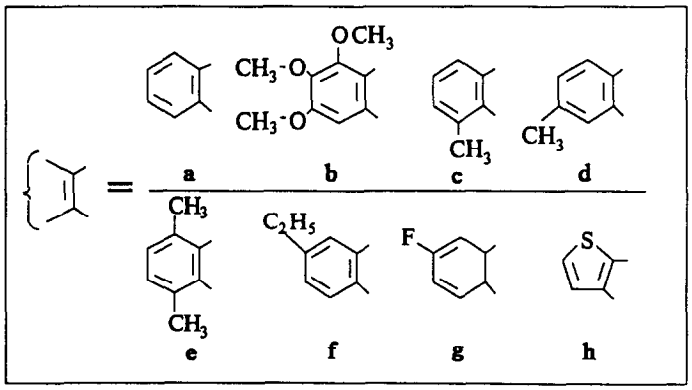
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C2H5
F
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e
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g
h

3

Scheme 1.

Pharmacology

The realization of the SCR test in normobaric hypoxic conditions was permitted by the use of a cell in which the O_2 rate was decreased to 7.5% by introduction of a nitrogen gas flow. Under these conditions, the SCR was decreased, *ie* the rise in palmar skin conductance (PSC) due to the photostimulus was lower than under normoxic conditions [4]. The average rise in PSC in the control group was 1.8 μA ± 0.3 in hypoxia compared with 3.5 μA ± 0.4 in normoxia.

The SCR hypoxia test reveals the antihypoxic activity of some compounds which are able to produce the recovery of this parameter. This recovery has been demonstrated and quantified for reference compounds such as naftidrofuryl, piracetam, piribedil, nicergoline and vincamine, which are known as cerebroprotective drugs in ischemia [4, 13–15].

Results and discussion

Statistically significant recovery doses and corresponding recovery percentages at these doses of reference compounds and aminocyclopentanones **8b–h**, **9a**, **9h**, **10a** and **10h** have been listed in the table I. Owing to the fact that these recovery percentages were not proportional to doses, it was not possible to calculate the ED_{50}. Also, minimal recovery dose (MRD), which was the lowest dose (mg/kg) from which the SCR recovery percentage was statistically significant, represented the reference comparison dose between compounds.

These results (table I) show that the activities of compounds **10h**, **8h**, **9h**, **8d**, **10a** and **9a** are comparable to the activity of naftidrofuryl at lower doses (≈ 2- to 25-fold) and that compounds **9a** and **9h**, at very weak doses (0.5 and 5 mg/kg respectively), bring about nearly complete recovery of the SCR which cannot be obtained with reference compounds at same doses.

Results show that the most active compounds are the mono- or dichloro derivatives, and that the thiophenic derivatives are more active than their benzologues. These results prompt us to synthesize new compounds with increased lipophilicity in each series.

Conclusion

The SCR-hypoxia test demonstrates the antihypoxic activity of a new family of aminocyclopentanones. These compounds seem more active than naftidrofuryl or piracetam. Further studies on their biological action mechanism are in progress with the aim of determining if it proceeds from a vasodilatory effect.

Table I. SCR recovery with aminocyclopentanones and reference compounds.

