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1-Oxa-3,8-diazaspiro[4.5]decan-2-one derivatives with a potent inhibitory effect on neural Ca-uptake and protecting action against TET-induced brain edema and memory and learning deficits†

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Summary — A series of novel 1-oxa-3,8-diazaspiro[4.5]decan-2-one derivatives **8–71** were synthesized. Several representatives were examined for their in vitro inhibitory action on ^{45}Ca-uptake into cerebrocortical synaptosomes depolarized by potassium and veratrine and on triethyltin-induced brain edema. Of the compounds displaying most potent inhibitory action on veratrine-induced ^{45}Ca-uptake into cerebrocortical synaptosomes and outstanding protection against triethyltin chloride (TET) induced brain edema in rats, four were tested for their antihypoxic action and prevention of learning and memory deficits elicited by various agents (eg, electroshock, diazepam, scopolamine, carbon dioxide and normobaric hypoxia). In some of these tests the four compounds showed remarkable protecting/restoring activity. It is assumed that the beneficial effects of these compounds in brain edema formation are probably related to their actions on intracellular Ca^{2+}- and Na^{+}-movements. These cellular effects may also play role in their antiamnesic actions, but other mechanisms may also be involved. On the basis of results obtained in the tests used, the pharmacological profile of the novel 1-oxa-3,8-diazaspiro[4.5]decan-2-one derivatives seems to differ from that of known Ca^{2+}-antagonists such as flunarizine or nimodipine and Na^{+}-channel blocker, phenytoin. Out of the four most active compounds tested, one (**44**) was selected for further investigation and this compound is currently under preclinical development with the code name of RGH-2716 or TDN-345.

1-oxa-3,8-diazaspiro[4.5]decan-2-one / neural Ca^{2+}-uptake inhibition / TET edema / antiamnesic action

Introduction

Brain injury of either ischemic or traumatic type rapidly causes disturbances in cellular energy production, profoundly alters many processes related ion homeostasis (eg, it greatly increases intracellular Ca^{2+} concentration and Na^{+}-influx) and leads to enhanced water permeability (ie, edema) [1] and thus may produce functional consequences manifested in deterioration of cognitive performance (ie, various deficits in learning and memory functions). Compounds interfering with one or more components of the above pathological cellular events or their sequence could have therapeutical potential as neuroprotective agents.

1-Oxa-3,8-diazaspiro[4,5]decan-2-ones substituted at different positions have been described as compounds possessing various pharmacological effects, eg, bronchodilatory, adrenolytic, antihistamine [2, 3], antihypertensive [4] and tachykinin NK_2 receptor antagonistic [5] activity. We have previously found that 4-methylene- or 4-hydroxy-4-methyl-1-oxa-3,8-diazaspiro[4.5]decan-2-ones remarkably inhibited ^{45}Ca uptake into depolarized cerebrocortical synaptosomes and they possessed antihypoxic action in various experimental situations [6]. In order to obtain more potent neural ^{45}Ca uptake inhibitory compounds with beneficial actions on brain metabolism and with memory enhancing or restoring potency, further modifications of these structures were carried out that resulted in novel compounds having potent inhibitory action on depolarization-induced ^{45}Ca uptake into rat cerebrocortical synaptosomes. Some representatives of these compounds (eg, **12**, **44**, **54**, **61**) offered complete protection against triethyl-tin chloride (TET) induced brain edema in rats and they showed prevention of learning and memory functions in mice against deleterious effects of different interventions (eg, administration of diazepam, scopolamine, inhalation of carbon dioxide or hypobaric hypoxia) known to cause learning and memory deficits.

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†This paper is dedicated to the memory of the first author, E Tóth, who died after submission of the manuscript.

Chemistry

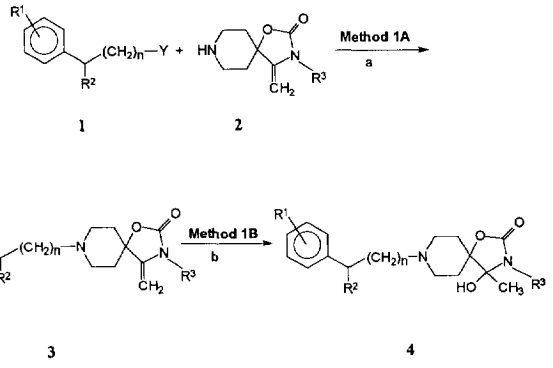
The novel 1-oxa-3,8-diazaspiro[4.5]decan-2-ones described in this paper can be obtained by several different ways (eg, schemes 1–3). The structures and physical data of the compounds are listed in table I.

Method 1 (A,B) (scheme 1) involved the condensation of 4-methylene-1-oxa-3,8-diazaspiro[4.5]decan-2-one derivatives **2** [6] with a phenylalkane derivative of formula **1**, wherein Y is a leaving group (eg, a mesyl, tosyl group or a halogen (chlorine or bromine)) in the presence of a base (eg, inorganic or tertiary organic bases may be used) in an inert organic solvent at reflux, optionally in the presence of an alkali metal iodide catalyst. This gave compound(s) **3** which, upon treatment with aqueous mineral and/or organic acids, could be hydrated to the corresponding 4-hydroxy-4-methyl-1-oxa-3,8-diazaspiro[4.5]decan-2-one derivatives **4**.

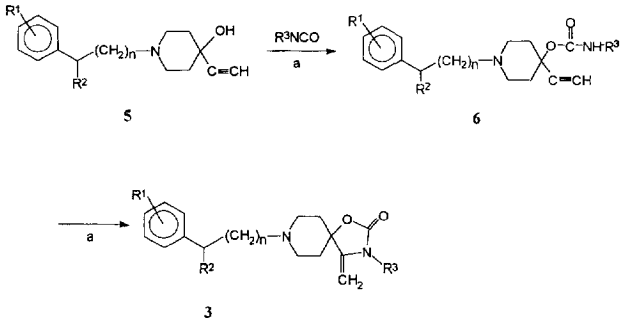
The phenylalkane derivatives **1** were either commercially available or synthesized by standard methods [2, 7].

Method 2 (scheme 2) involved the reaction of 4-ethynyl-4-piperidinol derivatives **5** with the appropriate isocyanates performed with CH_3ONa in toluene or K_2CO_3 in DMF or CH_3COOK in 2-picoline, which afforded the corresponding 4-carbamoyloxy-4-ethynylpiperidine derivatives **6**. Under the basic reaction conditions compounds **6** were cyclized intramolecularly (without isolation) to the corresponding 1-oxa-3,8-diazaspiro[4.5]decan-2-one compound **3**. The ethynylcarbinols **5** were prepared by ethynylation of the suitably substituted 4-piperidone derivatives as described elsewhere [8]. The isocyanates were commercially available.

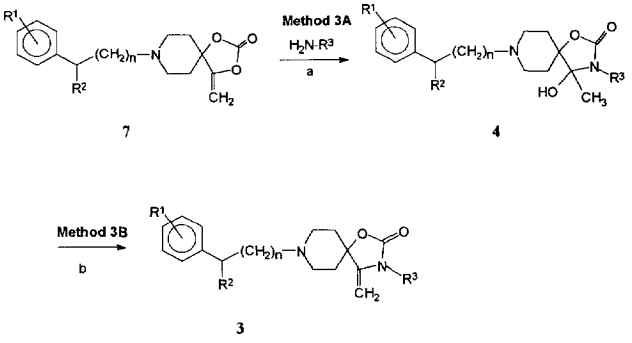
In *Method 3* (A,B) (scheme 3), the reaction of the appropriate 1,3-dioxolan-2-ones **7** with primary



Scheme 1. *Method 1A*. Reagents: a) 4-methyl-2-pentanone, K_2CO_3, KI, reflux; 32–89%. *Method 1B*. Reagents: b) HCOOH/3 M HCl; 5 °C; 94–99%.



