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Synthesis and pharmacological investigation of 9-methyl-1,2,3,4,6,7,12,12b-octahydro-7-oxo-indolo[2,3-*a*]quinolizine

Teresa Grandi [a], Fabio Sparatore [a,*], Anna Sparatore [b]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV 3, 16132 Genoa, Italy*
[b] *Istituto di Chimica Farmaceutica e Tossicologica, Università di Milano, Viale Abruzzi 42, 20131 Milan, Italy*

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Abstract

The title ketone, a supposed metabolite of 9-methyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine (MIQ), was prepared and found different on chromatographic comparison from the isolated metabolite **M_4**. However, the pharmacological screening of this compound evidenced, among others, some interesting properties as inhibition of arachidonic acid induced platelet aggregation and protection from both hypobaric and cyanide induced hypoxia in mice. These activities suggest a possible cerebroprotective action to be further investigated. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: 7-Oxo-indolo[2,3-*a*]quinolizidine; Anti-aggregating agents; Cerebroprotective agents

1. Introduction

The Fischer indole cyclization applied to several para-substituted phenylhydrazones of quinolizidin-1-one afforded 9-substituted-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizines, whose pharmacological properties were reported some years ago [1]. The 9-methyl derivative (MIQ) at a dose of 5 mg/kg exhibited a strong inhibition of carrageenan-induced edema in rat hind paw and anti-hypertensive activity in rats become hypertensive after DOCA s.c. implantation, as well as a peculiar biphasic (stimulating and then depressive) activity on spontaneous motility in rats.

In an attempt to explain this behaviour, the metabolic fate of this compound was investigated [2] and four metabolites (**M_1**–**M_4**) were isolated. The structures for three of them were characterized by several mass spectrometry techniques and confirmed by synthesis, while for the fourth (**M_4**) the structure of 9-methyl-1,2,3,4,6,7,12,12b-octahydro-7-oxo-indolo[2,3-*a*]quinolizine (**1**) was tentatively suggested on the basis of the available data (Scheme 1).

Therefore, compound **1** has been synthesized through an unequivocal procedure and its chromatographic behaviour was compared with that of metabolite **M_4**, while its pharmacological profile was investigated thoroughly (Scheme 2).

2. Chemistry

For the synthesis of **1**, the sequence followed by Rosenmund et al. [3–5] to prepare the corresponding demethylated compound was applied firstly with only minor modifications.

Thus, the 5-methyl-2-nitrophenylacetic acid [6] was converted to the chloride and reacted with *N*-cyclopentenylmorpholine to obtain 2-[(5-methyl-2-nitrophenyl)-acetyl]cyclopentanone, whose reduction gave the 2-(5-methylindol-2-yl)cyclopentanone. Schmidt rearrangement converted the cycloketone into a piperidone derivative whose reduction gave the 5-methyl-2-(2-piperidinyl)-1*H*-indole. Alkylation with ethyl bromoacetate in the presence of Hünig base, followed by alkaline hydrolysis furnished the 2-(5-methyl)-2(1*H*)-indolyl-1-piperidineacetic acid, which was finally cyclized with PPA to the required **1**.

* Corresponding author. Tel.: +39-010-512 721; fax: +39-010-353 8358.
E-mail address: sparator@unige.it (F. Sparatore)

