Eur J Med Chem (1991) 26, 403–413

Amnesia-reversal activity of a series of 5-alkoxy-1-arylsulfonyl-2-pyrrolidinones

E Toja, C Gorini, C Zirotti, F Barzaghi, G Galliani

Chemistry and Pharmacology Departments, Roussel Pharma SpA V le Gran Sasso 18, 20131 Milan, Italy

(Received 19 June 1990; accepted 15 November 1990)

Summary — A series of 5-alkoxy-1-arylsulfonyl-2-pyrrolidinones were prepared by condensation of arylsulfonyl chlorides with 5-alkoxy-2-pyrrolidinones. Most compounds reversed electroconvulsive shock-induced amnesia in mice, showing the typical inverted U-shaped dose–response curve. The results for 58 compounds indicate that the potency is maximal when there is a 5-ethoxy group and progressively declines as the ether alkyl chain is either elongated or shortened. Substitution on the phenyl ring or its replacement with heterocyclic rings or its hydrogenation decreases the activity. The most promising compounds, with anti-amnesic properties superior in many respects (greater potency, greater efficacy and broader active dose–range) to those of piracetam and aniracetam were further evaluated for reversing scopolamine-induced amnesia and for their anti-hypoxic activity. 5-Ethoxy-1-phenylsulfonyl-2-pyrrolidinone (**1**) and 5-(1-methylethoxy)-1-/3-(trifluoromethyl) phenylsulfonyl/-2-pyrrolidinone (**41**) were selected for further evaluation because of their potent anti-amnesic and/or antihypoxic activity.

Résumé — Propriétés antiamnésiques d'une série de 5-alkoxy-1-arylsulfonyl-2-pyrrolidinones. *Une série de 5-alkoxy-1-arylsulfonyl-2-pyrrolidinones a été préparée par condensation d'arylsulfochlorures avec des 5-alkoxy-2-pyrrolidinones. La plupart des composés antagonisaient l'amnésie induite par l'électrochoc chez la Souris, montrant la courbe dose–réponse typique en forme de cloche. Les résultats pour 58 composés montrent que la puissance est maximale quand il y a un groupement 5-éthoxy et elle diminue progressivement quand la chaîne alkyle est soit allongée, soit raccourcie. La substitution sur le noyau phényle ou le remplacement du phényle par un noyau hétérocyclique ou son hydrogénation diminuent l'activité. Les composés les plus prometteurs, dotés de propriétés anti-amnésiques supérieures sous différents aspects (plus grande puissance, plus grande réversibilité de l'amnésie et plus large éventail de doses efficaces) à celles du piracétam et de l'aniracétam, ont été évalués ultérieurement pour la réversibilité de l'amnésie induite par la scopolamine et pour les effets anti-hypoxides. La 5-éthoxy-1-phénylsulfonyl-2-pyrrolidinone (**1**) et la 1-/(3-trifluorométhyl) phenylsulfonyl/-5-isopropoxy-2-pyrrolidinone (**41**) ont été sélectionnées pour une évaluation plus approfondie en raison de leur puissante activité anti-amnésique et/ou antihypoxique.*

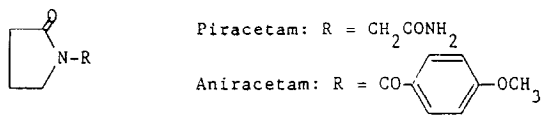
5-alkoxy-1-arylsulfonyl-2-pyrrolidinones / anti-amnesic activity / anti-hypoxic activity

One of the goals of geriatric psychopharmacology is to find new drugs for the treatment of various forms of cognitive dysfunction and dementia in the elderly. There are two important reasons for this. The first is that, although a wide variety of drugs or drug combinations has been tested in patients with impairment of memory and cognition, no treatment has been effective enough to satisfy physicians [1]. The second is that epidemiological surveys in developed countries show that 25% of people over 65 have mental health problems, and, with the present and projected demographic shift, the number of the elderly will rise considerably in the future [2].

A number of pharmaceutical companies around the world are actively engaged in the search for new drugs to combat senile memory disorders and, during recent years, novel classes of active substances have been discovered [3–5]. Among them, compounds containing the 2-pyrrolidinone moiety have been shown to facilitate learning and memory in various animal models of impaired cognition [6–7]. Piracetam is the prototype of this relatively new class of psychoactive drugs, known as 'nootropics', that are reported to have little or no behavioral effect under normal conditions, but to increase cognitive functions in experimentally-induced brain dysfunction. In addition to pro-mnemonic properties, these substances can protect the brain against various offenses, especially hypoxia and drug intoxication. Their mechanism(s) of action is not fully understood [8].

From a methodological point of view, a salient property of piracetam-like nootropic agents is their ability in mice and rats to protect against or to reverse experimentally-induced alterations of acquisition, retention or retrieval in passive avoidance procedures [4]. Compounds which are effective in these simple rodent experimental models were also found to be effective in other memory tests in both lower animal species and non-human primates. The validity of the findings obtained with the various animal behavioural models, including models of passive avoidance in rodents, seems to be confirmed by clinical studies [7]. Despite some disagreements in the literature, patients with dementia or alcoholic mental disorders, geriatric patients with mental and behavioral problems associated with cerebrovascular insufficiency, patients with ECS-induced amnesia, dyslexic children and patients with post-traumatic disturbances of consciousness appear to benefit from therapy with piracetam or nootropics of the piracetam type. Therefore, piracetam and other chemically related analogues, *eg* aniracetam, can be regarded as lead compounds for designing new pyrrolidinone derivatives with improved nootropic properties (scheme 1).

As part of our Cognition Activators program, we have synthesized and tested about three hundred pyrrolidinones characterized by the presence of an alkoxy group in position five. In this paper we describe the first series carrying arylsulfonyl groups on the ring nitrogen.

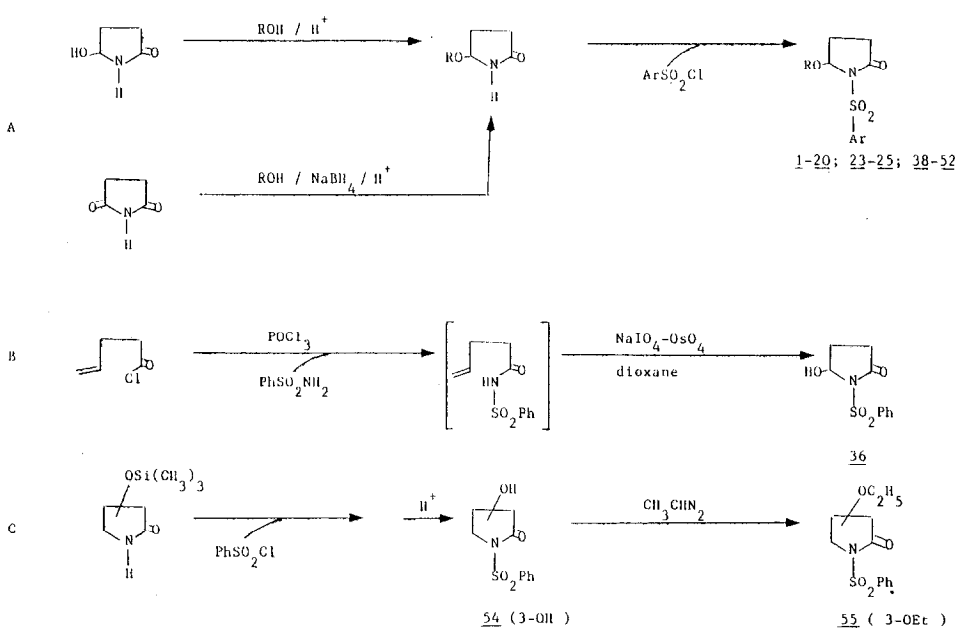


Scheme 1. Reference drugs.

