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Synthesis and anticonvulsant activity of some 1,2,3,3a-tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones and pyrrolo[2,1-*b*]thiazole analogues

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Summary — Tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones **2a–l** and its analogues **3a–d** were synthesized and tested as anticonvulsant agents. Some of the compounds were effective against bicuculline-induced seizures in mice. Binding studies with the prepared compounds using [^{3}H]flunitrazepam as a ligand for the benzodiazepine receptor and [^{3}H]muscimol for the γ-aminobutyric acid (GABA) receptor, demonstrate that such compounds have no affinity for these recognition sites. On the contrary, some of these compounds reduced, at very high concentrations, the binding of [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) to recognition sites located at the GABA-coupled chloride channel. However, a lack of correlation between the anticonvulsant activity of these compounds and their capability to displace [^{35}S]TBPS binding was observed. Furthermore, an *in vitro* study on the most active compound **2a** indicates that this compound may act intact on its target(s) rather than as a hydrolyzed compound **9**.

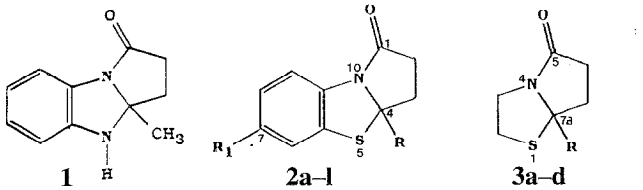
1,2,3,3a-tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones / 3,4,5,6,7,7a-hexahydro-2*H*-pyrrolo[2,1-*b*]thiazol-5-ones / anticonvulsant activity / [^{3}H]flunitrazepam / [^{3}H]muscimol / [^{35}S]TBPS / stability study

Introduction

Epilepsy is a cerebral disease characterized by seizure disorders. Different types of seizures require specific pharmacotherapeutic treatments [1]. However, currently marketed anticonvulsants do not often provide complete control of seizures, and are associated with a wide range of unwanted effects. Therefore, there is a need for new anticonvulsants with greater efficacy, specificity and lower toxicity [2]. In particular, novel agents allowing the treatment of complex types of seizures, such as those involved with the partial onset, are required.

In the mammalian brain, man included, the $GABA_A$ receptors play a major role in the pharmacology and physiopathology of epilepsy [3, 4]. Accordingly, a drug able to decrease the GABA content in the brain of rats and mice elicits a tonic-clonic seizure [5]. On the contrary, compounds known to enhance the concentration of GABA in the brain of the same animals induce a potent and efficacious anticonvulsant action [6]. Moreover, drugs which directly enhance the function of the GABA ionophore–receptor complex also induce a potent antiepileptic action [7–9].

Mono- and polycyclic 2-pyrrolidinone derivatives have potent anticonvulsant activity [10, 11]. In particular, the 3a-methyl-2,3,3a,4-tetrahydro-1*H*-pyrrolo-[1,2-*a*]benzimidazol-1-one **1** possesses anticonvulsant activity comparable to that of diphenylhydantoin and greater than that of valproate against sound-induced and maximal electroshock-induced seizures [12]. In an effort to obtain new anticonvulsant agents, we then investigated whether the replacement of the benzimidazole nucleus of **1** with the benzothiazole nucleus to give **2a** could be a favourable modification.

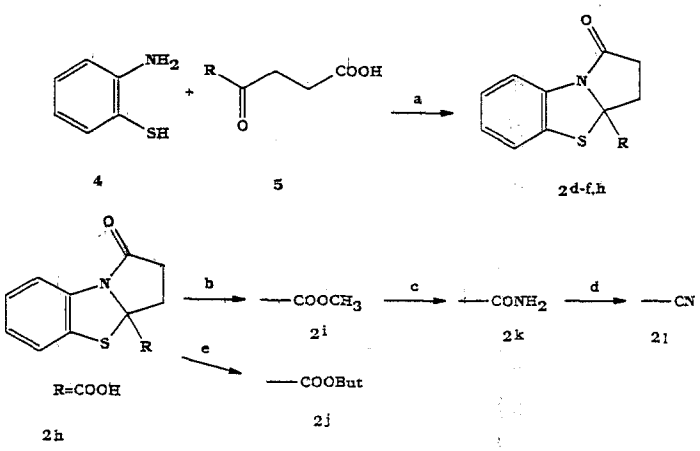


Structures **1**, **2**, **3**. **2a**: R = CH_3, R_1 = H.