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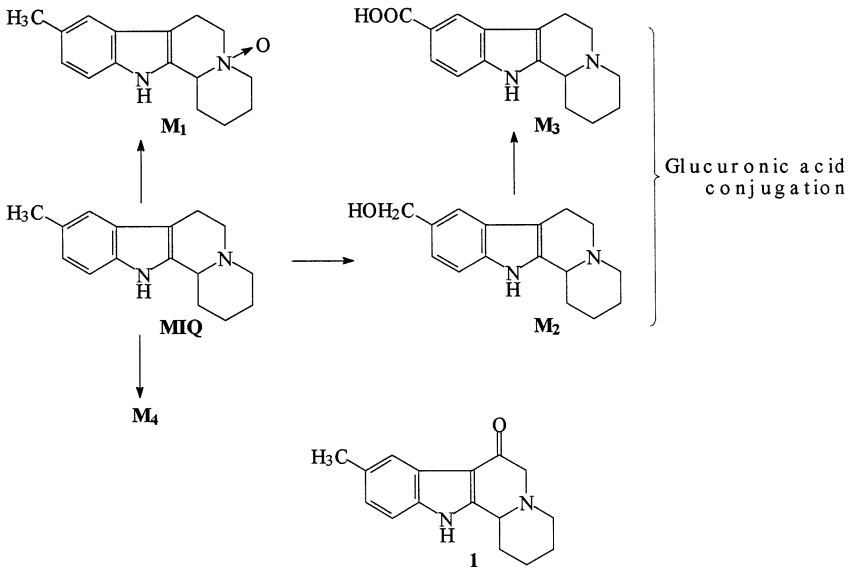
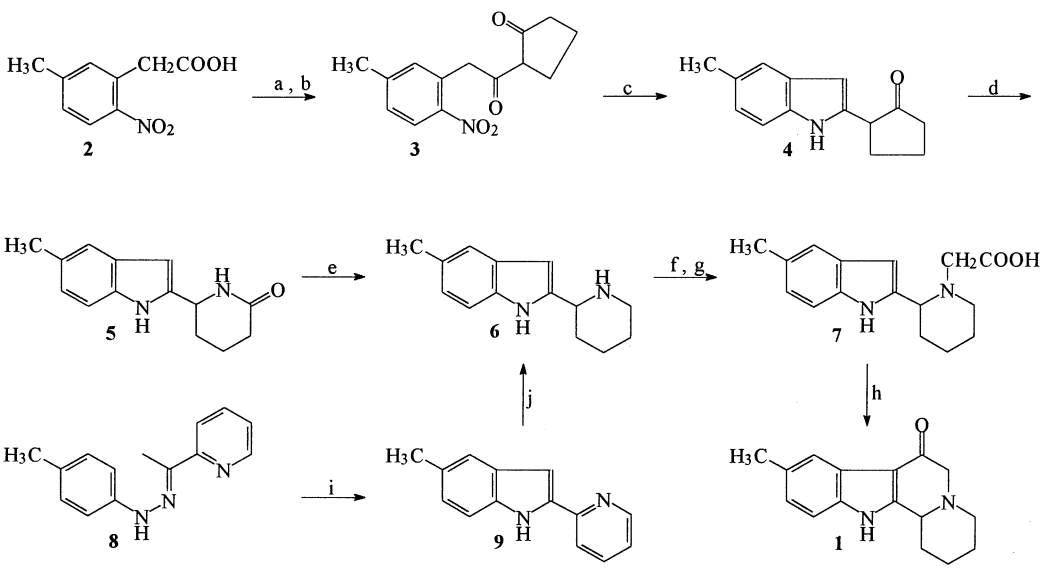


Scheme 1.



Scheme 2. Reagents: (a) $SOCl_2/CHCl_3$; (b) *N*-(1-cyclopentenyl)morpholine/TEA; (c) $SnCl_2$/ether; (d) NaN_3, H_2SO_4, $CHCl_3/C_6H_6$; (e) $LiAlH_4$/THF; (f) $BrCH_2COOC_2H_5$, *N*-ethyldicyclohexylamine/CH_3OH; (g) KOH, CH_3OH; (h) PPA, N_2, 75–100°C; (i) PPA, N_2, 180°C; (j) H_2/PtO_2, HCl, CH_3OH.

Since the overall yield of this synthetic sequence was only 3.5%, an alternative route was sought. Actually, the 5-methyl-2-(2-piperidinyl)-1*H*-indole was obtained more easily by catalytic hydrogenation [7] of 5-methyl-2-(2-pyridyl)indole that was produced through the Fischer cyclization of 2-acetylpyridine *p*-tolylhydrazone in analogy with known methods [8,9]. Starting from the last compound the overall yield of **1** was doubled.

3. Experimental

3.1. Chemistry

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected.

The elemental analyses were performed at the Microanalytical Laboratory of the 'Dipartimento di Scienze Farmaceutiche', University of Genoa, and the analyti-

cal results for the indicated elements were within ± 0.3% of the calculated values.