Chemistry

1-Arylsulfonyl-5-alkoxy-2-pyrrolidinones **1–20**, **23–35** and **38–52** were prepared by condensation of arylsulfonyl chlorides with 5-alkoxy-2-pyrrolidinones promoted by butyl lithium in tetrahydrofuran (method A) or by sodium bis-(trimethylsilyl)-amide in diethyl ether (method B) (scheme 2A). In the search for optimization of reaction conditions in the preparation of **1**, we found that either 55% NaH in DMF or t-BuOK in THF at – 20°C were less efficacious than BuLi in promoting the condensation (about 30% yield). Phase-transfer conditions, *ie* cetyltrimethylammonium bromide / 50% NaOH / C_6H_6 or tetrabutylammonium bromide / KOH / THF at room temperature were unsatisfactory (15% yield of **1**). Intermediate 5-alkoxy-2-pyrrolidinones were prepared either according to the Speckamp's procedure [9] by reduction of succinimide in lower alkyl alcohols or by acid-catalyzed exchange of 5-hydroxy-2-pyrrolidinone [10] with the appropriate alcohol.

Cation exchange resins were effective catalysts and this exchange reaction worked satisfactorily for



Scheme 2.

primary and secondary alcohols but not with t-BuOH. In contrast to a reported procedure [10], concentrated HCl did not shown advantages as a catalyst. 5-Ethoxy-1-(4-hydroxyphenylsulfonyl)-2-pyrrolidinone (**21**) and 1-(4-aminophenylsulfonyl)-5-ethoxy-2-pyrrolidinone (**22**) were obtained by hydrogenation of the corresponding 4-benzyloxyphenylsulfonyl (**20**) and 4-nitrophenylsulfonyl (**7**) derivatives.

5-Hydroxy-1-phenylsulfonyl-2-pyrrolidinone (**36**) was prepared (scheme 2B) by condensation of benzenesulfonamide with 4-pentenoyl chloride [11] in phosphorus oxychloride, followed by cleavage of the double bond of the intermediate imide by $NaIO_4$–OsO_4 in dioxane and spontaneous cyclization of the aldehyde group so formed [12]. The ^{1}H-NMR spectrum of **36** indicates the presence of only one cyclized product and excludes a ring-chain tautomerism. The hydroxyl group of **36** was readily acylated by acetic anhydride to give **37**, while it was resistant to alkylation under various experimental conditions and to an exchange reaction with EtOH and amberlyst 15. Treatment of **36** with a slight excess of triethyloxonium tetrafluoroborate in methylene chloride at room temperature for 72 h finally provided **1** in only 15% yield; therefore **36** was discarded as a possible intermediate for the preparation of final compounds. The alkylation of 3- and 4-hydroxy-1-phenylsulfonyl-2-pyrrolidinones (**54** and **56**) was similarly troublesome and acceptable yields of 3- and 4-ethoxy derivatives (**55** and **57**) were obtained only by employing an ether solution of diazoethane and Lewis acid catalysts ($AlCl_3$ or BF_3). Compounds **54** and **56** were prepared by the general condensation procedure of benzenesulfonyl chloride with 3-trimethylsilyloxy-2-pyrrolidinone [13] or 4-trimethylsilyloxy-2-pyrrolidinone [14] and removal of the silyl protective group (scheme 2C).

All substituted pyrrolidinones described here are racemic mixtures.

Pharmacology

Many behavioral models of artificially created or naturally occurring memory deficits have been proposed for use in the search for drugs to treat geriatric cognitive disfunctions. Comprehensive reviews of their application have been published [15–18].

All our compounds were first evaluated in mice by standard behavioral tests and for acute toxicity up to 1000 mg/kg, *po*. Only compounds without CNS effects were tested further for potential anti-amnesic properties.

In our primary screening test (PA-1), we investigated the ability of the compounds to reverse ECS-induced amnesia in mice submitted to a one-trial step-through passive avoidance task. The principle of this behavioral task is that animals submitted to aversive stimulation, such as footshock in a particular place, will avoid going there on a second occasion. Mice were treated orally immediately following ECS and the retention test was performed 3 h later. The most promising compounds in the PA-1 test were tested for their ability to reverse ECS-induced amnesia after oral treatment, 1.5 h before the retention test; the interval between training and retention was 24 h (PA-2 test). In addition, the ability of the compounds to reverse scopolamine-induced amnesia was investigated. The amnesic agent was administered intraperitoneally 15 min prior to training, mice were treated orally immediately after training and the retention test was performed 24 h later (PA-3 test) [19]. The potential anti-hypoxic properties of the compounds were evaluated through two models of chemical hypoxia, namely anemic ($NaNO_2$-induced) and histotoxic (KCN-induced) hypoxia in mice, using survival time as the evaluation parameter [20].

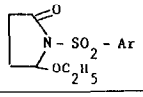
Results and structure–activity relationships

In table I, the compounds with the ethoxy group in position five are grouped by the type of substituent on the phenyl ring and the type of aryl or heterocyclic ring. In table II, the phenyl ring is unsubstituted (except **41–44**) and the alkoxy group is modified. In table III, the phenyl ring of **1** is replaced by a cyclohexyl group, the alkoxy group is placed in position three or four or removed. Piracetam and aniracetam are included as reference drugs.

The typical inverted U-shaped dose-response curve can be observed repeatedly in tables I and II. In this context it should be noted that when evaluating the efficacy of an anti-amnesic agent one must consider both the percentage of retention and the range of active doses. The broader the latter, the larger the 'therapeutic window' expected to appear in clinical assays of a promising candidate. Compound **1** is the best compound of this series, with greater potency and efficacy than piracetam and aniracetam. It shows a minimal effective oral dose of 12.5 mg/kg and an optimal retention test performance at the dose of 100 mg/kg. From the data collected in tables I–III, some structure–activity generalizations can be derived:

— any substituent on the phenyl ring has a detrimental effect and disubstitution (**24–28**) is even worse. Each substituent (*eg* Cl, NO_2, CF_3) modulates the activity in a different way, depending on whether it is in the *ortho*, *meta* or *para* position (compare **2–4**; **5–7**; **8–10**);

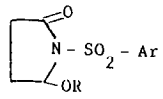
Table I. Amnesia-reversal activity (PA-1 test) of 1-arylsulfonyl-5-ethoxy-2-pyrrolidinones.