This paper describes the synthesis of the 1,2,3,3a-tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones (table I) **2a–l** and their evaluation as anticonvulsants. The **2a** congeners **2b–l** were selected in order to explore whether the substitution at the 3a- and 6-positions of **2a** might affect the biological response. In addition, the bicyclic compounds **3a–d** were prepared in order to assess whether a benzene ring is necessary for optimal anticonvulsant activity.

Chemistry

The 1,2,3,3a-tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones **2d–f** (table I) were prepared by reaction of 2-aminobenzenethiol **4** with the required 3-acylpropionic acids **5**. Compound **2h** was obtained by reacting **4** with 2-ketoglutaric acid. Treatment of the acid **2h** with *n*-butanol or methanol in the presence of sulfuric acid yielded the corresponding esters **2j** and **2i**, respectively. Reaction of this last compound with ammonia gave the amide **2k**, which, in turn, by treatment with trifluoroacetic anhydride in dry pyridine (1:1 v/v) [15] yielded the nitrile **2l** (scheme 1).



Scheme 1. (a) *p*-Toluenesulfonic acid; (b) CH_3OH, H_2SO_4; (c) NH_3; (d) TFA, dioxane, pyridine; (e) n-C_4H_9OH, H_2SO_4.

Table I. Physical properties of 1,2,3,3a-tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones **2** and 3,4,5,6,7,7a-hexahydro-2*H*-pyrrolo[2,1-*b*]thiazol-5-ones **3**.

Compd	*R*	R_1	*Yield (%)*	*mp (°C)*	*Formula*[a]	*Recrystallization solvent*	*log P*[b]	R_M[c]
2a	CH_3	H	–	oil[d]	$C_{11}H_{11}NOS$		2.01	0.33
2b	CH_3	Cl	50	oil	$C_{11}H_{10}ClNOS$		2.57	0.63
2c	CH_3	OCH_3	77	75–77	$C_{12}H_{13}NO_2S$	EtOH/petroleum ether	1.74	0.35
2d	C_6H_5	H	47	98–100	$C_{16}H_{13}NOS$	$CHCl_3$/petroleum ether	3.35	0.63
2e	4-Cl-C_6H_4	H	44	123–125	$C_{16}H_{12}ClNOS$	2-Propanol	3.87	c
2f	4-OCH_3-C_6H_4	H	57	117–118	$C_{17}H_{15}NO_2S$	MeOH	3.04	0.52
2g	C_6H_5	OCH_3	57	120–122	$C_{17}H_{15}NO_2S$	2-Propanol/petroleum ether	3.04	0.55
2h	COOH	H	25	200 (dec)	$C_{11}H_9NO_3S$	$CHCl_3$	1.11	e
2i	$COOCH_3$	H	62	103–104	$C_{12}H_{11}NO_3S$	MeOH/petroleum ether	1.39	e
2j	$COOC_4H_9$	H	65	90–92	$C_{15}H_{17}NO_3S$	$CHCl_3$/petroleum ether	2.18	e
2k	$CONH_2$	H	78	264–266	$C_{11}H_{10}N_2O_2S$	MeOH	0.70	–0.25
2l	CN	H	76	108–109	$C_{11}H_8N_2OS$	EtOH/$CHCl_3$	1.02	0.25
3a	CH_3			oil[d]	$C_7H_{11}NOS$		0.53	–0.14
3b	C_6H_5		67	69–70	$C_{12}H_{13}NOS$	$CHCl_3$/petroleum ether	2.03	0.27
3c	COOH		86	153–154	$C_7H_9NO_3S$	MeOH/H_2O	–0.40	f
3d	$COOCH_3$		15	110–112	$C_8H_{11}NO_3S$	MeOH/petroleum ether	–0.07	–0.18
l							1.19	–0.035

[a]All compounds gave satisfactory C, H, N analyses (± 0.4%). [b]Log *P* calculated according to reference [13]. [c]R_M measured by reversed-phase thin-layer chromatography. A linear correlation relating log *P* and R_M values has been derived (s = 0.12; r^2 = 0.867; n = 11). [d]Prepared according to reference [14]. [e]It should be noted that only R_f values between 0.3–0.8 are reliable. Consequently, R_M values outside the –0.3–0.6 range should be considered close to methodological limits. [f]Not determined.