Scheme 2. *Method 2*. Reagents: a) CH_3ONa/toluene; 50 °C; 65–88%.



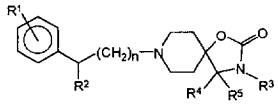
Scheme 3. *Method 3A*. Reagents: a) an excess of R^3-NH_2 as solvent, or xylene; 60–89%. *Method 3B*. Reagents: b) 4-methylbenzenesulphonic acid, xylene, reflux; 88–96%.

amines provided the 4-hydroxy-4-methyl-1-oxa-3,8-diazaspiro[4.5]decane derivatives **4**. Subsequent dehydration of the carbinol **4** to give **3** was accomplished by refluxing and stirring a mixture of **4** and 4-methylbenzenesulphonic acid catalyst in xylene under a water separator. The dioxolanes **7** were obtained by acid catalysed cyclization of the 4-carbamoyloxy-4-ethynylpiperidine derivatives of the formula **6**, eg, with dry hydrogen chloride gas, and subsequent hydrolysis of the intermediate 2-imino-1,3-dioxolane hydrochlorides as described elsewhere [8]. The appropriate amines were commercially available.

Pharmacology

Thirty members of the substituted 1-oxa-3,8-diazaspiro[4.5]decan-2-ones **8–71** falling into three major structural groups were tested for their inhibitory effects on ^{45}Ca-uptake into cerebrocortical synapto-

Table I. Physical data of 1-oxa-3,8-diazaspiro[4.5]decan-2-one derivatives.



Compound	*R¹*	*R²*	*R³*	*R⁴*	*R⁵*	*n*	*Mp (°C)*	*Formula*	*Yield (%)*	*Method*
8	H	H	CH_3	=CH_2		1	119–120	$C_{17}H_{22}N_2O_2$	79	1A
9	4-Cl	H	CH_3	=CH_2		1	118–119	$C_{17}H_{21}ClN_2O_2$	81	1A
10	4-F	H	CH_3	=CH_2		1	74–75	$C_{17}H_{21}FN_2O_2$	91	3B
11	H	H	CH_3	=CH_2		2	35–36	$C_{18}H_{24}N_2O_2$	68	2
12	4-F	4-F-Phenyl	CH_3	=CH_2		3	90–91	$C_{25}H_{28}F_2N_2O_2$	76	1A
13	H	H	CH_3	OH	CH_3	1	184–185	$C_{17}H_{24}N_2O_3$	98	1B
14	4-F	H	CH_3	OH	CH_3	1	> 300	$C_{17}H_{23}FN_2O_3$·HCl	97	1B
15	4-F	4-F-Phenyl	CH_3	OH	CH_3	3	220–223[a]	$C_{25}H_{30}F_2N_2O_3$·HCl	78	3A
16	H	H	C_2H_5	=CH_2		1	121–122	$C_{18}H_{24}N_2O_2$	85	1A
17	4-Cl	H	C_2H_5	=CH_2		1	106–107	$C_{18}H_{23}ClN_2O_2$	87	1A
18	4-F	H	C_2H_5	=CH_2		1	83–84	$C_{18}H_{23}FN_2O_2$	92	3B
19	H	Phenyl	C_2H_5	=CH_2		2	93–94	$C_{25}H_{30}N_2O_2$	58	1A
20	4-F	4-F-Phenyl	C_2H_5	=CH_2		2	152–154	$C_{25}H_{28}F_2N_2O_2$·maleate	55	1A
21	4-F	4-F-Phenyl	C_2H_5	=CH_2		3	111–112	$C_{26}H_{30}F_2N_2O_2$	88	2
22	4-F	H	C_2H_5	OH	CH_3	1	> 300	$C_{18}H_{25}FN_2O_3$·HCl	98	1B
23	4-F	4-F-Phenyl	C_2H_5	OH	CH_3	3	231–235[a]	$C_{26}H_{32}F_2N_2O_3$·HCl	96	1B
24	H	H	*n*-C_3H_7	=CH_2		1	97–98	$C_{19}H_{26}N_2O_2$	77	2
25	4-Cl	H	*n*-C_3H_7	=CH_2		1	82–83	$C_{19}H_{25}ClN_2O_2$	81	2
26	4-F	H	*n*-C_3H_7	=CH_2		1	78–79	$C_{19}H_{25}FN_2O_2$	86	1A
27	4-F	4-F-Phenyl	*n*-C_3H_7	=CH_2		3	107–108	$C_{27}H_{32}F_2N_2O_2$	89	1A
28	4-F	H	*n*-C_3H_7	OH	CH_3	1	246–248[a]	$C_{19}H_{27}FN_2O_3$·HCl	82	3A
29	4-F	4-F-Phenyl	*n*-C_3H_7	OH	CH_3	3	134–136[a]	$C_{27}H_{34}F_2N_2O_3$·HCl	97	1B
30	4-Cl	H	*i*-C_3H_7	=CH_2		1	102–103	$C_{19}H_{25}ClN_2O_2$	77	2
31	4-F	H	*i*-C_3H_7	=CH_2		1	103–104	$C_{19}H_{25}FN_2O_2$	88	1A
32	4-F	4-F-Phenyl	*i*-C_3H_7	=CH_2		3	118–119	$C_{27}H_{32}F_2N_2O_2$	74	1A
33	4-F	H	*i*-C_3H_7	OH	CH_3	1	> 300	$C_{19}H_{27}FN_2O_3$·HCl	60	3A
34	4-F	4-F-Phenyl	*i*-C_3H_7	OH	CH_3	3	251–253[a]	$C_{27}H_{34}F_2N_2O_3$·HCl	96	1B
35	H	H	*n*-C_4H_9	=CH_2		1	70–71	$C_{20}H_{28}N_2O_2$	78	2
36	4-Cl	H	*n*-C_4H_9	=CH_2		1	86–87	$C_{20}H_{27}ClN_2O_2$	75	2
37	4-F	H	*n*-C_4H_9	=CH_2		1	91–92	$C_{20}H_{27}FN_2O_2$	96	3B
38	4-F	4-F-Phenyl	*n*-C_4H_9	=CH_2		3	94–95	$C_{28}H_{34}F_2N_2O_2$	80	2
39	4-F	H	*n*-C_4H_9	OH	CH_3	1	> 300[a]	$C_{20}H_{29}FN_2O_3$·HCl	89	3A
40	4-F	4-F-Phenyl	*n*-C_4H_9	OH	CH_3	3	218–221[a]	$C_{28}H_{36}F_2N_2O_3$·HCl	87	3A
41	H	H	*t*-C_4H_9	=CH_2		1	106–107	$C_{20}H_{28}N_2O_2$	83	1A
42	4-Cl	H	*t*-C_4H_9	=CH_2		1	104–105	$C_{20}H_{27}ClN_2O_2$	68	2
43	4-F	H	*t*-C_4H_9	=CH_2		1	93–94	$C_{20}H_{27}FN_2O_2$	76	2
44	4-F	4-F-Phenyl	*t*-C_4H_9	=CH_2		3	90–92	$C_{28}H_{34}F_2N_2O_2$	85	1A
45	4-F	H	*t*-C_4H_9	OH	CH_3	1	286–288[a]	$C_{20}H_{29}FN_2O_3$·HCl	98	1B
46	4-F	4-F-Phenyl	*t*-C_4H_9	OH	CH_3	3	218–220[a]	$C_{28}H_{36}F_2N_2O_3$·HCl	95	1B
47	4-F	4-F-Phenyl	*n*-C_7H_{15}	=CH_2		3	121–122	$C_{31}H_{40}F_2N_2O_2$·maleate	92	3B
48	4-F	4-F-Phenyl	*n*-$C_{10}H_{21}$	=CH_2		3	106–107	$C_{34}H_{46}F_2N_2O_2$·maleate	82	1A
49	4-F	4-F-Phenyl	*n*-C_7H_{15}	OH	CH_3	3	107–109	$C_{31}H_{42}F_2N_2O_3$·HCl	88	3A