UV spectra were recorded on a Perkin–Elmer 550 S spectrophotometer; IR spectra were recorded on a Perkin–Elmer spectrometer Paragon FT-100. ^{1}H NMR spectra were taken on Varian Gemini 200 or Bruker 300 spectrometers, using $CDCl_3$ or DMSO-d_6 as solvents, with TMS as internal standard.

3.1.1. 2-[(5-Methyl-2-nitrophenyl)acetyl]cyclopentanone (3)

A mixture of 5-methyl-2-nitrophenylacetic acid [6] (8.00 g, 0.041 mol), thionyl chloride (5.94 g, 0.050 mol), DMF (1 ml) and anhydrous chloroform (50 ml) was heated for 4 h at 40°C under magnetic stirring. The solvent was removed, the residue was dissolved in dry chloroform and it was slowly added to a solution of *N*-(1-cyclopentenyl)morpholine (6.13 g, 0.040 mol) and triethylamine (6.07 g, 0.060 mol) in anhydrous chloroform (50 ml). The mixture was stirred for 2 h at room temperature (r.t.), then the solvent was removed at reduced pressure. The resulting oil was stirred overnight with 37% HCl (15 ml) and water (15 ml). Finally, water was added (50 ml) and the solution was extracted with chloroform. After removal of the solvent the viscous oil was chromatographed on a silica gel column ($CHCl_3$ as eluent). The first fractions were pooled and evaporated to afford **3** as a yellow oil which gave a positive colour test with $FeCl_3$ and was used without further characterization (6.30 g, 59%).

3.1.2. 2-(5-Methyl-1H-indol-2-yl)cyclopentanone (4)

Dry hydrogen chloride was bubbled for 30 min into a solution of **3** (3.40 g, 0.013 mol) and anhydrous stannous chloride (7.40 g, 0.039 mol) in dry ether (100 ml). The mixture was stirred at r.t. for 1 h, then the solvent was removed under reduced pressure to give a yellow residue which, in turn, was treated at 0°C with 15% NaOH solution (70 ml). After 15 min the solution was extracted with $CHCl_3$, the extracts were dried (Na_2SO_4), the solvent was removed and the residue was purified by chromatography on a silica gel column (eluent $CHCl_3$). The first fractions gave a solid which was crystallized from ethanol to give **4**, m.p. 147–148°C (1.20 g, 43%). IR (KBr, cm^{-1}): 3330 (NH), 1726 (CO). ^{1}H NMR ($CDCl_3$): 9.93 (br s, 1H, H(1′)); 7.34 (d, 1H, $J = 2.0$ Hz, H(4′)); 7.25 (d, 1H, $J = 8.5$ Hz, H(7′)); 6.97 (dd, 1H, $J_1 = 8.5$ Hz, $J_2 = 2.0$ Hz, H(6′)); 6.22 (m, 1H, H(3′)); 3.48 (m, 1H, H(2)); 2.70–1.92 (m, 9H, H(CH_3) + aliphatic protons). *Anal.* ($C_{14}H_{15}NO$) C, H, N.

3.1.3. 6-(5-Methyl-1H-indol-2-yl)piperidin-2-one (5)

A mixture of **4** (1.2 g, 0.0056 mol), sodium azide (0.75 g, 0.011 mol) and conc. H_2SO_4 (10 ml) in chloroform (60 ml) and benzene (60 ml) was stirred at 0°C for 2 h. The solution was then poured into ice/water, extracted with chloroform and the solvent was removed under reduced pressure. The resulting solid was crystallized from methanol to yield **5**, m.p. 235°C (dec.) (0.84 g, 66%). IR (KBr, cm^{-1}): 3264 (NH indole), 3180 (NH piperidinone), 1667 (CO). ^{1}H NMR (DMSO-d_6): 10.95 (br s, 1H, H(1′)); 7.84 (br s, 1H, H(1)); 7.30–7.19 (m, 2H, H(4′) + H(7′)); 6.88 (dd, 1H, $J_1 = 7.9$ Hz, $J_2 = 2.0$ Hz, H(6′)); 6.17 (s, 1H, H(3′)); 4.68 (br.t 1H, *J* about 6 Hz, H(6)); 2.35 (s, 3H, CH_3); 2.23 (t, 2H, $J = 6.2$ Hz, H(3)); 2.15–1.62 (m, 4H, H(4) + H(5)). *Anal.* ($C_{14}H_{16}N_2O$) C, H, N.