No	Ar	Formula[a]	mp(°C)	Recrystn[b] solvent	Yield %[c] (method)	% retention vs controls[d] at the following mg/kg oral doses						
						6.25	12.5	25	50	100	200	400
1	C_6H_5	$C_{12}H_{15}NO_4S$	116-119	A	68(A) 57(B)	+21	+79*	+95*	+118*	+132*	+109*	+ 92*
2	2-ClC_6H_4	$C_{12}H_{14}ClNO_4S$	100-101	B	19(A)	-	-18	- 5	+ 38	+ 53*	+ 52	-
3	3-ClC_6H_4	$C_{12}H_{14}ClNO_4S$	75-77	B	28(A)	-	-	+25	+ 30	+ 16	+ 91*	+ 50
4	4-ClC_6H_4	$C_{12}H_{14}ClNO_4S$	120-122	B	27(A)	-	-	-11	- 15	+ 31	+ 38	-
5	2-$NO_2C_6H_4$	$C_{12}H_{14}N_2O_6S$	98-100	B	23(A)	-	-	+11	+ 4	+ 38	+ 69*	-
6	3-$NO_2C_6H_4$	$C_{12}H_{14}N_2O_6S$	83-84	B	23(A)	-	+ 6	+27	+ 31	+ 52*	+108*	-
7	4-$NO_2C_6H_4$	$C_{12}H_{14}N_2O_6S$	125-126	A	51(A)	-	- 2	+42	+ 47*	+ 99*	+ 98*	+ 24
8	2-$CF_3C_6H_4$	$C_{13}H_{14}F_3NO_4S$	93-94	B	29(A)	-	-29	- 3	- 2	- 10	- 28	+ 35
9	3-$CF_3C_6H_4$	$C_{13}H_{14}F_3NO_4S$	58-59	C	23(A)	-	+24	-12	+ 41	+ 53*	+ 75*	+ 29
10	4-$CF_3C_6H_4$	$C_{13}H_{14}F_3NO_4S$	99-101	B	26(A)	-	-	- 1	- 29	+ 4	+ 26	+ 21
11	3-FC_6H_4	$C_{12}H_{14}FNO_4S$	57-59	B	34(A)	-	-	-28	+ 10	+ 12	+ 41*	+ 57*
12	4-FC_6H_4	$C_{12}H_{14}FNO_4S$	113-115	B	40(A)	-	-	- 2	+ 21	+ 18	+ 65*	+103*
13	3-$CH_3OC_6H_4$	$C_{13}H_{17}NO_5S$	63-64	C	43(A)	-	-	-16	- 37	+ 1	- 13	+ 74*
14	4-$CH_3OC_6H_4$	$C_{13}H_{17}NO_5S$	112-113	B	35(A)	-	-	+22	+ 25	+ 64*	+ 23	-
15	3-$C_6H_5C_6H_4$	$C_{18}H_{19}NO_4S$	89-90	B	36(A)	-	-13	-11	+ 14	- 13	± 0	- 28
16	4-$C_6H_5C_6H_4$	$C_{18}H_{19}NO_4S$	118-120	B	36(A)	-	- 4	+31*	+ 65*	+ 79*	+ 75*	+ 66*
17	3-$CH_3COC_6H_4$	$C_{14}H_{17}NO_5S$	98-99	B	19(A)	-	-	+22	- 10	+ 39	+ 40	-
18	3-$CH_3OCOC_6H_4$	$C_{14}H_{17}NO_6S$	101-102	B	29(A)	-	+14	-25	+ 8	- 25	+ 11	-
19	3-$CH_3SO_2C_6H_4$	$C_{13}H_{17}NO_6S_2$	153-154	A	18(A)	-	-	- 9	- 29	- 10	- 20	-
20	4-$C_6H_5CH_2OC_6H_4$	$C_{19}H_{21}NO_5S$	103-105	B	33(A)	-	-	+20	+ 17	+ 22	+ 14	-
21	4-HOC_6H_4	$C_{12}H_{15}NO_5S$	162-163	A	52[e]	-	+10	-27	+ 7	+ 14	+ 4	-
22	4-$H_2NC_6H_4$	$C_{12}H_{16}N_2O_4S$	185-186	A	57[e]	-	-	+23	+ 28	+ 72*	- 11	-
23	4-$CH_3C_6H_4$	$C_{13}H_{17}NO_4S$	147-149	B	29(A)	-	-	-12	+ 8	+ 21	+ 59*	-
24	2,6-$(CH_3)_2C_6H_3$	$C_{14}H_{19}NO_4S$	106-107	B	26(A)	-	- 9	+13	- 21	+ 15	+ 16	+ 44
25	3,5-$(CH_3)_2C_6H_3$	$C_{14}H_{19}NO_4S$	100-102	B	35(A)	-	+19	- 5	+ 21	+ 11	± 0	+ 5
26	3,4-$Cl_2C_6H_3$	$C_{12}H_{13}Cl_2NO_4S$	104-105	B	23(A)	-	-	-22	+ 30	+ 24	+ 47	-
27	3-CF_3-4-ClC_6H_3	$C_{13}H_{13}ClF_3NO_4S$	110-111	B	42(A)	+ 9	+28	+11	- 3	+ 44	+ 75*	- 6
28	3,5-$(CF_3)_2C_6H_3$	$C_{14}H_{13}F_6NO_4S$	77-79	B	34(A)	-	-	-16	- 34	- 12	+ 18	-
29	α-naphthyl	$C_{16}H_{17}NO_4S$	139-140	B	39(A)	-	-	-21	+ 20	+ 23	+ 47*	-
30	β-naphthyl	$C_{16}H_{17}NO_4S$	110-111	A	28(A)	-	-	+14	- 12	+ 39	+ 64*	-
31	2-pyridyl	$C_{11}H_{14}N_2O_4S$	59-60	B	10(A)	-	-	+21	+ 36	+ 49*	+ 88*	-
32	3-pyridyl	$C_{11}H_{14}N_2O_4S$	59-61	B	46(A)	-	- 2	+ 5	+ 1	+ 58*	+ 7	-
33	2-thienyl	$C_{10}H_{13}NO_4S_2$	100-102	B	28(A)	-	-	+11	+ 48*	+ 92*	+125*	-
34	3-thienyl	$C_{10}H_{13}NO_4S_2$	98-99	B	35(A)	-	+20	- 6	+ 20	+ 25	- 1	-
35	2-furyl	$C_{10}H_{13}NO_5S$	74-76	B	29(A)	-	+21	-29	- 1	- 23	- 8	-

[a]C, H, N analyses were within ± 0.4% of the calculated values. [b]A, 95% EtOH; B, i-PrOH; C, cyclohexane. [c]Yields were not optimized except for **1**. [d]*Significantly different from controls. $P < 0.05$ Dunnett *t*-test. [e]Prepared as described in the experimental section.

Table II. Amnesia-reversal activity (PA-1 test) of 5-alkoxy-1-arylsulfonyl-2-pyrrolidinones.



No	R	Ar	Formula[a]	mp(°C)	Recrystn[b] solvent	Yield %[c] (method)	% retention vs controls[d] at the following mg/kg oral doses					
							12.5	25	50	100	200	400
36	H	C_6H_5	$C_{10}H_{11}NO_4S$	121-122	B	41[e]	-19	+26	+ 11	+ 42	+ 46*	-
37	$COCH_3$	C_6H_5	$C_{12}H_{13}NO_5S$	153-155	A	72[e]	-16	-14	+ 9	+ 2	+ 30	-
38	CH_3	C_6H_5	$C_{11}H_{13}NO_4S$	121-123	B	28(A)	-	- 9	+ 23	+ 69*	+105*	+63
39	$(CH_2)_2CH_3$	C_6H_5	$C_{13}H_{17}NO_4S$	63-65	C	61(A)	+ 7	+50*	+ 96*	+ 72*	+ 82*	-
40	$CH(CH_3)_2$	C_6H_5	$C_{13}H_{17}NO_4S$	158-160	A	61(A)	+ 4	+54*	+ 95*	+ 72*	+ 65*	+42*
41	$CH(CH_3)_2$	$3\text{-}CF_3C_6H_4$	$C_{14}H_{16}F_3NO_4S$	101-102	B	49(A)	-	+ 6	+ 55*	+ 89*	+110*	+43*
42	$CH(CH_3)_2$	$4\text{-}C_6H_5C_6H_4$	$C_{19}H_{21}NO_4S$	135-137	A	43(A)	-	+31	+ 38	+ 73*	+ 50*	-
43	$CH(CH_3)_2$	$4\text{-}NO_2C_6H_4$	$C_{13}H_{16}N_2O_6S$	153-154	A	43(A)	-17	+34	+ 82*	+ 95*	+198*	+58*
44	$CH(CH_3)_2$	$4\text{-}CH_3OC_6H_4$	$C_{14}H_{19}NO_5S$	97-98	B	53(A)	-	+13	+ 17	+ 10	+ 43*	-
45	$CH_2CH{=}CH_2$	C_6H_5	$C_{13}H_{15}NO_4S$	54-56	C	13(B)	-	+ 4	+ 66*	+ 61*	- 12	-
46	$(CH_2)_3CH_3$	C_6H_5	$C_{14}H_{19}NO_4S$	46-48	B+i-Pr_2O	32(A)	-	+ 7	+ 18	+112*	+ 68*	-
47	$CH_2CH(CH_3)_2$	C_6H_5	$C_{14}H_{19}NO_4S$	70-71	B	67(A)	-	- 7	+ 17	+ 71*	+101*	-
48	$(CH_2)_4CH_3$	C_6H_5	$C_{15}H_{21}NO_4S$	oil[f]	-	76(A)	-10	+ 34	+ 48	+ 56*	+123*	-
49	$(CH_2)_5CH_3$	C_6H_5	$C_{16}H_{23}NO_4S$	oil[f]	-	32(A)	-14	+ 27	+ 14	+ 76*	+ 49*	-
50	cyclopentyl	C_6H_5	$C_{15}H_{19}NO_4S$	96-97	A	55(A)	-13	- 15	- 1	- 32	+ 25	-24
51	cyclohexyl	C_6H_5	$C_{16}H_{21}NO_4S$	108-109	B	28(A)	+30	+ 18	+ 39	+ 48*	+ 96*	+67*
52	$CH_2C_6H_5$	C_6H_5	$C_{17}H_{17}NO_4S$	81-82	B	27(B)	-	- 3	- 6	- 5	+ 22	+46

a,b,c,d,eSee footnotes in table I. [f]Purified by chromatography on silica gel and eluting with *n*-hexane - EtOAc (2:1).