Table I. *Continued.*

Compound	R^1	R^2	R^3	R^4	R^5	*n*	*Mp (°C)*	*Formula*	*Yield (%)*	*Method*
50	4-F	4-F-Phenyl	n-$C_{10}H_{21}$	OH	CH_3	3	109–111	$C_{34}H_{48}F_2N_2O_3$·HCl	97	1B
51	H	H	Cyclohexyl	$=CH_2$		1	152–153	$C_{22}H_{30}N_2O_2$	75	2
52	4-Cl	H	Cyclohexyl	$=CH_2$		1	134–135	$C_{22}H_{29}ClN_2O_2$	92	3B
53	4-F	H	Cyclohexyl	$=CH_2$		1	125–126	$C_{22}H_{29}FN_2O_2$	83	2
54	4-F	4-F-Phenyl	Cyclohexyl	$=CH_2$		3	122–123	$C_{30}H_{36}F_2N_2O_2$	81	1A
55	4-Cl	H	Cyclohexyl	OH	CH_3	1	310–315[a]	$C_{22}H_{31}ClN_2O_3$·HCl	94	1B
56	4-F	H	Cyclohexyl	OH	CH_3	1	> 315	$C_{22}H_{31}FN_2O_3$·HCl	87	3A
57	4-F	4-F-Phenyl	Cyclohexyl	OH	CH_3	3	258–260[a]	$C_{30}H_{38}F_2N_2O_3$·HCl	99	1B
58	H	H	Phenyl	$=CH_2$		1	137–138	$C_{22}H_{24}N_2O_2$	72	1A
59	4-Cl	H	Phenyl	$=CH_2$		1	134–135	$C_{22}H_{23}ClN_2O_2$	85	2
60	4-F	H	Phenyl	$=CH_2$		1	146–147	$C_{22}H_{23}FN_2O_2$	78	1A
61	4-F	4-F-Phenyl	Phenyl	$=CH_2$		3	125–126	$C_{30}H_{30}F_2N_2O_2$	81	2
62	4-F	H	Phenyl	OH	CH_3	1	> 300	$C_{22}H_{25}FN_2O_3$·HCl	97	1B
63	4-F	4-F-Phenyl	Phenyl	OH	CH_3	3	274–276[a]	$C_{30}H_{32}F_2N_2O_3$·HCl	97	1B
64	4-F	H	Benzyl	$=CH_2$		1	100–101	$C_{23}H_{25}FN_2O_2$	32	1A
65	4-F	4-F-Phenyl	Benzyl	$=CH_2$		3	81–82	$C_{31}H_{32}F_2N_2O_2$	88	3B
66	4-F	H	Benzyl	OH	CH_3	1	290–295[a]	$C_{23}H_{27}FN_2O_3$·HCl	98	1B
67	4-F	4-F-Phenyl	Benzyl	OH	CH_3	3	177–179[a]	$C_{31}H_{34}F_2N_2O_3$·HCl	82	3A
68	4-F	H	1-Naphthyl	$=CH_2$		1	160–161	$C_{26}H_{25}FN_2O_2$	65	2
69	4-F	4-F-Phenyl	1-Naphthyl	$=CH_2$		3	127–128	$C_{34}H_{32}F_2N_2O_2$	78	1A
70	4-F	H	1-Naphthyl	OH	CH_3	1	288–290[a]	$C_{26}H_{27}FN_2O_3$·HCl	97	1B
71	4-F	4-F-Phenyl	1-Naphthyl	OH	CH_3	3	180–182[a]	$C_{34}H_{34}F_2N_2O_3$·HCl	94	1B

[a]Decomposed.

somes depolarized by 60 mM K^+ or 20 μM veratrine. Further, 13 representatives of the three groups were also examined for their protective effect against the formation of brain edema in rats intoxicated with subchronic administration of TET. The most active compounds in these tests (**12**, **44**, **54**, **61**) were examined for antihypoxic actions (in hypobaric hypoxia) using SH rats and in various experimental situations where deficits in learning and memory processes were produced by various agents such as diazepam, scopolamine, carbon dioxide, electroshock in mice or by normobaric hypoxia in SH rats. The four selected compounds were more active in most of these tests than flunarizine, nimodipine (Ca^{2+}-antagonists) and phenytoin (Na^+-channel blocker) that were used as reference substances.

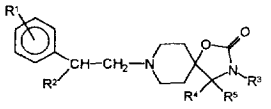
Results and discussion

Based on the general formula given in table I the most characteristic representatives of 1-oxa-3,8-diazaspiro[4.5]decan-2-ones included in this report were selected for pharmacological experiments. These compounds were divided into three major groups (groups 1, 2 and 3) according to the number of *n* (table I).

The effects of these selected compounds on the ^{45}Ca-uptake into cerebrocortical synaptosomes depolarized by different depolarizing agents (ie, by 60 mM K^+ or 20 μM veratrine) are summarized in tables II–IV. Flunarizine (Ca^{2+}-antagonist of diphenylpiperazine type), nimodipine (dihydropyridine type Ca^{2+}-antagonist) and phenytoin (a prototype Na^+-channel blocker) were also tested for comparison.

The examples given in group 1 (table II), where $n = 1$ and R^1 = F and R^2 = H in all cases, did not or only slightly inhibited ^{45}Ca-uptake induced by potassium depolarization whereas their action on veratrine-induced ^{45}Ca-uptake was somewhat more expressed especially when R^3 was a bulky, aromatic group (eg, 1-naphthyl, compound **68**). Replacement of methylene group at positions R^4 and R^5 with R^4 = OH and R^5 = CH_3 in these analogues resulted in a fairly potent inhibitor of veratrine-induced ^{45}Ca-uptake with IC_{50} of 1.34 μM (compound **70**). Reference compounds, flu-

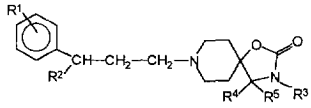
Table II. Effects of compounds belonging to group 1 and reference compounds on the in vitro synaptosomal $^{45}Ca^{2+}$-uptake stimulated with 60 mM K^+ or 20 μM veratrine.



Compound	*R¹*	*R²*	*R³*	*R⁴*	*R⁵*	*^{45}Ca-uptake inhibition (IC_{50})[a]*	
						K^+-stimulation	*Veratrine stimulation*
10	4-F	H	CH_3	=CH_2		≫ 30	> 30
37	4-F	H	*n*-C_4H_9	=CH_2		≫ 30	19.0
64	4-F	H	Benzyl	=CH_2		> 30	16.0
68	4-F	H	1-Naphthyl	=CH_2		≫ 30	9.0
14	4-F	H	CH_3	OH	CH_3	≫ 30	> 30
39	4-F	H	*n*-C_4H_9	OH	CH_3	≫ 30	> 30
66	4-F	H	Benzyl	OH	CH_3	40.2	4.7
70	4-F	H	1-Naphthyl	OH	CH_3	48.3	1.34
Flunarizine						22.6	1.3
Nimodipine						208	4.7
Phenytoin						≈300	24[b]

[a]IC_{50} values are given in μM; [b]percentage inhibition at 50 μM.