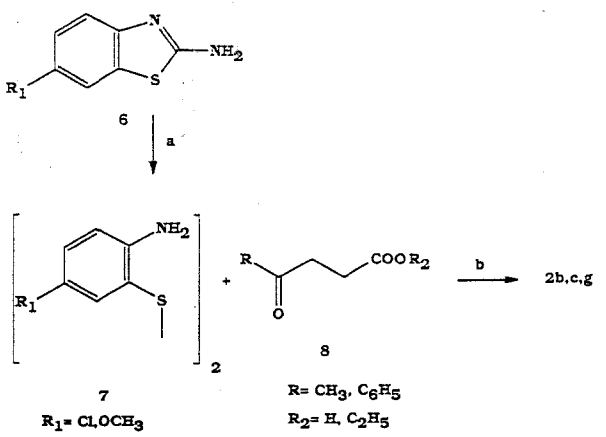
The 7-chloro or 7-methoxy aryl-substituted compounds **2b,c** and **2g** were conveniently obtained by reacting the appropriate 2,2'-diaryldithiodianilines **7** with the required ethyl 3-acetylpropionate and 3-benzoylpropionic acid, respectively (scheme 2). Compounds **7**, in turn, were prepared by alkaline hydrolysis of the corresponding commercially available 2-aminobenzothiazoles **6**. Compounds **3b–d** were prepared by reaction of cystamine with the appropriate 3-acylpropionic acid **5**.

The structures of the compounds **2b–l** and **3b–d** were confirmed by elemental analyses and spectral data (IR, ^{1}H-NMR and mass spectra). The physical characteristics of these compounds are summarized in table I.

Results and discussion

As reported in table II some of compounds **2a–l** and **3a–d** administered intraperitoneally to mice exerted a significant protection against the tonic-clonic convulsive pattern elicited by bicuculline. In particular, 9 compounds (**2a**, **c**, **e**, **g**, **h**, **j**, **l**, **3a**, **d**) exerted an anticonvulsant activity. Accordingly, some mice were protected or the onset of convulsions was delayed (latency time). The latency time is defined as the time elapsing between administration of the convulsant drug and the pattern of the onset of seizures (myoclonic and maximal seizures).

The most active compound was **2a**, which showed 44% protection in comparison with the 75% displayed by diphenylhydantoin. The toxicity profile of **2a** and **3a** was partially evaluated by determining the 24-h median lethal dose (LD_{50}, ip) in mice. Moreover, compounds **2a** and **3a** induced a marked sedation and loss of righting reflex. The LD_{50} values of **2a** and **3a** are reported together with those of reference compounds in table II. Furthermore, compounds **2a** and **3a** were found to be more potent than **1** and sodium valproate (table II). For **2a** and **3a** the maximal efficacy was obtained at 300 μmol/kg.



Scheme 2. (a) NaOH (aq); (b) *p*-toluenesulfonic acid.

In this series the structure–activity relationship can be summarized as follows: the anticonvulsant activity seems to decrease by introduction of substituents such as a chlorine atom or a methoxy group at the 6-position of **2a**. Within the set of 3a-*p*-substituted aryl compounds **2d–f**, a diminished activity compared with the parent compound **2a** was observed. When the methyl group of **2a** was replaced by a carboxylic, ester or amide function, a lower activity in terms of the number of mice protected was found (see entries **2h**, **j**, **k**). Interestingly, the butyl ester **2j** displayed a latency time as for myoclonic seizures which was longer than those of diphenylhydantoin and valproate. The observed anticonvulsant activity of **3a**, though lower than **2a**, is clearly indicative that the benzene ring is not an essential structural feature for anticonvulsant activity.