— replacement of the phenyl with a naphthyl group (**29**, **30**) or with a heterocyclic ring (**31–35**) did not give favourable results and was not further pursued;

— the potency is maximal with the ethoxy group on position 5. Replacement of ethoxy with *n*-propoxy (**39**) and isopropoxy (**40**) narrows the active dose range. This effect is more pronounced with elongation of the alkyl chain up to *n*-hexyloxy (**49**) or shortening of it (**38**), while branching of the alkyl chain (**39** *vs* **40**; **46** *vs* **47**) or ring formation (**51**) seems to have marginal effects, except for cyclopentyloxy (**50**). An aralkyl chain (**52**) removes all activity;

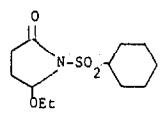
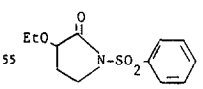
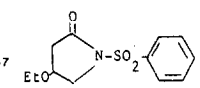
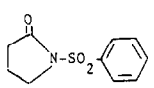
— compounds **36** and **37** have little or no activity probably reflecting their tendency to lose the 5-substituent and to form *N*-acyliminium ions, and so become hydrolytically labile;

— hydrogenation of the phenyl ring dramatically reduces the activity of the molecule (compare **53** *vs* **1**);

— the most surprising finding was that moving the ethoxy group to the 3 or 4 position (**55**, **57**) or its removal (**58**) abolished the activity. However, we and others [21] have observed that suitable derivatization of **58** could restore activity, albeit weak.

Some compounds with promising anti-amnesic profiles (**1**, **7**, **16**, **40**, **41**, **43**) were submitted for secondary evaluation. They proved to be more effective than piracetam and aniracetam in reversing ECS-induced amnesia in the PA-2 test (table IV) and scopolamine-induced amnesia (PA-3 test) (table V). Again **1** was the most potent compound, far superior to the reference drugs. Some interesting observations

Table III. Anti-amnesic activity in PA-1 test of miscellaneous compounds.

No	Structure	% retention vs controls* at the following mg/kg oral doses 25	50	100	200	400
53		-31	-39	+33	+53*	-
55		+20	+35	- 1	-19	-
57		-22	+12	-25	+ 2	-
58[b]		-17	+32	+15	+13	+3
PIRACETAM		+19	+10	+48*	+20	-
ANIRACETAM		+39	+77*	+88*	+32	-

a*Significantly different from controls. $P < 0.05$ Dunnett *t*-test. [b][21].

Table IV. Anti-amnesic activity in PA-2 test of selected compounds.

Compound	% retention vs controls[a] at the following mg/kg oral doses 6.25	12.5	25	50	100	200	400
1	+20	+70*	+104*	+137*	+187*	+115*	-
7	-	-	0	+ 40	+ 49*	+ 70*	-
16	-	-	+ 5	+ 43	+105*	+ 76*	-
40	-	-	+ 20	+ 26	+ 99*	+ 66*	-
41	-	-	+ 24	+ 87*	+145*	+ 93*	-
43	-	-	+ 10	+ 56*	+ 83*	+101*	-
PIRACETAM[b]	-	-	-	0	+ 16	+ 20	+11
ANIRACETAM	-	-	-	+ 11	+ 3	+ 92*	+15

a*Significantly different from controls. $P < 0.05$ Dunnett *t*-test. [b]+ 56% at 800 mg/kg *po*.

Table V. Anti-amnesic activity in PA-3 test of selected compounds.

Compound	% retention vs controls[a] at the following mg/kg oral doses 6.25	12.5	25	50	100	200	400
1	-	+38	+ 86*	+103*	+143*	+ 80*	-
7	-	+33	+ 61*	+ 88*	+111*	+100*	-
16	+14	+62*	+ 88*	+101*	+145*	+ 71*	-
40	+30	+43	+ 88*	+129*	+116*	+ 78*	-
41	-	+41	+ 93*	+125*	+148*	+ 49*	-
43	+12	+61*	+110*	+112*	+132*	+ 84*	-
PIRACETAM	-	-	+ 29	+ 76*	+ 2	- 13	-
ANIRACETAM	-	-	- 2	+ 58*	+ 94*	+ 30	-

a*Significantly different from controls. $P < 0.05$ Dunnett *t*-test.

can be made when the pharmacological results obtained in the three experimental models for the compounds **7**, **16**, **40**, **41**, **43** are compared with those of the leader compound **1** (table VI). First, in the PA-1 test when the compounds were given immediately after training, **1** and **40** were the most effective agents. Compounds substituted on the phenyl ring, *ie* 4-nitro (**7**) and 4-phenyl (**16**) derivatives of **1** and 4-nitro (**43**) and 3-trifluoromethyl (**41**) derivatives of **40**, were 1/2–1/4 as effective as the corresponding parent compound. Second, in ECS-treated animals the facilitatory effect of these nootropics on memory retrieval (PA-2 test) was substantially different from that on memory consolidation (PA-1 test). In fact, when the compounds were given 90 min before the retention test instead of immediately following training, compounds **7**, **16** and **40** showed relatively poor activity, 1/8–1/16 that of **1**. It is worth noting that aniracetam and also piracetam were poorly effective. Good pro-mnemonic activity was, on the contrary, observed with **41** and **43** and the former showed the best activity profile. When the interval between treatment and retention test was 45 min instead of 90 min, no substantial difference in activity was observed (data not shown). This suggests that the poor activity of **7**, **16** and **40** cannot be ascribed to the low bioavailability at the moment of the test session. Third, when the six compounds were administered immediately after training to scopolamine-treated animals, they showed very potent anti-amnesic activity and comparable activity profiles. These data indicate that structural modifications of 5-alkoxy-1-arylsulfonyl-2-

Table VI. Anti-amnesic profile of selected compounds and reference drugs. a. Approximate equipotent effective doses. b. Range of doses that significantly increase retention performance. c. Anti-amnesic index, *ie* sum of % retention of each dose causing statistically significant results.

Compound	PA-1 test			PA-2 test			PA-3 test		
	a	b	c	a	b	c	a	b	c
1	12.5	12.5-400	625	12.5	12.5-200	613	25	25-200	412
7	50	50-200	244	200	100-200	119	25	25-200	360
16	50	25-400	316	75	100-200	181	12.5	12.5-200	467
40	25	25-400	328	100	100-200	165	25	25-200	411
41	50	50-400	297	50	50-200	325	25	25-200	415
43	50	50-400	433	50	50-200	240	12.5	12.5-200	499
Piracetam	100	100	48	800	800	56	50	50	76
Aniracetam	50	50-100	165	200	200	92	50	50-100	152

Table VII. Anti-hypoxic activity of selected compounds.

Test	*Effective dose (mg/kg ip) for the following compounds*							
	1	*7*	*16*	*40*	*41*	*43*	*Pira-cetam*	*Anira-cetam*
$NaNO_2$-induced hypoxia[a]	100	[b]	200	200	80	200	1000	100
KCN-induced hypoxia[c]	200	200	200	200	75	150	500	400

[a]Activity expressed as 50% increase of survival time *vs* controls; [b]Inactive at the highest dose tested: 400 mg/kg *ip*; [c]Activity expressed as 30% increase of survival time *vs* controls.

pyrrolidinones induce changes in activity according to the experimental procedure. These changes can be due to a number of possible factors, including the different mechanisms by which ECS and scopolamine cause memory impairment and the different memory processes involved according to the schedule of treatment. As the most promising compounds are those with the lowest variation in activity, **1**, **41** and **43** possess the optimal structural requirements for minimizing task-dependent changes in the spectrum of anti-amnesic activity.

Beside their interesting anti-amnesic profile, compounds **1**, **7**, **16**, **40**, **41**, **43** also showed good anti-hypoxic properties. When administered intraperitoneally they protected the brain against chemically induced hypoxia better than piracetam and, on the whole, with a potency comparable to aniracetam (table VII). Compound **41** was the most effective anti-hypoxic agent. Biochemical and electrophysiological data confirmed that **41** protected the brain against experimental hypoxias of different origin [22].