Table III. Effects of compounds belonging to group 2 on the in vitro synaptosomal $^{45}Ca^{2+}$-uptake stimulated with 60 mM K^+ or 20 μM veratrine



Compound	*R¹*	*R²*	*R³*	*R⁴*	*R⁵*	*^{45}Ca-uptake inhibition (IC_{50})[a]*	
						K^+-stimulation	*Veratrine stimulation*
11	H	H	CH_3	=CH_2		> 100	> 30
19	H	Phenyl	C_2H_5	=CH_2		80.2	1.75
20	4-F	4-F-Phenyl	C_2H_5	=CH_2		18.1	0.97

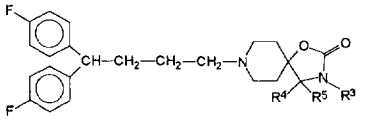
[a]IC_{50} values are given in μM.

narizine, nimodipine and phenytoin also displayed moderate or no inhibition in ^{45}Ca-uptake induced by potassium depolarization while the actions of flunarizine and nimodipine, but not that of phenytoin, in the inhibition of veratrine-induced ^{45}Ca-uptake were comparable to those of most active analogues in this group.

In group 2 (table III) where n = 2, introduction of R^2 = phenyl and especially that of R^2 = 4-F-phenyl (compound **20**) greatly improved the ability to inhibit veratrine-induced ^{45}Ca-uptake inhibitory activity (IC_{50} = 0.97 μM) even though it had a relatively small alkyl substituent at the R^3 position. Compound **20** was even active in inhibition of potassium depolarization induced ^{45}Ca-uptake (IC_{50} = 18.1 μM).

These results clearly indicate that at least three structural elements, ie, length of alkyl chain between the diazaspiro[4.5]decan-2-one and phenyl moieties,

Table IV. Effects of compounds belonging to group 3 on the in vitro synaptosomal $^{45}Ca^{2+}$-uptake stimulated with 60 mM K^+ or 20 μM veratrine.



Compound	R^3	R^4	R^5	^{45}Ca-uptake inhibition (IC_{50})[a]	
				K^+-stimulation	Veratrine stimulation
12	CH_3	=CH_2		17.7	1.60
21	C_2H_5	=CH_2		6.7	0.30
27	*n*-C_3H_7	=CH_2		8.2	0.37
32	*i*-C_3H_7	=CH_2		9.8	1.34
38	*n*-C_4H_9	=CH_2		5.8	0.32
44	*t*-C_4H_9	=CH_2		5.8	0.36
54	Cyclohexyl	=CH_2		5.3	0.14
61	Phenyl	=CH_2		2.1	0.26
69	1-Naphthyl	=CH_2		32.6	0.38
15	CH_3	OH	CH_3	11.3	0.84
23	C_2H_5	OH	CH_3	9.9	0.87
29	*n*-C_3H_7	OH	CH_3	24.4	0.71
34	*i*-C_3H_7	OH	CH_3	7.4	0.93
40	*n*-C_3H_7	OH	CH_3	6.1	1.66
46	*t*-C_4H_9	OH	CH_3	18.5	0.69
57	Cyclohexyl	OH	CH_3	31.4	0.59
63	Phenyl	OH	CH_3	40.4	0.93
67	Benzyl	OH	CH_3	15.2	0.71
71	1-Naphthyl	OH	CH_3	15.2	0.23

[a]IC_{50} values are given in μM.

substitutions on diazaspiro[4.5]decan-2-one part and R^2-substitution(s) could be important to obtain compounds with potent ^{45}Ca-uptake inhibitory activity.

Indeed, in group 3 (table IV) where length of alkyl chain was extended to 4 (ie, n = 3), R^1 = F and R^2 = 4-fluorophenyl in all examples, several potent inhibitors of synaptosomal ^{45}Ca-uptake were found (IC_{50} values varied from 0.23 and 1.66 μM for veratrine-induced uptake and for 2.1 and 40.4 μM for potassium-induced uptake, respectively). Compounds with R^4R^5 = methylene showed somewhat higher inhibitory activity in case of potassium depolarization compared to those where R^4 = OH and R^5 = CH_3, but no such a tendency could be observed in case of veratrine depolarization. Similarly, no clear-cut relationship could be seen between the quality and bulkiness of substitutions at position of R^3 and the ^{45}Ca-uptake inhibitory activity of the compounds.

Three members of compounds belonging to group 1 were examined for their protecting action against edema formation induced by TET intoxication. Flunarizine, nimodipine and phenytoin were again used as reference compounds. These results are depicted in table V. Out of these compounds only **70**, which possesses a 1-naphthyl substitution at position R^3, produced protection of 55%. This might correspond well to the inhibitory activity seen in the in vitro Ca^{2+}-uptake experiments. On the other hand, however, compounds from group 2 (**19**, **20**) with IC_{50} values in micromolar range for veratrine-induced uptake of Ca^{2+} (0.97 and 1.75 μM, respectively) did not show any protecting activity against TET-induced alterations. The poor absorption of the compounds might explain the lack of effect but the toxicity observed with **20** apparently contradicts to this assumption.

However, all compounds selected from group 3 as potent inhibitors of in vitro synaptosomal Ca^{2+} uptake proved to be very effective against TET-induced water content increase in rat brain; the compounds tested offered practically complete protection. This effect appeared in all compounds regardless of their substitution at positions R^3, R^4 or R^5. Moreover, these compounds not only protected against edema formation but the gross neurological deficits (ie, the greatly reduced righting and grasping reflex, reduction in motor activity) and body weight loss caused by TET administration also completely or almost completely disappeared. In case of compound **44** the above effects were greatly dose-dependent between 5 and 100 μmol/kg and already 5 μmol/kg produced a protection of 33% against TET-induced edema. The protecting activity in edema experiments also correlated with the change in brain Na^+ content. Namely, a 44.5% decrease was seen in brain Na^+ content already after administration of 5 μmol/kg (= 2.3 mg/kg) **44** compared to rats treated with TET, whereas higher doses (ie, 10, 25, 50 and 100 μmol/kg) resulted in a complete restoration in Na^+ content (data not shown). TET intoxication, like ischemia, is known to cause several cerebral metabolic changes in the brain accompanied with profound behavioural effects in addition to cerebral edema formation and increase in Na^+-content [17, 18]. Therefore, it is reasonable to assume that the beneficial effects of this particular group of 1-oxa-3,8-diazaspiro[4.5]decan-2-ones found in TET-edema experiments might be related mainly to their inhibitory effects on transmembrane Na^+-flux and/or Ca^{2+}-movements but their protective actions on intermediary metabolism of nerve cell may also contribute to the anti-edema effects. Regarding the inhibitory effects on Na^+- and Ca^{2+}-movements, the assumption is largely supported with the notion that out of reference substances phenytoin (an Na^+-channel blocker) and flunarizine, that is also known to directly

Table V. Effects of various 1-oxa-3,8-diazaspiro[4.5]decan-2-ones with different substitutions and reference compounds on TET-induced brain edema in rats.

Group	*Compound*[a]	R^1	R^2	R^3	R^4	R^5	*Inhibition (%)*
Group 1	**14**	4-F	H	CH_3	OH	CH_3	10.1
	39	4-F	H	n-C_4H_9	OH	CH_3	4.0
	70	4-F	H	1-Naphthyl	OH	CH_3	55.5[b]
Group 2	**11**	H	H	CH_3	$=CH_2$		8.6
	19	H	Phenyl	C_2H_5	$=CH_2$		7.1
	20	4-F	4-F-Phenyl	C_2H_5	$=CH_2$		1.9[c]
Group 3	**12**	4-F	4-F-Phenyl	CH_3	$=CH_2$		106.3[b]
	38	4-F	4-F-Phenyl	n-C_4H_9	$=CH_2$		92.3[b]
	44	4-F	4-F-Phenyl	t-C_4H_9	$=CH_2$		108.6[b]
	54	4-F	4-F-Phenyl	Cyclohexyl	$=CH_2$		113.7[b]
	61	4-F	4-F-Phenyl	Phenyl	$=CH_2$		95.0[b]
	69	4-F	4-F-Phenyl	1-Naphthyl	$=CH_2$		85.9[b]
	46	4-F	4-F-Phenyl	t-C_4H_9	OH	CH_3	94.3[b]
Flunarizine							67.6[b]
Nimodipine							–20.2
Phenytoin							79.8[b]

[a]All compounds were administered orally in a dose of 0.1 mmol/kg twice a day for 5 days, 1 and 6 h after 2.5 mg/kg TET; [b]significantly different from TET + vehicle-treated group, $P < 0.001$; [c]toxic at the dose investigated.

interfere with Na^+-channels [19], produced almost 80 and 67% protection, respectively, against TET-induced brain edema whereas nimodipine had no protective activity and rather slightly enhanced TET-induced edema and other neurological symptoms.