COMPOUNDS	DOSES mg/kg	RECOVERY %
8b	0.1	NS
	0.5	79 ± 52
	5	80 ± 40
	10	73 ±42
	20	80 ± 43
	50	NS
8c	0.5	NS
	1	40 ± 24
	4	37 ±26
	8	NS
	20	NS
8d	0.05	NS
	0.1	27 ± 18
	0.5	20 ± 17
	1	32 ± 30
	5	37 ± 25
	10	NS
8e	0.1	NS
	0.5	NS
	1	37 ± 30
	5	50 ± 27
	10	55 ± 26
	20	30 ± 29
	40	NS
8f	0.1	NS
	0.5	NS
	1	30 ± 26
	5	37 ± 29
	10	27 ± 27
	20	37 ± 30
	40	NS
8g	1	NS
	5	NS
	10	30 ± 24
	20	NS
8h	0.01	NS
	0.05	25 ± 18
	0.1	35 ± 24
	0.5	NS
	0.1	NS
9a	0.01	NS
	0.05	45 ± 30
	0.1	78 ± 46
	0.5	94 ± 37
	1	47 ± 38
	5	66 ± 49
	10	91 ± 50
	20	NS
9h	0.005	NS
	0.01	28 ± 25
	0.05	84 ± 42
	0.1	87 ± 37
	0.5	77 ± 46
	1	91 ± 42
	5	102 ± 47
	10	69 ± 26
	20	66 ± 35
	30	123 ± 48
	5	

COMPOUNDS	DOSES mg/kg	RECOVERY %
10a	0.05	NS
	0.1	58 ± 50
	5	76 ± 45
	10	89 ± 45
	20	38 ± 27
	50	NS
10h	0.005	NS
	0.01	42 ± 35
	0.05	25 ± 17
	0.1	32 ± 25
	0.5	52 ± 22
	1	52 ± 29
	5	50 ± 24
	10	62 ± 28
	20	52 ± 28
	50	NS
Naftidrofuryl	0.1	NS
	0.25	33 ± 33
	0.5	35 ± 34
	2.5	61 ± 38
	5	73 ± 32
	7.7	66 ± 26
	18.8	80 ± 43
	30	61 ± 31
	46	50 ± 28
	58	NS
Nicergoline	2.5	NS
	5	NS
	7.5	69 ± 54
	10	89 ± 44
	20	73 ± 57
	40	NS
Piracetam	0.15	NS
	0.31	NS
	1.25	47 ± 32
	2.5	56 ± 37
	5	58 ± 35
	10	52 ± 32
Vincamine	2	NS
	4	NS
	8	49 ± 46
	32	38 ± 29
	64	NS
Piribedil	1	NS
	2	38 ± 21
	4	62 ± 32
	8	70 ± 34

NS: non significant.

Experimental protocols

Chemistry

Melting points were determined on a Kofler block and are uncorrected. Elemental analysis agreed with theoretical values to within ± 0.4%. IR spectra were recorded with a Philips PU-9716 spectrophotometer. ^{1}H-NMR spectra were recorded for solutions in $(CD_3)_2SO$ at 200 MHz with tetramethylsilane as an internal standard using a Jeol JMN-FX 200 spectrometer.

Table II. Melting point, yield, spectral and analytical data.

*No**	*mp (°C)*	*Yield (%)*	*IR (cm^{-1}) (KBr)*	*^{1}H-NMR (ppm/TMS) (DMSO–d_6)*
8b	>260	87	1685 (CO) 3100–2600 (NH_3)	8.77 (NH_3); 7.63 (H_4); 4.86 (H_3); 3.94 (CH_3); 3.91 (CH_3); 3.78 (CH_3); 3.08 (H_2); 26.60 (H_2)
8c	>260	63	1710 (CO) 3100–2600 (NH_3)	8.58 (NH_3); 7.5 (arom); 5.08 (H_3); 3.15 (H_2); 2.70 (H_2); 2.54 (CH_3)
8d	>260	67	1690 (CO) 3100–2600 (NH_3)	9.62 (NH_3); 7.86 (H_4); 7.58 (H_7); 7.34 (H_6); 4.89 (H_3); 3.13 (H_2); 2.60 (H_2); 2.57 (CH_3)
8e	>260	70	1700 (CO) 3100–2600 (NH_3)	8.53 (NH_3); 7.45 (H_5); 7.30 (H_6); 4.98 (H_3); 3.12 (H_2); 2.67 (H_2); 2.35 (CH_3); 2.47 (CH_3)
8f	>260	66	1720 (CO) 3100–2600 (NH_3)	8.77 (NH_3); 7.98 (H_4); 7.70 (H_5); 7.56 (H_7); 4.91 (H_3); 3.06 (H_2); 2.73 (CH_2); 2.54 (H_2); 1.20 (CH_3)
8g	>260	63	1720 (CO) 3100–2600 (NH_3)	8.86 (NH_3); 8.11 (H_4); 7.50 (H_5, H_7); 4.91 (H_3); 3.17 (H_2); 2.68 (H_2)
8h	>260	90	1695 (CO) 2960–2680 (NH_3)	9.03 (NH_3); 8.38 (H_2); 7.63 (H_3); 4.90 (H_4); 3.43 (H_5); 2.97 (H_5)
9a	>260	63	1730 (CO) 3200–2600 (NH_3)	9.20 (NH_3); 7.90 (arom); 5.05 (H_3 and H_2)
9h	>260	87	1715 (CO) 3200–2640 (NH_3)	9.30 (NH_3); 8.56 (H_2); 7.61 (H_3); 5.23 (H_5); 5.00 (H_4)
10a	>260	91	1730 (CO) 3200–2640 (NH_3)	9.45 (NH_3); 7.9 (arom); 5.09 (H_3)
10h	>260	83	1720 (CO) 3200–2560 (NH_3)	9.59 (NH_3); 8.69 (H_2); 7.68 (H_3); 5.58 (H_4)