In conclusion, two of the most promising compounds of this series have nootropic properties superior in many respects to those of piracetam and aniracetam: **1**, coded RU 35929 and **41**, coded RU 47067. They have been chosen for further pharmacological and toxicological evaluation.

Experimental protocols

Chemistry

Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. IR spectra were recorded in nujol mull with a Perkin-Elmer model 881 spectrophotometer. NMR spectra were determined on Varian T-60 or Varian VXR-200 s spectrometers; chemical shifts are reported in δ units downfield from Me_4Si; the coupling constants are expressed in Hz; standard abbreviations are used. Thin layer chromatography was carried out on silica gel 60 F 254 precoated glass plates (Merck, 0.25 mm). Column chromatography was performed on silica gel 60 (Merck, 230–400 mesh). Microanalyses were performed by the Analytical Laboratory Service of the Department of Industrial Chemistry of the University of Milan, and agreed with theoretical values to within ± 0.4% except where indicated. Piracetam was kindly donated by Istituto Chemioterapico Italiano Fine Chemicals, Lodi, Italy and aniracetam was synthesized according to a patented procedure [23].

General procedure for the preparation of 5-alkoxy-2-pyrrolidinones

Method A: by reduction of succinimide

5-Isopropoxy-2-pyrrolidinone. Sodium borohydride (24 g, 634 mmol) was added in one portion at – 5°C to a stirred solution of succinimide (43 g, 434 mmol) in isopropanol (1300 ml). The reaction mixture was stirred at –5–0°C for 4 h while adding 10 drops of 2 N HCl in isopropanol at regular intervals of 15 min. The pH of the mixture was finally brought to 2 by the addition of a further 2 N HCl in isopropanol and stirring was continued at 0°C for 2 h. After neutralization with a saturated solution of KOH in isopropanol at –5–0°C, the solvent was evaporated under reduced pressure and the residue repeatedly extracted with chloroform. The solution was filtered through celite, washed with water, dried over Na_2SO_4 and evaporated to yield 48 g (77%) of a white powder, used in the next step. mp = 68–71°C. A sample recrystallized from benzene-hexane melted at 70–72°C; IR 3183 and 3112 (NH), 1705 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (d, 6H, *J* = 6 Hz, CH_3), 1.60–2.72 (m, 4H, CH_2-CH_2), 3.67 (m, 1H, *J* = 6 Hz, $CHCH_3$), 4.98 (dd, 1H, *J* = 6 Hz, 2 Hz, 5-CH), 7.92 (br, 1H, NH). Anal $C_7H_{13}NO_2$ (C, H, N).

Reduction of succinimide in the appropriate alcohol following this procedure afforded: 5-*n*-propoxy-2-pyrrolidinone, mp = 51–52°C after recrystallization from *n*-hexane; 66% yield. Anal $C_7H_{13}NO_2$ (C, H, N). 5-Methoxy-2-pyrrolidinone [24] bp = 111–118°C, 0.1 mbar in the kugelrohr; 42% yield. 5-Ethoxy-2-pyrrolidinone [9] mp = 48–51°C; 75% yield. 5-*n*-Butoxy-2-pyrrolidinone [24] mp = 36–38°C; 33% yield. All 5-alkoxy-2-pyrrolidinones were used in the next step without purification. Succinimide was the only impurity detected in the NMR spectrum.

Method B: from 5-hydroxy-2-pyrrolidinone

A solution of 5-hydroxy-2-pyrrolidinone [10] (7.07 g, 70 mmol) in the appropriate absolute alcohol (175 ml) was stirred at 60–65°C for 3 h in the presence of amberlite IR - 120

(H^+ form, 3.5 g). The resin was filtered off and the solvent was cautiously evaporated under vacuum (0.3 mbar). The residue was purified by chromatography on silica gel eluting with ethyl acetate. Solid compounds were recrystallized from the solvent indicated to obtain analytical samples. Oily compounds decomposed on attempted distillation and were analyzed after thorough elimination of ethyl acetate. The following compounds were prepared by method B: 5-isobutyloxy-2-pyrrolidinone, mp = 31–32°C (Et_2O-pentane) 74% yield. Anal $C_8H_{15}NO_2$ (C, H, N). 5-*n*-pentyloxy-2-pyrrolidinone, mp = 42–43°C (Et_2O-pentane) 63% yield. Anal $C_9H_{17}NO_2$ (C, H, N). 5-cyclopentyloxy-2-pyrrolidinone, mp = 90–91°C (i-Pr_2O) 62% yield. Anal $C_9H_{15}NO_2$ (C, H, N). 5-*n*-hexyloxy-2-pyrrolidinone, mp = 35–37°C (hexane) 68% yield. Anal $C_{10}H_{19}NO_2$ (C, H, N). 5-cyclohexyloxy-2-pyrrolidinone, mp = 95–96°C (i-Pr_2O) 41% yield. Anal $C_{10}H_{17}NO_2$ (C, H, N). 5-benzyloxy-2-pyrrolidinone, mp = 80–82°C (benzene-hexane) 72% yield. Anal $C_{11}H_{13}NO_2$ (C, H, N). 5-allyloxy-2-pyrrolidinone, pale yellow oil which should be used immediately. Unstable at room temperature. 26% yield. Anal $C_7H_{11}NO_2$. C: calcd, 59.56; found, 57.81; N: calcd, 9.92; found 9.10.

The use of amberlyst 15 ion exchange resin allows this exchange reaction to be run at about 21°C for 3 h without a substantial variation of the reported yields.

Arylsulfonyl chlorides. Intermediate chlorides were purchased (Aldrich Chemical Co) or synthetized as described. 4-Phenylmethoxybenzenesulfonyl chloride, to our knowledge, has never been previously described. 2-Chlorobenzenesulfonyl chloride [25]; 3-chlorobenzenesulfonyl chloride [25]; 2-trifluoromethylbenzenesulfonyl chloride [26]; 3-trifluoromethylbenzenesulfonyl chloride [27]; 4-trifluoromethylbenzenesulfonyl chloride [28]; 3-fluorobenzenesulfonyl chloride [29]; 3-methoxybenzenesulfonyl chloride [30]; 3-phenylbenzenesulfonyl chloride [31]; 4-phenylbenzenesulfonyl chloride [32]; 3-acetylbenzenesulfonyl chloride [33]; methyl ester of 3-chlorosulfonylbenzoic acid [34];3-chlorosulfonylphenylmethylsulfone [35]; 2,6-dimethylbenzenesulfonyl chloride [29]; 3,5-dimethylbenzenesulfonyl chloride [29]; 3,4-dichlorobenzenesulfonyl chloride [36]; 4-chloro-3-trifluoromethylbenzenesulfonyl chloride [37]; 3,5-ditrifluoromethylbenzenesulfonyl chloride [38]; 2-pyridinesulfonyl chloride [39]; 3-pyridinesulfonyl chloride [40]; 2-thiophenesulfonyl chloride [41]; 3-thiophenesulfonyl chloride [42]; 2-furansulfonyl chloride [43].

4-phenylmethoxybenzenesulfonyl chloride

A solution of benzyl bromide (27.95 g, 163 mmol) in 95% ethanol was added to a stirred solution of sodium salt of 4-hydroxybenzenesulfonic acid dihydrate (30 g, 129 mmol) in 15% aqueous sodium hydroxide (48 ml). The reaction mixture was heated at reflux for 5 h, left overnight at room temperature and the solid collected by filtration was dried under vacuum over phosphorus pentoxide. The sodium salt of 4-phenylmethoxybenzenesulfonic acid so obtained (34.3 g) was made to react with phosphorus pentachloride (25 g, 120 mmol) in methylene chloride (430 ml) for 7 h at reflux. The solvent was evaporated and the residue was taken up in toluene (250 ml), filtered through celite and the resulting solution evaporated under reduced pressure. The solid was recrystallized from diisopropyl ether to give 23.95 g (65%) of the expected chloride, mp = 101–103°C ^1H NMR ($CDCl_3$) δ 5.08 (s, 2H, CH_2), 6.97 (d, 2H, J = 8 Hz, aromatic meta to sulfonyl group), 7.27 (s, -5H, phenyl), 7.82 (d, 2H, J = 8 Hz, aromatic ortho to sulfonyl group). Anal $C_{13}H_{11}ClO_3S$ (Cl).