Compounds found to be most effective in TET experiments either in prevention of brain edema or neurological deficits (ie, **12**, **44**, **54** and **61**) were selected for further studies. The relatively low acute toxicity of these compounds (oral $LD_{50} > 1000$ mg/kg in all four cases) also contributed to their selection.

Table VI shows the protecting actions of the selected and reference compounds against hypobaric hypoxia-induced lethality in SH rats. Out of the four, two (ie, compounds **12** and **44**) showed antihypoxic actions manifested in the increase of survival rate and these actions were apparently dose-dependent in the dose range studied. In this test only nimodipine proved to be active and no significant protection was seen in case of flunarizine and phenytoin.

Out of the four compounds, three demonstrated significant antiamnesic effects in the diazepam-induced anterograde amnesia model in mice (table VII). In case of **12** and **61** these effects were already apparent in doses as low as 0.1 mg/kg. Neither of the reference compounds showed protecting activity against diazepam-induced anterograde amnesia, rather a tendency to further deteriorate was observed. The antiamnesic actions of the four selected compounds

Table VI. Effects of test compounds **12**, **44**, **54** and **61**, and reference compounds on hypobaric hypoxia-induced lethality in SH-rats.

Compound	*Dose*[a] *(mg/kg)*	*Protected animals (%)*
12	10	20
	50	70[b]
44	5	10
	10	20
	50	60[b]
54	10	10
	50	20
61	10	0
	50	0
Flunarizine	10	20
	50	40
Nimodipine	5	30
	10	60[b]
Phenytoin	10	0
	50	20

[a]Compounds were given orally 1 h before hypoxia challenge; [b]significantly different from control, $P < 0.05$.

Table VII. Antiamnesic effects of compounds **12**, **44**, **54** and **61** and reference compounds against diazepam-induced anterograde amnesia.

Treatment	*Dose*[a] *(mg/kg)*	*Retention latency time (s) (mean ± SEM)*	*Prevention (%)*	*Retention time > 200 s*[b] *(%)*
Control	–	195 ± 41.0	–	60
DIAZ + vehicle	3	60 ± 14.6	–	0
DIAZ + **12**	3 + 0.1	226 ± 31.5[c]	123[c]	60
	3 + 10.0	188 ± 31.5	95[c]	50
DIAZ + **44**	3 + 0.1	168 ± 35.4	80	40
	3 + 10.0	217 ± 35.7[c]	116[c]	70
DIAZ + **54**	3 + 0.1	105 ± 36.1	33	22
	3 + 10.0	184 ± 30.8	92	33
DIAZ + **61**	3 + 0.1	230 ± 34.4[c]	126[c]	80
	3 + 10.0	294 ± 5.8	173[c]	100
Control	–	186 ± 38.2	–	60
DIAZ + vehicle	3	76.7 ± 27.4	–	0
DIAZ + flunarizine	3 + 0.1	43.7 ± 27.4	–30	10
	3 + 10.0	46.4 ± 14.8	–28	0
DIAZ + nimodipine	3 + 0.1	97.1 ± 35.7	19	20
	3 + 10.0	17.1 ± 3.4	–54	0
DIAZ + phenytoin	3 + 0.1	25.3 ± 4.7	–47	0
	3 + 10.0	67.3 ± 26.9	–9	10

[a]Diazepam (DIAZ) was administered ip whereas reference and test compounds were given orally (for other details see *Experimental protocols*); [b]number of animals (given in percentage) not entering into the black compartment of the box in 200 s; [c]significantly different from DIAZ + vehicle group, $P < 0.05$.

were further studied in scopolamine-induced anterograde amnesia, electroshock and carbon dioxide induced retrograde amnesia models. Neither of them produced significant protecting activity in scopolamine-induced anterograde amnesia model. However, **51** showed significant prevention at the dose of 0.1 mg/kg in carbon dioxide induced retrograde amnesia and **61** had the same action at the dose of 10 mg/kg in electroshock-induced retrograde amnesia model (data not shown). Table VIII shows the results obtained with the four selected and three reference compounds in hypoxia-induced memory disturbances. In the dose tested (ie, 10 mg/kg) only **54** and **61** gave significant protecting activity and no such an action was found in case of reference compounds.

The actions of the compounds in TET-induced brain edema may well be related to their inhibitory effects on veratrine-induced alterations in cerebrocortical synaptosomes. Veratrine is known to prevent inactivation of Na^+-channels, a phenomenon leading

Table VIII. Effects of compounds **12**, **44**, **54** and **61**, and reference compounds against normobaric hypoxia-induced memory impairment in SH rats.

Compound	*Dose*[a] *(mg/kg)*	*Prevention (%)*
12	10	0
44	10	24
54	10	84[b]
61	10	74[b]
Flunarizine	10	0
Nimodipine	10	0
Phenytoin	10	0

[a]Compounds were given orally 1 h before normobaric hypoxia challenge; [b]significantly different from control + vehicle: $P < 0.05$.

to greatly increased Na^+-influx and, as a consequence, to the depolarization of intracellular organelles, such as mitochondria and endoplasmic reticulum, and thus causing a massive release of Ca^{2+} from these sites. The potent and preferential inhibitory effects of some of the 1-oxa-3,8-diazaspiro[4.5]decan-2-one derivatives described in this paper lends support to the hypothesis that they interact with membrane Na^+-channels and, by preventing stimulus evoked Na^+-influx, they inhibit intracellular Ca^{2+}-release rather than transmembrane Ca^{2+}-flux. Although no such data are available at present their direct effects on intracellular Ca^{2+}-release cannot be ruled out. Prevention by these compounds against intracellular Na^+-increase could be directly demonstrated in TET experiments. Although the above cellular mechanism (ie, inhibition of Na^+-influx and intracellular Ca^{2+}-release) may partly contribute to the antihypoxic and antiamnesic actions of the four compounds found in various models, detailed investigations (not shown here) indicate the participation of other mechanism(s), too. Regarding the likely interactions of the four selected compounds with neuronal Na^+- and Ca^{2+}-movements and their related (or unrelated) pharmacological (eg, antihypoxic or antiamnesic) actions, data presented here indicate that their (biochemical and pharmacological) profile may be somewhat different from those of known Ca^{2+}- and Na^+-channel blockers (ie, flunarizine, nimodipine and phenytoin) included in our experiments as reference substances.

According to our data obtained so far the pharmacological actions are possibly unrelated to direct involvement of cholinergic system as neither of the four compounds possessed effects on either M_1 or M_2 cholinergic receptors (data not shown). In vitro binding experiments demonstrated, however, moderate interactions between the two compounds **44** and **61** and α_1-adrenergic receptors (IC_{50} = 180 and 289 nM) while all the four compound showed slight interaction with S-2 serotoninergic receptors (IC_{50} = 118, 204, 195 and 194 nM, respectively) and with dopaminergic D_2 (IC_{50} = 89, 60, 115 and 91 nM, respectively) receptors. In addition, **44** had an IC_{50} of 230 nM in the in vitro D_1 dopaminergic binding assay. In vivo experiments confirmed the slight accelerating effects of **44** on brain serotoninergic and dopaminergic systems but these effects were manifested only in doses higher than those offering antiamnesic and antiedema actions. Despite the mild in vitro interactions with α_1-adrenergic receptors no significant cardiovascular effects (eg, alterations in mean arterial blood pressure, heart rate, cerebral and femoral blood flow) in anesthetised dogs were found with the four compounds (data not demonstrated here). Although they interacted with dopaminergic and serotoninergic receptors no behavioural changes related to these monoaminergic systems were seen at pharmacological doses. Moreover, no hypothermizing effects of the compounds that could, at least partly, explain the antihypoxic effects of the compounds were observed.