3.1.4. 5-Methyl-2-(2-piperidinyl)-1H-indole (6)

A suspension of **5** (0.84 g, 0.0037 mol) in anhydrous THF (20 ml) was refluxed with $LiAlH_4$ (1.68 g, 0.044 mol) for 2 h. Water was added slowly (3 ml) and the product was extracted with ether. The organic phases were evaporated and the white solid was crystallized from cyclohexane, m.p. 148–150°C (0.56 g, 71%). IR (KBr, cm^{-1}): 3283 (NH indole), 3143 (NH piperidine). ^{1}H NMR (DMSO-d_6): 0.79 (br s, 1H, H(1)); 7.27–7.14 (m, 2H, H(4) + H(7)); 6.84 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 =$ 2.0 Hz, H(6)); 6.13 (s, 1H, H(3)); 3.75 (dd, 1H, $J_1 =$ 10.0 Hz, $J_2 = 3.0$ Hz, H(2′)); 3.03 (br d, 1H, $J = 12.8$ Hz); 2.79–2.60 (m, 2H); 2.38 (s, 3H, CH_3); 2.00–1.87 (m, 1H + NH); 1.79–1.32 (m, 4H). *Anal.* ($C_{14}H_{18}N_2$) C, H, N.

3.1.5. 2-(5-Methyl-2(1H)-indolyl)-1-piperidineacetic acid (7)

A solution of **6** (1.00 g, 0.0047 mol), ethyl bromoacetate (7.0 ml), *N*-ethyldicyclohexylamine (7.0 ml) in methanol (40 ml) was stirred at r.t. for 48 h. The solution was evaporated to dryness and the residue treated with benzene/pentane (1:1) to allow the precipitation of the *N*-ethyldicyclohexylamine hydrobromide. After filtration, the organic phase was extracted with 1 N HCl and the acid extracts made alkaline with NH_4OH 2 N up to pH 10. Finally, the aqueous phase was extracted with benzene, the organic phase was dried (Na_2SO_4) and evaporated to give an oil which was dissolved in 7 ml of 20% KOH methanolic solution. The mixture was stirred at r.t. for 20 h and added with water (20 ml). The pH value was adjusted to 5.5, the white solid which precipitated was filtered, washed with water and finally crystallized from methanol to yield **7**, m.p. 217–219°C (dec.) (0.67 g, 52%). IR (KBr, cm^{-1}): 3600–2600 (very broad, NH indole + =NH$^+$), 1616 and 1368 (COO$^-$). ^{1}H NMR (DMSO-d_6, 300 MHz): 10.87 (br s, 1H, H(1′)); 7.23–7.18 (m, 2H, H(4′) + H(7′)); 6.84 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz, H(6′)); 6.18 (d, 1H, $J = 2.0$ Hz, H(3′)); 3.78 (dd, 1H, $J_1 = 11.0$ Hz, $J_2 = 3.0$ Hz, H(2)); 3.11–3.04 (m, 1H); 3.10 (d, 1H, $J = 16.0$ Hz, H(α)); 2.80 (d, 1H, $J = 16.0$ Hz, H(α)); 2.65–2.54 (m, 1H); 2.33 (s, 3H, CH_3); 1.86–1.30 (m,

water protons allows no ^{1}H NMR assignment for the NH^+. *Anal.* ($C_{16}H_{20}N_2O_2$) C, H, N.