General procedure for the synthesis of 5-alkoxy-1-arylsulfonyl-2-pyrrolidinones

5-Ethoxy-1-(phenylsulfonyl)-2-pyrrolidinone(1)

Method A. A 1.6 M solution of *n*-butyllithium in hexane (43.7 ml, 70 mmol) was added slowly at – 70°C to a stirred solution of 5-ethoxy-2-pyrrolidinone [9] (9 g, 70 mmol) in freshly distilled, dry tetrahydrofuran (280 ml) under nitrogen. After 45 min of stirring, a solution of freshly distilled benzenesulfonyl chloride (12.4 g, 70 mmol) in the same solvent (20 ml) was added dropwise and stirring was continued at – 70°C for 2 h. The cooling bath was removed and the reaction mixture was stirred until room temperature was attained. The solvent was evaporated under reduced pressure, the residue was triturated with water and collected by filtration. Recrystallization from ethanol gave 12.8 g (68%) of **1**. IR 1740 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7 Hz, CH_3), 2.2–2.76 (m, 4H, CH_2-CH_2), 3.54–3.84 (m, 2H, CH_2O), 5.65 (d, 1H, J = 4.9 Hz, 5-CH), 7.48–7.68 (m, 3H, meta and para aromatic), 8.06–8.11 (m, 2H, ortho aromatic).

Method B. A solution of 5-ethoxy-2-pyrrolidinone [9] (2 g, 15.5 mmol) in dry diethyl ether (20 ml) was added under argon at 0°C to a solution of 95% sodium bis (trimethylsilyl) amide (3.3 g, 17 mmol) in the same solvent (300 ml). After 30 min of vigorous stirring, a solution of freshly distilled benzenesulfonyl chloride (3 g, 17 mmol) in dry diethyl ether (6 ml) was added dropwise at 0°C. The reaction mixture was then stirred for 2 h at room temperature. The solvent was evaporated, the residue was partitioned between chloroform and water, the organic layer was filtered through celite, dried (Na_2SO_4) and evaporated. The residue was recrystallized from ethanol to give 2.4 g (57.5%) of **1**.

Compounds listed in tables I and II were prepared from the corresponding 5-alkoxy-2-pyrrolidinones and arylsulfonyl chlorides following either method A or B as shown.

*5-Ethoxy-1-(4-hydroxyphenylsulfonyl)-2-pyrrolidinone (**21**)*

A solution of **20** (6.3 g, 16.7 mmol) in 95% ethanol (150 ml) was hydrogenated in the presence of 10% palladium on carbon (1.2 g) at room temperature and atmospheric pressure. The reaction mixture was filtered through celite, the solvent was evaporated and the residue recrystallized from ethanol to give 2.5 g (52%) of **21**. IR 3450 (OH), 1720 (CO) cm^{-1}; ^1H NMR ($CDCl_3$ + 20% DMSO-d_6) δ 1.17 (t, 3H, J = 7 Hz, CH_3), 1.67–2.80 (m, 4H, CH_2-CH_2), 3.37–3.83 (m, 2H, CH_2O). 5.52 (d, 1H, J = 3 Hz, 5-CH), 6.77 (d, 2H, J = 9 Hz, meta aromatic), 7.72 (d, 2H, J = 9 Hz, ortho aromatic), 9.87 (s, 1H, OH).

*1-(4-Aminophenylsulfonyl)-5-ethoxy-2-pyrrolidinone (**22**)*

A solution of **7** (4.5 g, 14.3 mmol) in 95% ethanol (200 ml) was hydrogenated in the presence of 10% palladium on carbon (0.5 g) at room temperature and atmospheric pressure. Chloroform was added to dissolve the precipitate and the reaction mixture was filtered through celite. Evaporation of solvents left a residue which was recrystallized from ethanol to give 2.32 g (57%) of **22**. IR 3500 and 3400 (NH_2), 1720 (CO) cm^{-1}; ^1H NMR ($CDCl_3$-DMSO-d_6 1:1) δ 1.15 (t, 3H, J = 7 Hz, CH_3), 1.83–2.63 (m, 4H, CH_2-CH_2), 3.32–3.75 (m, 2H, CH_2O), 5.50 (d, 1H, J = 3 Hz, 5-CH), 5.72 (br, 2H, NH_2), 6.50 (d, 2H, J = 8 Hz, meta aromatic), 7.47 (d, 2H, J = 8 Hz, *ortho* aromatic).

*5-Hydroxy-1-phenylsulfonyl-2-pyrrolidinone (**36**)*

4-Pentenoyl chloride [11] (3.36 g, 28 mmol) was added to a dispersion of benzenesulfonamide (3.47 g, 22 mmol) in phos-

phorus oxychloride (5 ml) and stirring was continued at room temperature for 20 h. The solution obtained was cautiously evaporated under reduced pressure and the oily residue (5 g) dissolved in 1,4-dioxane (150 ml) and water (50 ml). Osmium tetroxide (51 mg, 0.2 mmol) and after 20 min sodium periodate (5.1 g, 23.8 mmol) were added and the reaction mixture was stirred for 1 h. A second amount of sodium periodate (5.1 g) was added and stirring was continued for a further 2 h. The inorganic salts were filtered off, the resulting solution decolorized with charcoal, filtered and evaporated under reduced pressure. The oily residue was triturated with diethyl ether to afford 2.07 g (39% based on sulfonamide) of a white powder, homogeneous on TLC (silica gel, EtOAc-*n*-hexane 1:1) with mp = 114–116°C. The analytical sample was recrystallized from 2-propanol. mp = 121–122°C. IR 3308 (OH), 1710 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.87–2.88 (m, 4H, CH_2-CH_2), 4.10 (br, 1H, OH), 5.90 (dd, 1H, *J* = 6 Hz and 2 Hz, 5-CH), 7.23–7.53 (m, 3H, *meta* and *para* aromatic), 7.80–8.00 (m, 2H *ortho* aromatic).

*5-Acetoxy-1-phenylsulfonyl-2-pyrrolidinone (**37**)*
A solution of **36** (2.9 g, 12 mmol) in acetic anhydride (58 ml) was heated at reflux for 90 min, then cooled to room temperature and concentrated under reduced pressure. Acetic anhydride was completely removed by azeotropic distillation with toluene. The solid residue was recrystallized from 95% EtOH to give 2.45 g (72%) of **37**: mp = 153–155°C. IR 1760 (OCO), 1740 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.02 (s, 3H, CH_3), 2.07–2.70 (m, 4H, CH_2-CH_2), 6.60 (d, 1H, *J* = 4 Hz, 5-CH), 7.35–7.97 (m, 5H, aromatic).

*1-Cyclohexylsulfonyl-5-ethoxy-2-pyrrolidinone (**53**)*
Condensation of cyclohexylsulfonyl chloride [44] (7.63 g, 41.8 mmol) with 5-ethoxy-2-pyrrolidinone (5.4 g, 41.8 mmol) according to the procedure described for compound **1** (method A) afforded 3.4 g (29.5%) of **35** as a pale yellow oil, after chromatography on a silica gel column eluting with *n*-hexane: ethyl acetate 8:2. The analytical sample was obtained after elimination of residual solvents at 45°C, 0.13 mbar for 4 h. IR 1740 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.08–3.04 (m, 17H, CH_3 and cyclohexyl CH_2), 3.46–3.88 (m, 3H, CH_2O and cyclohexyl-CH), 5.42 (d, 1H, *J* = 5 Hz, 5-CH).