Experimental protocols

Chemistry

Melting points were determined on Büchi 510 apparatus and are uncorrected. IR spectra were obtained with a Nicolet 20DXC FTIR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian VXR-300 and UNITY plus 500 NMR spectrometer using tetramethylsilane as an internal standard. The chemical shifts marked 'a' correspond to the numerical middle of the relevant fluorine-coupled ^{13}C multiplet. Elemental analysis (C, H, N and halogen) were in agreement with calculated values (within ± 0.4%).

Method 1A. 8-[4,4-Bis(4-fluorophenyl)butyl]-3-(1,1-dimethylethyl)-4-methylene-1-oxa-3,8-diazaspiro[4.5]decan-2-one ***44***

A mixture of 3-(1,1-dimethylethyl)-4-methylene-1-oxa-3,8-diazaspiro[4.5]decan-2-one (22.43 g, 0.10 mol), 1,1'-(4-chlorobutylidene)bis(4-fluorobenzene) (30.88 g, 0.11 mol), anhydrous potassium carbonate (15.2 g, 0.11 mol) and potassium iodide (1.82 g, 0.011 mol) in 4-methyl-2-pentanone (224 mL) was vigorously stirred and refluxed for 18–20 h and monitored by TLC. After cooling, water (50 mL) was added to the reaction mixture, the organic phase was separated, washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulphate and evaporated under reduced pressure. Consecutive crystallization from isopropyl ether and isopropanol gave the title compound in 85.0% yield, mp 91–93 °C.

IR (cm^{-1}) 1756 (CO), 1641 (C=C), 1230 (C-O-C), 1219 (Ar-F), 1600 (Ar-skeletal), 834 (Ar-H). ^{1}H-NMR (500 MHz, $CDCl_3$, 30 °C, δ [ppm]): 1.44 (2H, m, $NCH_2CH_2CH_2CH$), 1.58 (9H, s, ^{t}Bu(CH_3)), 1.76 (2H, m, H_e-6,10), 1.80 (2H, m, H_a-6,10), 2.00 (2H, m, $NCH_2CH_2CH_2CH$), 2.29 (2H, m, H_a-7,9), 2.34 (2H, m, $NCH_2CH_2CH_2CH$), 2.72 (2H, m, H_e-7,9), 3.87 (1H, t, $NCH_2CH_2CH_2CH$), 4.09 and 4.46 (2H, d and d, $=CH_2$), 6.96 (4H, m, fluoro-phenyl H-3,5), 7.15 (4H, m, fluorophenyl H-2,6). ^{13}C-NMR (125 MHz, $CDCl_3$, 30 °C, δ [ppm]): 25.5 ($NCH_2CH_2CH_2CH$), 28.3 (^{t}Bu(CH_3)), 33.9 ($NCH_2CH_2CH_2CH$), 36.8 (C-6,10), 49.2 (C-7,9), 49.8 ($NCH_2CH_2CH_2CH$), 57.0 (^{t}Bu(C)), 58.4 ($NCH_2CH_2CH_2CH$), 79.1 (C-5), 85.2 ($=CH_2$), 115.3 (fluoro-phenyl C-3,5)[a], 129.1 (fluorophenyl C-2,6)[a], 140.5 (fluoro-phenyl C-1)[a], 149.7 (C-4), 154.5 (C-2), 161.4 (fluoro-phenyl C-4)[a].

Compound **44** is currently under development with code numbers of **RGH-2716** and **TDN-345** in Chemical Works of Gedeon Richter Ltd and Takeda Chemical Industries Ltd, respectively.

Method 1B. 8-[4,4-Bis(4-fluorophenyl)butyl]-3-cyclohexyl-4-hydroxy-4-methyl-1-oxa-3,8-diazaspiro[4.5]decan-2-one•HCl ***57***

To a stirred solution of 8-[4,4-bis-(4-fluorophenyl)butyl]-3-cyclohexyl-4-methylene-1-oxa-3,8-diazaspiro[4.5]decan-2-one (4.9 g, 0.01 mol) in 10 mL formic acid 50 mL of 1 M hydrochloric acid was added and the reaction mixture was then stirred for additional 30 min at 5 °C. The crystals precipitated were filtered, washed with water and dried to afford the hydrochloride in 98.5% yield; decomposes at 260 °C.

IR (cm^{-1}) 3220 (OH), 2750–2200 ($^+$NH), 1735 (CO), 1232 (Ar-F), 1133 (C-O-C), 1607 (Ar-skeletal), 833 (Ar-H). ^{1}H-NMR (500 MHz, DMSO-d_6, 30 °C, δ [ppm]: 1.05 and 1.57 (2H, m, cyclohexyl H-4), 1.24 and 1.73 (2H, m, cyclohexyl H-3, interchangeable), 1.26 and 1.73 (2H, m, cyclohexyl H-5), interchangeable), 1.31 (3H, s, 4-CH_3), 1.59 (2H, m, NCH_2CH_2-CH_2CH), 1.60 and 1.90 (2H, m, cyclohexyl H-2), 1.68 and 2.03 (2H, m, cyclohexyl H-6), 1.71 and 2.25 (2H, m, H-10), 1.99 and 2.14 (2H, m, C-6), 2.05 (2H, m, $NCH_2CH_2CH_2CH$), 2.92 and 3.43 (2H, m, H-7), 2.95 and 3.34 (2H, m, C-9), 3.07 (1H, m, cyclohexyl H-1), 3.10 (2H, m, $NCH_2CH_2CH_2CH$), 4.01 (H, t, $NCH_2CH_2CH_2CH$), 6.19 (1H, s, OH), 7.11 (4H, m, fluoro-phenyl H-3,5), 7.35 (4H, m, fluoro-phenyl H-2,6), 10.80 (1H, br, HCl). ^{13}C-NMR (125 MHz, DMSO-d_6, 30 °C, δ [ppm]: 20.5 (4-CH_3), 21.8 ($NCH_2CH_2CH_2CH$), 24.9 (cyclohexyl C-4), 25.5 and 25.6 (cyclohexyl C-3,5), 26.1 (C-6), 29.0 (C-10), 29.8 (cyclohexyl C-6), 30.0 (cyclohexyl C-2), 32.0 ($NCH_2CH_2CH_2$-CH), 47.9 (C-9), 48.2 (C-7), 48.2 ($NCH_2CH_2CH_2CH$), 50.9 (cyclohexyl C-1), 55.3 ($NCH_2CH_2CH_2CH$), 79.8 (C-5), 89.3 (C-4), 115.1 (fluoro-phenyl C-3,5)[a], 129.2 (fluoro-phenyl C-2,6)[a], 140.6 (fluoro-phenyl C-1)[a], 153.5 (C-2), 160.6 (fluoro-phenyl C-4)[a].

Method 2. 8-[4,4-Bis(4-fluorophenyl)butyl]-4-methylene-3-phenyl-1-oxa-3,8-diazaspiro[4.5]decan-2-one ***61***

To a stirred suspension of 1-[4,4-bis(4-fluorophenyl)butyl]-4-ethynyl-4-piperidinol (7.38 g, 0.02 mol) and sodium methoxide (0.22 g, 0.004 mol) in 30 mL toluene, a solution of phenyl isocyanate (2.62 g, 0.022 mol) in 8 mL toluene was added at 50 °C under argon. After stirring for 1 h at the same temperature it was cooled, washed with water, dried (anhydrous magnesium sulphate) and concentrated under reduced pressure. The residue was crystallized from ethanol to give the title compound in 81% yield, mp 125–126 °C.