3.1.6. 9-Methyl-1,2,3,4,6,7,12,12b-octahydro-7-oxoindolo[2,3-a]quinolizine (1)

A mixture of **7** (0.3 g, 0.0011 mol) and polyphosphoric acid (25.57 g) was heated under nitrogen atmosphere for 0.5 h at 75°C, for 0.5 h at 85°C and for 3 h at 90–100°C. The reaction mixture was poured into ice/water, alkalinized to pH 10 with concentrated ammonia and extracted with chloroform. The organic phase was dried (Na_2SO_4) and the solvent removed to afford a solid which was purified by a chromatography on a silica gel column (ethyl acetate as eluent). After a fore-run which was discarded, the fractions were collected and evaporated to give **1** as a white solid which was crystallized from methanol, m.p. 240°C (dec.) (0.16 g, 57%). UV (CH_3OH) (λ_{max}, nm ($\log \varepsilon$)): 295 (4.00), 265 (4.06), 245 (4.25). IR (KBr, cm^{-1}): 3191 (NH), 1620 (CO). ^{1}H NMR (DMSO-d_6, 300 MHz): 11.80 (br s, 1H, H(12)); 7.84 (s, 1H, H(8)); 7.31 (d, 1H, $J = 8.4$ Hz, H(11)); 7.03 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 2.0$ Hz, H(10)); 3.55–3.48 (m, 1H); 3.31 (d, 1H, $J = 16.5$ Hz, H(6)); 3.10 (dd, 1H, $J_1 = 16.5$ Hz, $J_2 = 2.5$ Hz, H(6)); 2.98–2.89 (m, 1H); 2.50–2.36 (m, 2H); 2.42 (s, 3H, CH_3); 1.95–1.53 (m, 5H). *Anal.* ($C_{16}H_{18}N_2O$) C, H, N.

Compound **1** was converted into the hydrochloride by means of the stoichiometric quantity of 1 N ethanolic HCl; the solution was evaporated to dryness and the residue washed several times with dry ether. M.p. > 260°C (dec.).

3.1.7. 2-Acetylpyridine-p-tolylhydrazone (8)

A mixture of 2-acetylpyridine (4.83 g, 0.040 mol) and *p*-tolylhydrazine (4.88 g, 0.040 mol) in ethanol (3 ml) was heated for 1 h at 90–100°C under magnetic stirring. On cooling, a pale yellow crystalline solid precipitated and was crystallized from *n*-hexane, to give yellow needles, m.p. 95–97°C (8.70 g, 97%). IR (KBr, cm^{-1}): 3337 (NH). ^{1}H NMR ($CDCl_3$): 8.55 (m, 1H, H(6)); 8.19 (m, 1H, H(3)); 7.67 (td, 1H, $J_1 = 7.50$ Hz, $J_2 = 1.60$ Hz, H(4)); 7.48 (br s, 1H, NH); 7.21–7.08 (m, 5H, H(5) + phenylene protons); 2.46 (s, 3H, acetyl CH_3); 2.38 (s, 3H, CH_3 on the phenyl ring). *Anal.* ($C_{14}H_{15}N_3$) C, H, N.

3.1.8. 5-Methyl-2-(2-pyridyl)indole (9)

A mixture of **8** (4.50 g, 0.020 mol) and polyphosphoric acid (13.5 g) was heated slowly from 25 to 180°C under nitrogen. After 5 min at 180°C, the mixture was cooled to r.t., added to water (20 ml), 6 N NaOH (20 ml) and extracted with ether. The ethereal extracts were dried (Na_2SO_4), evaporated at reduced pressure and the residue was submitted to column chromatography on alumina (ratio 1:20) using ether as eluent to give **9**, which was crystallized from methanol, m.p. 125–126°C (1.80 g, 43%). IR (KBr, cm^{-1}): 3350 (NH). ^{1}H NMR ($CDCl_3$): 9.61 (br s, 1H, H(1)); 8.57 (m, 1H, H(6′)); 7.81 (m, 1H, H(3′)); 7.72 (td, 1H, $J_1 = 7.5$ Hz, $J_2 = 1.6$ Hz, H(4′)); 7.44 (s, 1H, H(4)); 7.30 (d, 1H, $J = 8.0$ Hz, H(7)); 7.16 (m, 1H, H(5′)), 7.05 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz, H(6)); 6.95 (s, 1H, H(3)); 2.46 (s, 3H, CH_3). *Anal.* ($C_{14}H_{12}N_2$) C, H, N.

Compound **9** was converted into the hydrochloride as described for **1**; m.p. 209–212°C (dec.).