With the aim of exploring whether the lipophilicity of the examined compounds might affect the anticonvulsant activity, the percent of protection has been plotted against the calculated log *P* [13] (values < 3.5). In this regard, no clear correlation was found. However, the most active compound possesses a log*P* ≈ 2. This finding is in agreement with the minimal hydrophobicity principle for drugs acting at the CNS level [17].

Taking into account that the GABA transmission, in many other systems, plays an important role in the etiology of epilepsy, in the present work we explored whether compounds **2** and **3** might interact with the GABA receptor.

The ability of compounds **2a–l** and **3a–d** to interact with the benzodiazepine recognition site was investigated by evaluating their capability to displace the binding of [^{3}H]flunitrazepam in homogenates of rat cerebral cortex. As shown in table III our compounds failed to significantly inhibit [^{3}H]flunitrazepam binding.

Consistent with the results reported in table III, these molecules also failed to change [^{3}H]muscimol binding. All together these results suggest that our compounds do not directly interact with the GABA and benzodiazepine recognition sites.

To further clarify the mechanism of action of these compounds at the level of the $GABA_A$ receptor complex, we studied their capability to interact with recognition sites located at the level of the chloride channel coupled to the $GABA_A$ receptor. To do so, we evaluated the capability of our compounds **2a**, **c–j**, **l**, **3a**, **b**, **d** to displace [^{35}S]TBPS binding in rat cortical membrane homogenates. In fact, [^{35}S]TBPS has been

Table II. Effect of 1,2,3,3a-tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones **2** and 3,4,5,6,7,7a-hexahydro-2*H*-pyrrolo[2,1-*b*]thiazol-5-ones **3** on seizures induced by bicuculline in mice and lethal dose of the most active and prototype drugs.

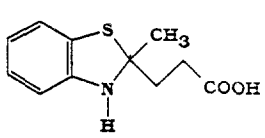
Compd	*Dose (μmol/kg)*	*N*[a]	*Latency time*[b] *(min)*		LD_{50}[c]	*Effect against myoclonic no protected/ no tested*	*Effect against maximal*[e] *no protected/ no tested*	*Percent of protection*
			myoclonic seizures	*maximal seizures*				
Control		30	5.40 ± 0.65 (30)	10.14 ± 1.20 (28)		0/30	2/30	
2a	150	6	3.83 ± 1.28 (6)	8.00 ± 2.20 (6)	336 ± 27	0/6	0/6	
	300	9	7.22 ± 1.53 (8)*	16.33 ± 4.35 (5)*		1/9	4/9*	44.4
	600	6	7.42 ± 0.82 (6)	11.60 ± 1.76 (5)		0/6	1/6	
2b	300	3	6.81 ± 1.03 (3)	11.83 ± 2.54 (3)		0/3	0/3	
2c	300	3	6.95 ± 2.13 (3)	12.63 ± 5.38 (2)		0/3	1/3	
2d	300	3	6.00 ± 2.08 (3)	11.33 ± 1.45 (3)		0/3	0/3	
2e	300	3	6.33 ± 0.88 (3)	7.00 ± 1.00 (2)		0/3	1/3	
2f	300	3	6.67 ± 0.33 (3)	14.67 ± 3.18 (3)		0/3	0/3	
2g	300	8	7.75 ± 0.94 (8)*	16.29 ± 2.23 (7)*		0/8	1/8	12.5
2h	300	3	3.33 ± 0.88 (2)	7.13 ± 0.13 (3)		0/3	1/3	
2i	300	8	5.50 ± 1.16 (8)	10.38 ± 2.24 (8)		0/8	0/8	
2j	300	7	14.71 ± 3.63 (7)**	24.40 ± 6.19 (5)**		0/7	2/7	28.5
2k	300	3	4.00 ± 1.15 (3)	10.00 ± 4.36 (3)		0/3	0/3	
2l	300	3	9.33 ± 0.88 (3)*	15.00 ± 3.00 (2)		0/3	1/3	
3a	150	6	4.67 ± 1.48 (6)	9.00 ± 2.80 (6)	1126 ± 24	0/6	0/6	
	300	9	10.25 ± 1.70 (8)**	21.17 ± 1.99 (6)**		1/9	3/9***	33.3
	600	12	6.54 ± 0.96 (12)	14.36 ± 1.42 (11)		0/12	1/12	
3b	300	3	7.00 ± 1.73 (3)	12.33 ± 1.86 (3)		0/3	0/3	
3c	300	3	2.67 ± 0.67 (3)	8.00 ± 0.58 (3)		0/3	0/3	
3d	300	3	7.67 ± 2.33 (3)	13.50 ± 4.50 (2)		0/3	1/3	
1	300	3	10.33 ± 1.76 (3)*	15.00 ± 2.89 (3)		0/3	0/3	
Diphenyl-hydantoin	100	8	3.86 ± 1.09 (7)	24.50 ± 12.5 (2)**	229.61[d]	1/8	6/8**	75
Valproate	2000	3	8.00 ± 3.00 (3)	15.30 ± 3.84 (3)	1104.62[d]	0/3	0/3	