IR (cm^{-1}) 1771, 1765 (CO), 1687, 1647 (C=C), 1231 (COC), 1225, 1217 (Ar-F), 1601 (Ar- skeletal), 825, 769, 698 (Ar-H). ^{1}H-NMR (300 MHz, $CDCl_3$, 24 °C, δ [ppm]): 1.47 (2H, m, $NCH_2CH_2CH_2CH$), 1.99 (4H, m, H-6,10), 2.01 (2H, m, NCH_2-CH_2CH_2CH), 2,38 (2H, m, H_a-7,9), 2.43 (2H, m, NCH_2CH_2-CH_2CH), 2.82 (2H, m, H_e-7,9), 3.88 (H, t, $NCH_2CH_2CH_2CH$), 4.07 and 4.16 (2H, d and d, = CH_2), 6.97 (4H, m, fluoro-phenyl H-3,5), 7.17 (4H, m, fluoro-phenyl H-2,6), 7.33 (2H, m, phenyl H-2,6), 7.39 (1H, m, phenyl H-4), 7.47 (2H, m, phenyl H-3,5). ^{13}C-NMR (75 MHz, $CDCl_3$, 24 °C, δ [ppm]): 25.4 (NCH_2CH_2-CH_2CH), 33.7 ($NCH_2CH_2CH_2CH$), 36.8 (C-6,10), 49.0 (C-7,9), 49.7 ($NCH_2CH_2CH_2CH$), 58.4 ($NCH_2CH_2CH_2CH$), 82.0 (C-5), 82.0 (=CH_2), 115.2 (fluoro-phenyl C-3,5)[a], 127.0 (phenyl C-2,6), 128.3 (phenyl C-4), 129.0 (fluoro-phenyl C-2,6)[a], 129.5 (phenyl C-3,5), 133.8 (phenyl C-1), 140.4 (fluoro-phenyl C-1)[a], 150.5 (C-4), 154.4 (C-2), 161.3 (fluoro-phenyl C-4)[a].

Method 3A. 3-Butyl-8-[2-(4-fluorophenyl)ethyl]-4-hydroxy-4-methyl-1-oxa-3,8-diazaspiro[4.5]decan-2-one HCl ***39***

Twenty-five millimoles (7.2 g) of 8-[2-(4-fluorophenyl)ethyl]-4-methylene-1,3-dioxa-8-azaspiro[4.5]decan-2-one was dissolved in 36 mL of *n*-butylamine. When the reaction had subsided and returned to 24–25 °C the reaction mixture was stirred at room temperature for 20 h, then evaporated under reduced pressure. The resulting solid was recrystallized from benzene, and melted at 155–156 °C. The yield was 89%. The melting point of the HCl salt is greater than 300 °C.

IR (cm^{-1}) 3280 (OH), 1730 (CO), 1232 (Ar-F), 1120 (C-O-C), 1598 (Ar-skeletal), 832 (Ar-H). ^{1}H-NMR (500 MHz, DMSO-d_6, 30 °C, δ [ppm]): 0.90 (3H, t, $NCH_2CH_2CH_2CH_3$), 1.29 (2H, m, $NCH_2CH_2CH_2CH_3$), 1.34 (3H, s, 4-CH_3), 1.51 (2H, m, $NCH_2CH_2CH_2CH_3$), 1.80 (1H, m, H_e-10), 2.07 (1H, m, H_e-6), 2.21 (1H, m, H_a-6), 2.31 (1H, m, H_a-10), 3.06 (1H, m, H_a-7), 3.07 (1H, m, H_a-9), 3.11 (2H, m, -CH_2CH_2N), 3.12 (2H, m, $NCH_2CH_2CH_2CH_3$), 3.31 (2H, m, -CH_2CH_2N), 3.53 (1H, m, H_e-9), 3.63 (1H, m, H_e-7), 6.26 (1H, s, OH), 7.17 (2H, m, fluoro-phenyl H-3,5), 7.33 (2H, m, fluoro-phenyl H-2,6), 11.3 (1H, br, HCl). ^{13}C-NMR (125 MHz, DMSO-d_6, 30 °C, δ [ppm]): 13.6 ($NCH_2CH_2CH_2CH_3$), 19.5 ($NCH_2CH_2CH_2CH_3$), 20.4 (4-CH_3), 26.1 (C-6), 28.5 (-CH_2CH_2N), 29.2 (C-10), 31.1 ($NCH_2CH_2CH_2CH_3$), 39.2 ($NCH_2CH_2CH_2CH_3$), 47.9 (C-9), 48.2 (C-7), 56.0 (-CH_2CH_2N), 80.4 (C-5), 88.5 (C-4), 115.3 (fluoro-phenyl C-3,5)[a], 130.4 (fluoro-phenyl C-2,6)[a], 133.2 (fluoro-phenyl C-1)[a], 155.4 (C-2), 161.0 (fluoro-phenyl C-4)[a].

Method 3B. 3-Butyl-8-[2-(4-fluorophenyl)ethyl]-4-methylene-1-oxa-3,8-diazaspiro-[4.5]decan-2-one ***37***

A mixture containing 3-butyl-8-[2-(4-fluorophenyl)ethyl]-4-hydroxy-4-methyl-1-oxa-3,8-diazaspiro[4.5]decan-2-one (7.3 g, 0.02 mol) and 4-methylbenzene sulphonic acid monohydrate (1.5 g, 0.008 mol) in xylene (72 mL) was boiled under stirring while the water formed in the reaction was azeotropically distilled off. The reaction was monitored by TLC. After termination of the reaction the mixture was cooled, treated with $NaHCO_3$ (sat aq sol), and the organic phase was washed with water, dried over anhydrous magnesium sulphate, and evaporated to dryness under reduced pressure. Crystallization from diisopropyl ether provided the product, mp 91–92 °C, yield 96%.

IR (cm^{-1}) 2809, 2792, 2771 (N-CH_2), 1757 (C=O), 1677 (C=C), 1222 (Ar-F), 1134 (COC), 827 (=CH_2), 1599 (Ar-skeletal), 808 (Ar-H). ^{1}H-NMR (500 MHz, $CDCl_3$, 30 °C, δ [ppm]): 0.95 (3H, t, $NCH_2CH_2CH_2CH_3$), 1.35 (2H, m, $NCH_2CH_2CH_2$-CH_3), 1.59 (2H, m, $NCH_2CH_2CH_2CH_3$), 1.85 (2H, m, H_e-6,10), 1.92 (2H, m, H_a-6,10), 2.46 (2H, m, H_a-7,9), 2.63 (2H, m, -CH_2CH_2N), 2.78 (2H, m, -CH_2CH_2N), 2.90 (2H, m, H_e-7,9), 3.45 (2H, m, $NCH_2CH_2CH_2CH_3$), 4.03 and 4.12 (2H, d and d, =CH_2), 6.97 (2H, m, fluoro-phenyl H-3,5), 7.15 (2H, m, fluoro-phenyl H-2,6). ^{13}C-NMR (125 MHz, $CDCl_3$, 30 °C, δ [ppm]): 13.7 ($NCH_2CH_2CH_2CH_3$), 19.9 ($NCH_2CH_2CH_2CH_3$), 28.4 ($NCH_2CH_2CH_2CH_3$), 32.9 (-CH_2CH_2N), 36.9 (C-6,10), 41.2 ($NCH_2CH_2CH_2CH_3$), 49.1 (C-7,9), 60.4 (-CH_2CH_2N), 80.0 (=CH_2), 81.4 (C-5), 115.1 (fluoro-phenyl C-3,5)[a], 130.0 (fluoro-phenyl C-2,6)[a], 135.8 (fluoro-phenyl C-1)[a], 149.8 (C-4), 155.5 (C-2), 161.4 (fluoro-phenyl C-4)[a].