*3-Hydroxy-1-phenylsulfonyl-2-pyrrolidinone (**54**)*
The solid residue obtained by condensation of benzenesulfonyl chloride (10.19 g, 57.7 mmol) with 3-trimethylsilyloxy-2-pyrrolidinone [13] (10 g, 57.7 mmol) according to the procedure described for compound **1** (method A) was dissolved in 1 N HCl (10 ml) and THF (30 ml) and stirred for 15 min to remove the trimethylsilyl group. The solution was poured into ice water (400 ml) and extracted with ethyl acetate (100 ml x 3). The combined extracts were dried (Na_2SO_4), the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel with *n*-hexane: ethyl acetate 1:1 as eluent to give 5.7 g (41%) of **54** after recrystallization from ethanol. mp = 111–112°C. IR 3380 (OH), 1743 and 1717 (CO); ^1H NMR ($CDCl_3$) δ 1.88–2.08 and 2.42–2.56 (2 m, 2H, 4-CH_2), 2.85 (s, 1H, OH), 3.72 (dt, *J* = 10 and 6.3 Hz, 5-CH), 3.90–4.01 (m, 1H, 5-CH), 4.32 (dd, 1H, *J* = 10 and 8.2 Hz, 3-CH), 7.51–7.73 (m, 3H, *meta* aromatic), 8.02–8.08 (m, 2H, *ortho* aromatic). Anal $C_{10}H_{11}NO_4S$ (C, H, N).

*3-Ethoxy-1-phenylsulfonyl-2-pyrrolidinone (**55**)*
Ethereal diazoethane was prepared from *N*-ethyl *N*-nitrosourea as reported by Marshall and Partridge [45] and immediately used. Titration was carried out with ethereal benzoic acid and back titration with 0.1 N NaOH to the phenolphthalein end point. A 0.37 M solution of diazoethane in diethyl ether (10 ml, 3.7 mmol) was added at – 10°C to a stirred solution of **54** (0.95 g, 3.9 mmol) in methylene chloride (35 ml) containing a small amount (≈ 20 mg) of aluminium chloride as catalyst. The rate of addition was controlled in order to avoid an excess of diazoethane (yellow color). A second small amount of aluminium chloride was added followed by dropping of the ethereal solution of diazoethane (5 ml), while keeping the temperature at – 10°C. The mixture was allowed to come to room temperature, washed with 5% $NaHCO_3$ and the organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography on silica gel with *n*-hexane: ethyl acetate 1:1 as eluent to give 0.42 g (39%) of **55** as a pale yellow oil. IR 1744 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (t, 3H, *J* = 7 Hz, CH_3), 1.90–2.14 and 2.28–2.44 (2 m, 2H, 4-CH_2), 3.44–4.08 (m, 5H, OCH_2, 3-CH and 5-CH_2), 7.45–7.76 (m, 3H, *meta* aromatic), 8.02–8.07 (m, 2H, *ortho* aromatic). Anal $C_{12}H_{15}NO_4S$ (C, H, N).

*4-Hydroxy-1-phenylsulfonyl-2-pyrrolidinone (**56**)*
The solid residue obtained by condensation of benzenesulfonyl chloride. (7 g, 40 mmol) with 4-trimethylsilyloxy-2-pyrrolidinone [14] (7 g, 40 mmol) according to the procedure described for compound **1** (method A) was triturated for 15 min, with 8% HCl (50 ml) to remove the trimethylsilyl group. The solid was collected by filtration, dried under vacuum and recrystallized from benzene: ethyl acetate 10:1 to give 4.5 g (47%) of **56** as yellow powder. mp = 119–120°C. IR 3480 (OH), 1708 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.41 (dd, 1H, *J* = 17.6 and 2.4, 3-CH), 2.67 (dd, 1H, *J* = 17.6 and 5.9, 3-CH), 3.89 (dd, 1H, *J* = 10.7 and 2.3, 5-CH), 3.99 (dd, 1H, *J* = 10.7 and 4.6, 5-CH), 4.42 (br, 1H, 4-CH), 5.09 (d, 1H, *J* = 3.7, OH), 7.51–7.70 (m, 3H, *meta* aromatic), 8.00–8.06 (m, 2H *ortho* aromatic). Anal $C_{10}H_{11}NO_4S$ (C, H, N).

*4-Ethoxy-1-phenylsulfonyl-2-pyrrolidinone (**57**)*
A 0.3 M solution of diazoethane in diethyl ether (15 ml, 4.5 mmol; see preparation of **55**) was added at – 10°C to a stirred solution of **56** (2.8 g, 11.6 mmol) in methylene chloride (150 ml) containing six drops of boron trifluoride etherate as catalyst. The addition of ethereal diazoethane and boron trifluoride etherate was repeated 5 times for 5 h while keeping the temperature between – 12°C and – 7°C. The solution was allowed to come to room temperature, washed with 5% $NaHCO_3$ and the organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography on silica gel with *n*-hexane: ethyl acetate 1:1 as eluent to give 0.7 g (22%) of **57** after recrystallization from ethanol. mp = 110–112°C. IR 1743 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (t, 3H, *J* = 7 Hz, CH_3), 2.42–2.75 (m, 2H, 3-CH_2) 3.45 (dq, 2H, *J* = 7 and 2.2 Hz, CH_2O), 3.85–4.17 (m, 3H, 4-CH and 5-CH_2), 7.44–7.72 (m, 3H, *meta* aromatic), 8.02–8.07 (m, 2H, *ortho* aromatic). Anal $C_{12}H_{15}NO_4S$ (C, H, N).

Pharmacology

Antiamnesic activity in mice (A) ECS-induced amnesia (PA-1 test). Male CD1 mice, weighing 25–30 g, from Charles River were housed in air-conditioned rooms with a light-dark cycle of 12 h (light on at 7:00 am), acclimatized to laboratory conditions for 7–12 days before being randomly assigned to treatment groups. Standard chow and tap water were available '*ad libitum*' except during the experiment. Test compounds were suspended or dissolved (piracetam) in 0.25% methocel and

administered orally in a volume of 20 ml/kg. Controls were given vehicle only. Inhibitory avoidance training and testing were carried out in a step-through apparatus consisting of a small lighted 'safe' chamber (start box 10 x 10 x 12 cm) and a dark shock chamber (22 x 16 x 12 cm) separated by a guillotine door (5 x 5 cm). During the training trial each mouse was placed in the lit chamber and after a 5-s orientation period the door was raised to allow passage to the dark chamber. Once inside the dark compartment the door was lowered and an unavoidable footshock (1 mA x 1 s) was automatically delivered by a shock generator through the grid floor of steel rods (0.3 cm diameter) set 0.3 cm apart. Immediately after the footshock, the animal was gently but quickly removed from the punishment chamber and subjected to a single transcorneal electroconvulsive shock (ECS) of 22 mA for 0.2 s (train). This treatment produced full clonic-tonic convulsion in all animals. The time between footshock and ECS never exceeded 15 s (normally 8–11 s). Mice were treated orally with various doses of the test compound or with vehicle about 20 s after ECS. The maximal dose to be tested was established from observation of gross behaviour of mice. The range of doses was selected after a pilot dose-finding study to establish the minimal effective dose and the shape of the dose–response curve for each compound. To avoid order-of-testing, half of each group were trained in a counterbalanced order between 9:00 and 11:00 am. The retention test was given 3 h after treatment. Each animal was again placed in the starting chamber, in the same order as in the acquisition session, and its step-through latency was recorded as a measure of retention. Animals not crossing into the dark box within 180 s were removed and given a score of 180 s. Results are expressed as % increase of latency *vs* the corresponding controls, *ie* mean latencies to step through of treated animals — mean latencies of corresponding controls/mean latencies of controls x 100. The significance of differences between drug-treated and vehicle-treated animals was analysed by the Dunnett *t*-test. All experiments were performed at least twice, with 10 animals in each group at each replication. Under these experimental conditions, the mean latency to entering the dark box during training was about 10 s. In the retention test, re-entry latency for mice punished by footshock reached the maximum latency of the test session (180 s), indicating that they had learned to avoid the shock chamber, whereas the latency of animals that had been punished and subjected to ECS was markedly shorter (35–45 s), indicating that amnesia was induced to some extent.

(B) ECS-induced amnesia (PA-2 test). Mice, test apparatus, inhibitory avoidance training and testing, and evaluation of the results were as described in the PA 1 test. However, the intensity of ECS was 17 mA; the interval between training and test was 24 h and the treatment was given 1.5 h prior to retention testing. Under these experimental conditions, the mean latencies of controls at the test session were comparable to those obtained in the PA-1 test, *ie* about 40 s.