3.1.9. 5-Methyl-2-(2-piperidinyl)-1H-indole (6)

A solution of **9** as hydrochloride (1.80 g, 0.0073 mol) in methanol (60 ml) was reduced in a Parr apparatus using PtO_2 (0.21 g) as catalyst. After removal of the catalyst, the solvent was evaporated under reduced pressure, the residue dissolved in dichloromethane and was shaken with a 10% solution of Na_2CO_3. Evaporation of the organic phase yielded **6**, which was crystallized from cyclohexane. M.p. 148–150°C (0.88 g, 58%).

3.2. Chromatography

For mono- and bidimensional thin layer chromatography, precoated silica gel 60 F254 TLC plates (5 × 20 and 20 × 20 cm, thickness 0.25 mm), from Merck (Bracco, Milan, Italy) were used. Compound **1** was laid down on plates as methanolic solution. Mixtures of *iso*-propanol, ethyl acetate, benzene, 25% ammonia (30:30:30:9) or toluene, ethyl formate, formic acid (50:40:10) were used as eluents. Spots were visualized under UV light (254 nm) and by spraying the plates with Dragendorff's reagent.

3.3. Pharmacology

The pharmacological profile of compound **1** was explored through a preliminary broad screening performed by Panlabs Inc. (Bothell, WA, USA). This assay package, named PharmaScreen®, is used in the determination of the maximum tolerated dose (MTD, p.o. and i.p.), with simultaneous behavioural examination (Irvin test), and in 34 primary in vivo tests (using a suitable MTD fraction, depending on the test type) and in 26 in vitro tests concerning CNS, cardiovascular and gastrointestinal apparatuses, intermediary metabolism, allergy and inflammation.

Compound **1** was used as hydrochloride. For in vivo tests **1** was generally administered by oral route using a gastric tube, as aqueous solution or finely homogenized suspension in Tween 80 (2%) when the highest dose (300 mg/kg) was used. In a few cases it was introduced intraperitoneally as aqueous solution (10 ml/kg). Groups of three or five animals (rats or mice) were used. For in vitro assays, the dissolution of test compound in buffer or saline solution was speeded up by 4H). A broad water signal between 3.65 and 3.40 hides

Table 1
Some in vivo activities of compound **1** and reference drugs

Test [a]		Dose (mg/kg) route	Effect	Response	Ref. Drug	Dose (mg/kg) route	Response
Phenylquinone writhing (mice) [11]		100 p.o.	[b]	18	ibuprofen	25 p.o.	65
Tetrabenazine hypothermia (mice)		30 i.p.	[c]	15	imipramine	3 p.o.	50
					tranylcypromine	3 p.o.	50
Hypobaric hypoxia (mice)		100 i.p.	[d]	128; 113	diazepam	30 i.p.	120
		30		0			
Hypoxia cyanide induced (mice) [13]		100 p.o.	[e]	60	flunarizine	30 p.o.	60
		30		20			
Blood pressure (SH rats) [10,11]	(1 h)	100 p.o.	[f]	−6	nifedipine	5 p.o.	−18
	(4 h)			−8			−14
Heart rate (SH rats) [10,11]	(1 h)	100 p.o.	[f]	−10	nifedipine	5 p.o.	–7
	(4 h)			−8			−10
Bleeding time (mice) [13]		100 p.o.	[g]	88; 85	aspirin	30 p.o.	55
		30		42			
Anti-ulcer ethanol induced (rats)		100 p.o.	[h]	50; 50	carbenoxolone	300 p.o.	50
		30		38			
Anti-ulcer stress induced (rats) [10]		30 p.o.	[h]	25	chlorpromazine	20 p.o.	71
Urine volume change (rats) [10,11]		30 p.o.	[i]	+80	hydroflumethiazide	3 p.o.	+70
		10		+20			
Saluresis (rats) [10,11]		30 p.o.	[j]	+200	hydroflumethiazide	3 p.o.	+200
		10		+10			
Kaluresis (rats) [10,11]		30 p.o.	[j]	+40	hydroflumethiazide	10 p.o.	+200