[a]Number of animals. [b]The mean ± SEM of time to the onset of myoclonic or maximal seizures. The data were submitted to Student's *t*-test. *$P < 0.05$; **$P < 0.01$; the numbers in parentheses refer to the number of animals which displayed myoclonic or maximal seizures. [c]Median lethal dose in mg/kg measured after 24 h. [d]From reference [16]. [e]The data were submitted to χ^2 test (Fisher's exact test). *$P < 0.05$; **$P < 0.01$; ***$P = 0.05$.

shown to bind specifically to picrotoxin-sensitive recognition sites at the level of the GABA-coupled chloride channel [18].

The results reported in table III show that some of the examined compounds, at a concentration of 100 μM, reduced [^{35}S]TBPS binding. However, the lack of a correlation between the anticonvulsant activity of these compounds and their capability to displace [^{35}S]TBPS binding is also evident.

A number of factors may account for the absence of correlation between *in vivo* experiments and [^{35}S]-TBPS binding. In this regard, the different stability of these compounds in biological media might play an important role. Moreover, taking into account that some monocyclic 2-pyrrolidinone derivatives with anticonvulsant activity have been considered as GABA prodrugs [10], it seemed of interest to investigate whether the *in vivo* activity of **2a** could be ascribed to its hydrolyzed product **9**. The latter is a known compound [14] and may be considered as a 4-methyl-GABA derivative in which the nitrogen function and the C-4 are part of the heterocyclic moiety.



Structure **9**.

Table III. Effect of 1,2,3,3a-tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones **2** and 3,4,5,6,7,7a-hexahydro-2*H*-pyrrolo[2,1-*b*]thiazol-5-ones **3** on [^{3}H]flunitrazepam, [^{3}H]muscimol and [^{35}S]TBPS binding.