Pharmacology

General procedures

In diazepam-, scopolamine-, electroshock- or carbon dioxide-induced amnesia tests, male NMRI mice (Charles-River, Hungary) weighing 24–26 g were used after fasting of 16 h, while in the hypobaric hypoxia tests selectively bred, male, Wistar-derived spontaneously hypertensive rats (from our own breeding colony) weighing 200–220 g were used. In the TET-induced brain edema studies male, Hannover-Wistar rats weighing 180–200 g from our conventional breeding colony were used. Rats of the same strain were used for the in vitro ^{45}Ca-uptake experiments.

Drugs were suspended in 1% Tween-80 and given orally in a volume of 1.0 mL/100 g body weight (for mice) or 0.5 mL/100 g body weight (for rats), respectively.

TET was obtained from Merck-Schuchardt (FRG), phenytoin (free acid) and flunarizine dihydrochloride were from Sigma. Diazepam and nimodipine were synthesized in our Chemical Departments. $^{45}CaCl_2$ was purchased from Amersham. All other reagents were obtained from commercial sources and were of analytical grade.

Student's *t*-test (for TET experiments) and Mann–Whitney *U*-test (for amnesia experiments) was used for statistical comparisons.

$^{45}Ca^{2+}$-uptake into rat cerebrocortical synaptosomes [9]
Rats were killed by cervical dislocation. The brains were removed and the cerebral cortices were dissected rapidly. The cortices were weighted and homogenised in 10 volumes of ice-cold 0.32 M sucrose solution. The homogenates was centrifuged at 1000 *g* for 10 min at 4 °C. The supernatant was recentrifuged at 12 000 *g* for 20 min at 4 °C. The pellet obtained (P2 fraction) was suspended in 0.32 M sucrose solution. The synaptosomal fraction (20 mg protein/mL) were used immediately.

The incubation solution contained 112 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.2 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 10 mM glucose and 20 mM Tris base. This solution was bubbled with 95% O_2 and 5% CO_2 until its pH reached 7.4. Crude P2 fraction (1 mg protein) and test agents were added into tubes and preincubated at 37 °C for 20 min. When the depolarisation was induced by potassium ^{45}Ca-uptake was initiated by addition of 50 µL $^{45}CaCl_2$ (2.8 kBq) in 1.2 M KCl. Basal (unstimulated) ^{45}Ca-uptake was initiated by addition of 50 µL $^{45}CaCl_2$ in 1.2 M NaCl. The final volume of the incubation mixture was 1 mL and the incubation time was 20 s. The uptake was terminated by adding 5 mL stopping solution (120 mM NaCl, 5 mM KCl, 5 mM EGTA, 20 mM Tris, pH 7.4) which was followed by rapid filtration through Whatman GF/C glass fiber filters. The filters were washed twice with 5 mL washing solution (132 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.2 mM $CaCl_2$, 20 mM Tris, pH 7.4).

When veratrine was used as depolarising agent ^{45}Ca-uptake was initiated by addition of 50 µL $^{45}CaCl_2$ in 400 µM veratrine solution. The basal (unstimulated) ^{45}Ca-uptake was initiated by addition of 50 µL $^{45}CaCl_2$ in distilled water. Incubation, termination and filtration of samples was identical with that of described for K^+-induced ^{45}Ca-uptake.

The filters were then placed into vials and dried at 40 °C for about 1 h. Radioactivity was determined by liquid scintillation spectrometry. IC_{50} values (concentrations giving 50% inhibition) were calculated by using the data of two or three independent experiments from individual curves consisting of at least four different concentrations of the test compounds. Probit analysis was used for the calculation of IC_{50} values.

TET-induced edema in rat brain [10]
Rats were intoxicated by daily oral administration of 2.5 mg/kg TET. The test compounds were given orally in a dose of 100 µmol/kg 1 h after TET treatment and 6 h thereafter. This treatment protocol was repeated on five consecutive days and animals were decapitated 2 h after the last treatment with test compounds. The brains were rapidly removed, rinsed in saline, blotted on filter paper and their wet weights were immediately measured. Brain samples were dried for at least 48 h at 100 °C and the dried residues were re-weighed. The difference between the wet and dry weight represents the brain water content which is expressed as a percentage of wet weight.

Hypobaric hypoxia induced lethality in SH rats [11]
The method described by Nakanishi et al [11] was adapted to rats. One hour after the oral administration of the test compounds spontaneously hypertensive (SH) rats (ten per dose group) were placed (two at a time) into a desiccator of 6 L. The barometric pressure is then decreased to 22.66 kPa (170 mmHg) in 20 s. The survival time (ie, interval between reaching the low pressure and the last chest movement) of the animals was determined. By definition, those animals were considered as protected that survived 30% longer than the mean survival time of the control (ie, vehicle-treated) group. The mean survival time of vehicle-treated animals was 249 ± 28.8 s (n = 10).

Anterograde amnesia induced by diazepam or scopolamine [12, 13]
For the measurement of diazepam/scopolamine-induced anterograde amnesia an eight-channel computer-controlled step-through passive avoidance apparatus (consisting of dark and light compartments separated by a guillotine door) was used.

Exploration trial. Mice were pre-selected before learning experiment. Animals that did not enter the dark compartment in 30 s during the exploration trial were excluded from further study.

Acquisition trial. Next day pre-selected animals (ten in each group) were placed in the lit compartment and when they crossed the door between the dark and light compartments with all four limbs within 30 s they received a foot shock (1 mA for 3 s) through the bottom stainless-steel grid. Latency time to enter the dark compartment was registered.

Retention trial. Twenty-four hours after acquisition animals were placed again in the light compartment and the latency time to enter the dark compartment was registered up to 300 s. Test compounds were given orally 1 h prior to acquisition trial and 30 min or 1 h later the mice received diazepam (3 mg/kg, ip) or scopolamine (3 mg/kg, ip), respectively.

Carbon dioxide- and electroconvulsive shock-induced retrograde amnesia [14, 15]
The method was essentially same as those used for diazepam/scopolamine-induced anterograde amnesia but amnesia was induced in retrograde fashion either by carbon dioxide or electroshock (ECS).

In the former case immediately after the acquisition trial mice were placed in plastic boxes (20 × 20 × 20 cm) flushed with carbon dioxide. Twenty seconds later animals were taken from the boxes, reanimated by artificial respiration and returned to their home cages. Test compounds were administered 23 h after the acquisition trial and retention was tested 1 h later.

In case of ECS-induced amnesia, 1 h after acquisition trial mice received auricular ECS (20 mA for 0.2 s) and the test compounds were given orally 1 h later and retention was tested 24 h after the acquisition.

Hypoxia-induced memory impairment [16]
SH rats (six in each group) were trained in microprocessor-controlled shuttle boxes to develop two-way conditioned avoidance response (CAR). Animals performed daily 30 cycles consisting of a 15 s intercycle interval, a 15 s conditioned stimulus (periodical light, 1 Hz frequency) and a 10 s footshock (0.8 mA) as unconditioned stimulus in each day for 3 days. The animals had to change their compartment while the light was on otherwise they received a footshock. On the 4th day they were treated with the test compounds and 60 min later they were placed into the shuttle box where hypoxic conditions were maintained by perfusion of air and nitrogen (200 L/min/box) in a ratio giving 6% O_2 content in the inspired air. Following 20 min equilibration period the animals performed 30 cycles. The number of conditioned avoidance responses was recorded automatically by a computerised program and group means were calculated. In each experiment two control groups were used (placebo and placebo + hypoxia).

LD_{50} determinations
Compounds were administered orally to mice (ten animals per dose group) and the deaths were recorded for 5 days. They were tested in at least five different doses up to 1000 mg/kg.

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