[a] References for the methods employed (when no reference is cited, the method is described in the present paper).
[b] % Inhibition of number of writhes.
[c] % Reduction of tetrabenazine-induced hypothermic response.
[d] % Prolongation of survival time of mice placed in hypobaric (200 mmHg) chamber.
[e] % Surviving mice 60 min after i.v. administration of submaximal lethal dose of KCN.
[f] % Variation of blood pressure and heart rate in spontaneously hypertensive rats; variation higher than 10 and 20%, respectively, are considered highly significant.
[g] % Prolongation of bleeding time in mice after transection of the tip (0.5 mm) of each tail.
[h] % Reduction of scores attributed to ulcerative lesions, 1 h after the administration of 1 ml of absolute ethanol or after 4 h of forced partial immersion in water.
[i] % Variation of urine volume in test versus control animals 6 h from administration.
[j] % Variation of Na^+ or K^+ excretion expressed as μeq/100 g body weight 6 h from administration.

the H(5′) signals; moreover, the rapid exchange with means of DMSO; the final concentration of DMSO, not interfering with tests, was 0.1% for platelet aggregation and 0.5% for all others.

The procedures for most of these assays were already described [10–16]; the pertinent reference is indicated near each assay name in the Tables 1 and 2, which are relative only to the more significant ones. Procedures not previously described are given in the following section. A pre-established level of response which is high enough to suggest a significant activity is indicated for each assay. Doses (mg/kg) or concentrations (μg/ml or μM) indicated in the methods were the highest utilized routinely, depending on toxicity; when significant activity was detected, lower doses or concentrations were tested in order to define the minimal effective ones, and secondary tests were performed to provide some insight for the possible mechanism of action.

3.3.1. Tetrabenazine hypothermia [17]

Test sample is administered p.o. (30 mg/kg) to a group of three mice 30 min before injection of tetrabenazine methan sulfonate (50 mg/kg, i.p.) and body temperature is recorded 60 min later. Reduction of tetrabenazine-induced hypothermic response by more than 50% is considered significant and may indicate anti-depressant activity.

3.3.2. Hypobaric hypoxia [18]

The test substance is administered i.p. (100 mg/kg) to a group of three mice 30 min before being placed in a chamber at a hypobaric pressure of 200 mmHg. Prolongation of survival time relative to a vehicle-treated control group of animals by more than 100% in the absence of CNS depressant effect may indicate cerebroprotective activity.

Table 2
Some in vitro activities of compound **1** and reference drugs

Test [a]	Conc. (μM)	Effect	Response	Ref. drug	Conc. (μM)	Response
Adrenergic α_1 antagonism [15] (rat vas deferens)	30	[b]	15	prazosin	0.08	70
Cardiac inotropy (guinea pig left atria) [11]	30	[c]	−20			
Cardiac chronotropy (guinea pig right atria) [11]	30	[c]	−5	clonidine	3	−18
Rat portal vein spontaneously activated [11]	30	[d]	20	cromakalim	0.3	81
Tracheal contraction (guinea pig) [11]	30	[e]	27	epinephrine	0.3	79
Inhibition of platelet aggregation induced by:						
Arachidonic acid [11]	30	[f]	100	aspirin	14	100
	10		0	indomethacin	0.3	100
ADP [11]	30	[f]	0	2-chloroadenosine	3	74
PAF [11]	30	[f]	4	apafant	0.3	68
Tromboxane A_2 [12]	30	[f]	8	sulotroban	10	60
Bradykinin B_2 antagonism [11]	30	[g]	12	nifedipine	0.03	60
Cholecystokynin CCK_A antagonism [11]	30	[g]	18	devazepide	0.03	70
Leukotriene LTD_4 antagonism [12]	30	[g]	25	LY-171883	1	74

[a] References for the methods employed.
[b] % Reduction of the phenylephrine-induced contractile response.
[c] % Variation in contractile force or rate, variations higher than 40 and 10%, respectively, are considered highly significant.
[d] % Inhibition of spontaneous movements.
[e] % Inhibition of tracheal tone relative to relaxation induced by 0.3 μg/ml epinephrine.
[f] % Inhibition of maximum non reversible rabbit platelet aggregation induced by sodium arachidonate (50 μg/ml), ADP (0.8 μg/ml), PAF (10–20 ng/ml) and U-46619 (5 μg/ml).
[g] % Reduction of guinea pig ileum contractile response to the various agents.