Compd	*% of specific inhibition [^{3}H]flunitrazepam binding*[a]		*% of specific inhibition [^{3}H]muscimol binding*[a]			*% of specific inhibition [^{35}S]TBPS binding*[a]		
	1 μM	*10 μM*	*1 μM*	*10 μM*	*100 μM*	*1 μM*	*10 μM*	*100 μM*
2a	0	2 ± 0.05	0	0			7 ± 0.25	24 ± 1*
2b	[b]	[b]	[b]	[b]		[b]	[b]	[b]
2c	3 ± 0.15	6 ± 0.2	0	0			0	51 ± 2.3*
2d	10 ± 0.3	26 ± 0.9*		0	0	3 ± 0.05	31 ± 1*	96 ± 2.1*
2e	15 ± 0.5*	19 ± 0.9*		0	0	5 ± 0.08	10 ± 0.03	67 ± 1.5*
2f	8 ± 0.3	12 ± 0.3		0	7 ± 0.04	1 ± 0.02	46 ± 0.6*	96 ± 1.7*
2g	0	5 ± 0.2		0	0	0	10 ± 0.18	84 ± 4.8*
2h	0	7 ± 0.3	0	0			9 ± 0.2	16 ± 0.6*
2i	4 ± 0.1	4 ± 0.1	0	0			12 ± 0.3	38 ± 1.2*
2j	8 ± 0.2	17 ± 0.5*		0	6 ± 0.03	2 ± 0.03	35 ± 1.5*	85 ± 5.2*
2k	[b]	[b]	[b]	[b]		[b]	[b]	[b]
2l	2 ± 0.09	7 ± 0.3	0	1			17 ± 0.36*	46 ± 2.7*
3a	1 ± 0.02	0	0	2 ± 0.01			13 ± 0.3	10 ± 0.4
3b	0	8 ± 0.2	6 ± 0.03	8 ± 0.05			17 ± 0.6*	58 ± 2.6*
3c	[b]	[b]	[b]	[b]		[b]	[b]	[b]
3d	0	0	0	12 ± 0.1			15 ± 0.38*	11 ± 0.6
1	5 ± 0.1	8 ± 0.4		4 ± 0.03	5 ± 0.03		3 ± 0.1	8 ± 0.6
Diazepam							51 ± 3*	

[a]Biochemical data were analyzed using Student's *t*-test. *$P < 0.01$. Data are the mean ± SEM of 3 experiments performed in triplicate. [b]Not determined.

Thus, the stability of **2a** in Tris-HCl buffer, pH 7.4, with rat brain membranes as well as with rat plasma at 4 ± 0.5°C and 35 ± 0.5°C was evaluated. In such experimental conditions no conversion of compound **2a** to **9** was detected by HPLC within the sensivity limits of the method. Hence, it seems that **2a** may act intact on its target(s) rather than as the hydrolyzed compound **9**.

From the above results, taken together, it follows that there is no evidence that the examined compounds may act through a GABA mechanism.

In summary, the replacement of the benzimidazole moiety of **1** with the bioisoster benzothiazole moiety led to the design of a series of compounds, some of which exhibit anticonvulsant properties in bicuculline-induced seizures. Of the examined series, the parent compounds **2a** and **3a** emerged as promising candidates whose anticonvulsant overall profile must be further evaluated in other seizure paradigms and neurotoxic tests. In particular, the principle advantages of **2a** are related to the significant percentage of mice that it protected as well as to the protection against the myoclonic onset. At present, the mechanism(s) underlying the action of these anticonvulsant agents is (are) unknown and further experimental work is necessary for clarification of this aspect.

Experimental protocols

Chemistry

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. IR spectra were obtained on a Perkin–Elmer 283 spectrometer (KBr pellets unless otherwise stated). HPLC analyses (stability studies) were performed by using a Waters delivery system Mod 600 equipped with a variable-wavelength UV detector (Waters Mod 486) in reverse-phase mode. ^{1}H-NMR spectra were recorded on a Varian 390 or XL–200 instrument operating at 90 or 200 MHz, respectively. Chemical shifts are given in δ value downfield from Me_4Si as an internal standard. Mass spectra were recorded on a Hewlett–Packard 5995c GC–MS low resolution spectrometer. Silica gel 60 (Merck 70–230 mesh) was used for column chromatography. Elemental analyses were carried out with a Carlo Erba Mod 1106 analyzer and results were within ± 0.4% of the theoretical values. The following reactions were performed under a nitrogen atmosphere.

3a-Phenyl-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-1-one ***2d***

A solution of 2-aminobenzenethiol (1.4 g, 11.2 mmol) and 3-benzoylpropionic acid (2 g, 11.2 mmol) in *n*-butanol (50 ml) containing catalytic amounts of *p*-toluenesulfonic acid was refluxed for 31 h. Evaporation of the solvent under reduced pressure gave a residue which was purified by column chromatography (petroleum ether/ethyl acetate 1:1 v/v as eluent) to give 1.4 g of **2d**. IR ν_{max} 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.5–

3.2 (m, 4H, CH_2), 7.0–7.3 (m, 8H, ArH), 7.7–7.9 (m, 1H, ArH); MS, m/e 267 (M^+, 62), 190 (100). Anal $C_{16}H_{13}NOS$ (C, H, N). The following pyrrolobenzothiazolones were prepared in a similar way. The time of reflux and the solvent used are indicated.