*Analysis: C, H, N.

*Procedure for 3-amino-3-arylpropionic acids **2a–h** [5, 6]*
A solution of 1 mol arylaldehyde **3a–h** in 300 ml ethanol was added with 2 mol ammonium acetate and 1 mol malonic acid. The reaction mixture was refluxed for 6 h. The precipitate formed was filtered and washed with 250 ml boiling ethanol. Yield was ≈ 65%.

*Procedure for 3-trifluoroacetylamino-3-phenylpropionic acids **4a, 4c–4g** [6]*
A solution of 0.1 mol amino acid **2a, 2c–2g** in 50 ml trifluoroacetic anhydride was stirred at room temperature for 30 min. It was then evaporated to dryness and the oily residue crystallized. The solid was recrystallized from ether. Yield was ≈ 85%.

*Procedure for 3-trifluoroacetylamino-3-phenylpropionic acid chlorides **5a, 5c–5g** [6]*
A solution of 0.1 mol trifluoroacetylaminoacid **4a, 4c–4g** in 50 ml thionyl chloride was refluxed for 30 min and evaporated to dryness. The residual solid was then washed with petroleum ether, filtered and dried. Yield was ≈ 95%.

*Procedure for 3-trifluoroacetylaminoindan-1-ones **1a, 1c–1g** [6]*
A solution of 0.05 mol acid chloride **5a, 5c–5g** in 100 ml methylene chloride was added with 10 g aluminium chloride. The reaction mixture was refluxed for 20 min and evaporated to dryness. Residue was triturated with 100 ml H_2O and the insoluble part was filtered, dried and recrystallized from ether. Yield was ≈ 65%.

*Procedure for 5,6,7-trimethoxy-3-trifluoroacetylaminoindan-1-one **1b** [7, 8] and 4-trifluoroacetylamino-4,5-dihydrocyclopenta [b] thiophen-6-one **1h** [5]*
A solution of 0.1 mol amino acid **2b** or **2h** in 40 ml trifluoroacetic acid was stirred for 20 min at room temperature and then 40 ml of trifluoroacetic anhydride added. The reaction mixture was refluxed for 3 h and evaporated to dryness. The oily residue was triturated in 100 ml H_2O and the crystals formed were filtered, washed with water, dried and recrystallized from ether. Yield was ≈ 82% for **1b** and 66% for **1h**.