3.3.3. Anti-ulcer (ethanol-induced) activity [19]

The test substance is administered p.o. (100 mg/kg) to overnight-fasted rats 15 min before gavage with 1 ml of absolute ethanol. The animals are sacrificed 1 h later and gastric ulceration is scored subjectively concerning degree of haemorrhage and severity of ulcerative lesions. Inhibition of gastric ulcers by more than 50% suggests cytoprotective activity.

4. Results and discussion

The chromatographic behaviour of **1** on two different TLC systems (TCL_1, $R_f = 0.74$; TLC_2, $R_f = 0.06$) indicated that it is different from the MIQ metabolite **M_4** previously isolated [2] (TCL_1, $R_f = 0.04$; TLC_2, $R_f = 0.25$), whose structure, therefore, remains undefined.

In the general pharmacological screening, compound **1** resulted well tolerated up to the dose of 100 mg/kg i.p. or 300 mg/kg p.o. in mice, without any sign of central or autonomic effect during the 72 h of observation after administration.

Compound **1** actually exhibited an activity profile quite different from that of MIQ. On the one hand, the strong anti-inflammatory and anti-hypertensive activities observed in rats with MIQ at 5 mg/kg were, respectively, completely abolished or strongly reduced even when **1** was administered in rats at doses up to 100 mg/kg p.o. Moreover, the biphasic effect of MIQ on spontaneous motility in rats was not shown by **1** neither in rats (at 30 mg/kg) nor in mice at 300 mg/kg p.o. or 100 mg/kg i.p.

On the other hand, compound **1** exhibited the following significant activities: saluretic and moderate kaluretic activities associated with increased urine output; protection from ethanol-induced gastric ulcers in rats, unrelated to in vivo anti-cholinergic activity; inhibition of arachidonic acid-induced platelet aggregation in vitro, with strong prolongation of bleeding time in mice (although similar concentration failed to inhibit ADP and tromboxane A_2-induced aggregation).

Still more interesting was the capacity of compound **1** at 100 mg/kg i.p. or p.o. to prolong the survival time of mice placed in a chamber at a hypobaric pressure of 200 torr and to reduce the mortality of mice receiving a sub-maximal lethal dose ($LD_{95} = 2.4$ mg/kg i.v.) of potassium cyanide. No toxic or other untoward central or autonomic signs were seen at similar or higher dose levels; thus, both assays suggest a possible cerebroprotective activity for **1** deserving further investigations.

Although at only moderate level some other activities were seen both in vivo and in vitro: inhibition of phenylquinone writings, antagonism to tetrabenazine-induced hypothermia (indicating possible anti-depressive activity), inhibitions of stress-induced gastric ulcers; adrenergic α_1-antagonism on rat vas deferens, potassium channel activation (reduction of spontaneous myogenic movements of strips of rat portal-mesenteric veins), leukotriene LTD_4, bradykinin B_2 and cholecystokinin CCK_A antagonism on guinea pig ileum and relaxation of guinea pig tracheal strips.

Concluding, it is worth noting that the pharmacological profile of compound **1**, bearing a ketonic function

in position 7, is also quite different from that of octahydroindoloquinolizines bearing oxygenated function (ketonic or alcoholic) in position 2, which exhibited sedative and hypotensive actions [20] or inhibited the GABA uptake [21].

5. Conclusions

The prepared 9-methyl-1,2,3,4,6,7,12,12b-octahydro-7-oxo-indolo[2,3-*a*]quinolizine (**1**) was found to be different from the MIQ metabolite $\mathbf{M_4}$ whose structure remains unclear.

Nevertheless, compound **1** exhibited several interesting properties among which are the inhibition of arachidonic acid-induced platelet aggregation with increase of bleeding time and the capacity to protect mice from both hypobaric and cyanide-induced hypoxia. The simultaneous presence of these activities in the same molecule indicate compound **1** as an interesting new lead for the search of cerebroprotective agents useful in neurodegenerative diseases.

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