(C) Scopolamine induced amnesia (PA-3 test). Mice, test apparatus, inhibitory avoidance training and testing, preparation of compounds and evaluation of the results were as described in the PA 1 test. To produce amnesia for the inhibitory avoidance task the mice were treated with scopolamine hydrobromide. The antimuscarinic drug was administered intraperitoneally, dissolved in saline, at the dose of 0.7 mg/kg, 15 min prior to training. The dose of scopolamine and the time-interval before training were chosen on the basis of the results of earlier studies [19], in order to obtain considerable impairment of retention; *ie* mean latencies of about 50 s, in comparison with 180 s (cut-off time) for the mice not treated with scopolamine. For this test, compounds were administered immediately (≈ 10 s) after training and the retention test was given 24 h later.

Antihypoxic activity in mice. A. $NaNO_2$-induced hypoxia. Male CD 1 mice from Charles River, weighing 25–30 g, randomly distributed among treatment groups, were used. Test compounds or vehicle were administered ip 30 min before administration of 160 mg/kg, sc of $NaNO_2$ (dissolved in saline, 10 ml/kg). After $NaNO_2$ treatment each animal was observed individually, and the interval until death occurred was recorded. To avoid possible day-to-day variations, a control group was included in each experiment. All experiments were normally performed at least twice, with 6–8 animals in each group at each replication. The survival time, that is, the time between administration of $NaNO_2$ and cessation of respiratory movements, was used as the parameter for evaluation. The observation period was 2 h. The mean survival time for control animals given 160 mg/kg of $NaNO_2$ ip, ranged from 30 and 40 min.

B. KCN-induced hypoxia. For this purpose male CD 1 mice (see protocol A) were given vehicle or compounds *ip* 30 min before being treated with 20 mg/kg sc of KCN. The observation period was 10 min. Survival time was used as the parameter for evaluation. Control animals survived hystotoxic hypoxia induced by 20 mg/kg of KCN sc, for 160–190 s.

Acute toxicity in mice. Male CD 1 mice fasted 16 h, were randomly distributed with respect to treatment and cage assignement. The animals were treated *po* with the test compounds or vehicle. A total of 5–10 animals were used for each dose. Individual body weights were recorded after treatment every day, except Saturday and Sunday, and deaths were recorded daily. The observation period lasted 7 d. The approximate median lethal dose (LD_{50}) was determined. All compounds listed in tables I and II had LD_{50} > 1000 mg/kg *po*, except **33** (500 mg/kg) and **34** (400 mg/kg).

Statistical analysis. The results are expressed as means ± SD. The Dunnett *t*-test was used to calculate the significance of the differences between treated groups and corresponding control groups, as shown in the tables.

Acknowledgments

The authors acknowledge the expert technical assistance of C Parini (Chemistry Dept), A Viscardi, V Spada, A Tiengo and M Rosa Leccardi (Pharmacology Dept), A Butti and A Lettieri (Analytical Dept). We thank T Ranucci and C Brambilla who typed the manuscript.

References

1 Coper H, Herrmann WM (1988) *Pharmacopsychiatria* 21, 211–217
2 Brody JA (1984) *Publ Health Rep* 99, 468–474
3 Hershenson FM, Moos WH (1986) *J Med Chem* 29, 1125–1130
4 Moos W, Davis R, Schwarz R, Gamzu E (1988) *Med Res Rev* 8, 353–391
5 Mattson RJ, Moon SL (1988) *Annu Rep Med Chem* 23, 29–38
6 Heise G (1987) *Trends Pharmacol Sci* 8, 65–68
7 Gamzu ER, Hoover TM, Gracon SI, Ninteman MV (1989) *Drug Dev Res* 18, 177–189
8 Mondadori C, Petschke F, Hausler A (1989) *Pharmacopsychiatria* (Suppl) 22, 102–106
9 Hubert JC, Wijnberg JBPA, Speckamp WN (1985) *Tetrahedron* 31, 1437–1441

10 Farina F, Martin MV, Paredes MC (1984) *Heterocycles* 22, 1733–1739
11 Wuonola MA, Woodward RB (1976) *Tetrahedron* 32, 1085–1095
12 Barco A, Benetti S, Pollini GP, Baraldi PG, Simoni D, Vicentini CB (1979) *Synthesis* 68–70
13 Aschwanden W, Kyburz E (1981) *Ger Offen* 3029671; (1981) *Chem Abstr* 95, 61978 h
14 Pellegata R, Pinza M, Pifferi G, Gaiti A, Mozzi R, Tirillini B, Porcellati G (1981) *Il Farmaco Ed Sci* 36, 845–855
15 Mondadori C (1979) *Pharmacol Ther* 5, 423–425
16 Bartus RT, Flicker C, Dean RL (1983) *In: Assessment in geriatric psychopharmacology* (Mark Powley Associates Inc Madison CT) Chapter 24
17 Schindler U, Rush DK, Fielding S (1984) *Drug Dev Res* 4, 567–576
18 Gamzu E (1985) *Annu NY Acad Sci* 444, 370–393
19 Galliani G, Cesana R, Barzaghi F (1987) *Med Sci Res* 15, 313–314
20 Gibson GE (1985) *In: Cerebral energy metabolism and metabolic encephalopathy* (Candless Mc, ed) Plenum Press New York and London, Chapter 3
21 Roger P, Choay P, Fournier JP (1985) European Patent 138721
22 Barzaghi F, Formento ML, Nencioni A, Galliani G (1990) *Eur J Pharmacol* 183, 1475
23 Kyburz E, Aschwanden W (1982) European Patent 44088
24 Mitzlaff M, Warning K, Rehling H (1980) *Synthesis* 315–317
25 Meerwein H, Dittmar G, Göllner R, Hafner K, Mensch F, Steinfort O (1957) *Chem Ber* 90, 841–852
26 Ashton MJ, Cook DC, Daniels CJ, Loveless AD, Pratt JT, Bull BT (1984) Ger Offen DE 330 403; (1988) *Chem Abstr* 100, 209826b
27 Hoffman RV, Saucy G, Roth GP, Scott JW (1990) *Org Synthesis Coll* Vol 7, 508–511
28 Gadient F, Jucker E, Lindemann A, Taeschler M (1962) *Helv Chim Acta* 45, 1860–1870
29 Prinsen AJ, Cerfontain H (1965) *Recl Trav Chim Pays-Bas Belg* 84, 24–30
30 Perold GW, Van Lingen PFA (1959) *Chem Ber* 92, 293–298
31 Dannley RL, Gregg EC (1954) *J Am Chem Soc* 76, 2997–3000
32 Hiroshi N (1957) Tohoku Yakka Daigaku Kiyo 4, 19–29; (1958) *Chem Abstr* 52, 7234e
33 Troeltzsch C (1963) *J Prakt Chem* 22, 192–201
34 Wegscheider R, Furcht M (1902) *Monatsch Chem* 23, 1096–1120
35 Twist RF, Smiles S (1925) *J Chem Soc* 1248–1252
36 Leslie VJ, Rose JB (1974) US Patent 3830 781
37 Hempel J, Klauke E (1971) Brit Patent 1258557; (1972) *Chem Abstr* 76, 153338k
38 Mrozik HH (1972) Ger Pffen, 2218778; (1973) *Chem Abstr* 78, 43042t
39 Boggiano BG, Petrow V, Stephenson O, Wild AM (1961) *J Pharm Pharmacol* 13, 567–574
40 Machek G (1938) *Monatsh Chem* 72, 77–92
41 Cymerman-Craig J, Vaughan GN, Warburton WK (1956) *J Chem Soc* 4114–4118
42 Arcoria A, Maccarone E, Musumarra G, Tomaselli GA (1974) *J Org Chem* 39, 1689–1691
43 Arcoria A, Maccarone E, Musumarra G, Tomaselli GA (1974) *J Org Chem* 39, 3595–3598
44 Ziegler C, Sprague JM (1951) *J Org Chem* 16, 621–625
45 Marshall JA, Partridge JJ (1968) *J Org Chem* 33, 4090–4097