Procedure for cis 2-chloro-3-trifluoroacetylaminoindan-1-one ***6a*** *[10] and cis 5-chloro-4-trifluoroacetylamino-4,5-dihydrocyclopenta [b] thiophen-6-one* ***6h*** *[9]*
A solution of 0.05 mol trifluoroacetylaminocyclopentanone **1a** or **1h** in 50 ml chloroform was bubbled for 45 s at room temperature with chlorine. The precipitate formed was filtered, dried and recrystallized from ether. Yield was ≈ 50% for **6a** and 44% for **6h**.

Procedure for 2,2-dichloro-3-trifluoroacetylaminoindan-1-one ***7a*** *[10] and 5,5-dichloro-4-trifluoracetylamino-4,5-dihydrocyclopenta [b] thiophen-6-one* ***7h*** *[9]*
A solution of 0.05 mol trifluoroacetylaminocyclopentanone **1a** or **1h** in 50 ml chloroform was bubbled for 45 s at room temperature with chlorine. The reaction mixture was then refluxed for 20 min and evaporated to dryness. The solid residue was recrystallized from ether. Yield was ≈ 81% for **7a** and 82% for **7h**.

Procedure for 1-oxoindanyl-3-ammonium chlorides ***8a–g*** *[6],* ***9a*** *[10] and* ***10a*** *[10] and 6-oxocyclopenta [b] thienyl-4-ammonium chlorides* ***8h*** *[5],* ***9h*** *[11] and* ***10h*** *[11]*
0.05 mol trifluoroacetylaminocyclopentanone **1b–h**, **6a**, **6h**, **7a** or **7b** was dissolved in 50 ml of 6 N aqueous solution of hydrochloric acid. The reaction mixture was refluxed for 30 min, then cooled and filtered. The solution was evaporated to dryness and residual crystals were recrystallized from isopropanol (table II).

Pharmacology

Animals
Male and female IOPS OF1 mice weighing 18–24 g were randomized into groups of 20 animals.

Drugs
All drugs were administered ip. They were dissolved in a distilled water solution of 0.5% carboxymethylcellulose, and the concentration level adjusted in order to administer 0.4 ml per 20 g body weight.

Apparatus
Experiments were carried out in normobaric hypoxia with an O_2 rate of 7.5%. The unit allowed work in a constant atmosphere at normal pressure and was made of polyvinyl chloride which was flexible and transparent with: 4 cuffs with gloves to work inside the unit; gaseous nitrogen flow; an instrument for measuring oxygen rate; a palmar skin conductance meter and a 100 W glow lamp providing photostimulus.

The PSC meter consisted of resistances, a DC generator (a 4.5 V dry battery) and a galvanometer connected to 2 metallic electrodes. Their free ends were 2 mm apart, so that the circuit was open. A black cloth cover placed over the cell turned it into a darkroom suitable for eliciting the SCR in response to photostimulus.

Experimental procedure
Subjects in groups of 10 were treated in the darkroom outside the cell 20 min before the reading was taken. Then they were brought into the hypoxic atmosphere of the unit 10 min after treatment, *ie* 10 min before the reading was taken.

Readings
The mouse was taken by its skin in the nuchal region and positioned in front of the electrodes which were immediately placed. In this manner, the circuit was closed. The intensity of the current was proportional to the conductivity of the palmar skin of the animal. A first PSC reading (r_0) for the animal was carried out in the dark. Then the photostimulus was applied for 7–10 s. A new reading (r_1), corresponding to the variance in skin conductance level was then required. The difference between the 2 readings (r_1–r_0) represented the SCR.

Statistical analysis
The calculation r_1–r_0 represented the SCR after treatment. The SCR (r_1–r_0) was calculated for each animal (control or treated). On the basis of these results, SCR recovery percentage was calculated for individual animals.

$$\text{SCR recovery \%} = \frac{(r_1\text{–}r_0)\ \text{treated} - (r_1\text{–}r_0)\ \text{control}}{(r_1\text{–}r_0)\ \text{control}}$$

Then for each group SCR recovery percentage average, with SE at $P < 0.05$ was also calculated.

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