3a-(4-Chlorophenyl)-1,2,3,3a-tetrahydropyrrolo[2,1-b]-benzothiazol-1-one ***2e***
Reflux time in xylene 32 h. IR ν_{max} 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.5–3.2 (m, 4H, CH_2), 6.9–7.3 (m, 7H, ArH), 7.7–7.8 (m, 1H, ArH); MS, m/e 301 (M^+, 50), 190 (100). Anal $C_{16}H_{12}ClNOS$ (C, H, N).

3a-(4-Methoxyphenyl)-1,2,3,3a-tetrahydropyrrolo[2,1-b]-benzothiazol-1-one ***2f***
Reflux time in *n*-butanol 24 h. IR ν_{max} 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.5–3.2 (m, 4H, CH_2), 3.73 (s, 3H, OCH_3), 6.7–7.3 (m, 7H, ArH), 7.7–7.9 (m, 1H, ArH); MS, m/e 297 (M^+, 86), 242 (100). Anal $C_{17}H_{13}NO_2S$ (C, H, N).

1-Oxo-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-3a-carboxylic acid ***2h***
Reaction time at room temperature in ethyl ether 1 h. The precipitate was filtered off and washed with ethyl ether, was the pure α-benzothiazolin-2-yl glutaric acid **10** (14% yield). The filtrate evaporated under reduced pressure gave a residue which was purified by crystallization from chloroform to give the compound **2h** in 25% yield. Compound **10**: mp 164–166°C; IR ν_{max} 3400, 3100, 1720 cm^{-1}; ^{1}H-NMR (DMSO–d_6) δ: 2.1–2.3 (m, 2H, CH_2), 2.3–2.6 (m, 2H, CH_2), 6.8–7.3 (m, 4H, ArH). Anal $C_{11}H_{11}NO_4S$: Calc: C, 52.17; H, 4.38; N, 5.53; found: C, 51.89; H, 4.27; N, 5.30. Compound **2h**: IR ν_{max} 1730, 1660 cm^{-1}; ^{1}H-NMR (DMSO–d_6) δ: 2.4–2.8 (m, 4H, CH_2), 6.9–7.3 (m, 3H, ArH), 7.4–7.6 (m, 1H, ArH). Anal $C_{11}H_9NO_3S$ (C, H, N).

Methyl-1-oxo-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-3a-carboxylate ***2i***
The crude product obtained following the procedure to prepare **2h** was dissolved in methanol (50 ml) containing sulfuric acid (1 ml) and the mixture was refluxed for 5 h. Evaporation of the solvent under reduced pressure gave a residue which was dissolved in 50 ml dilute $NaHCO_3$ and extracted with chloroform (3 x 30 ml). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. Column chromatography of the residue (petroleum ether/ethyl acetate 1:1 v/v as eluent) gave compound **2i**. Compound **2j** was prepared in similar way. IR ν_{max} 1740, 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.5–3.0 (m, 4H, CH_2), 3.76 (s, 3H, OCH_3), 7.0–7.2 (m, 3H, ArH), 7.6–7.8 (m, 1H, ArH); MS, m/e 249 (M^+, 10), 190 (100). Anal $C_{12}H_{11}NO_3S$ (C, H, N).

Butyl-1-oxo-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-3a-carboxylate **2j**
IR ν_{max} 1740, 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.86 (t, 3H, CH_3), 1.0–1.7 (m, 4H, CH_2), 2.5–3.0 (m, 4H, CH_2), 4.13 (t, 2H, OCH_2), 7.0–7.2 (m, 3H, ArH), 7.6–7.7 (m, 1H, ArH); MS, m/e 291 (M^+, 1), 190 (100). Anal $C_{15}H_{17}NO_3S$ (C, H, N).

1-Oxo-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-3a-carboxamido ***2k***
A mixture of **2i** (0.3 g, 1.2 mmol) and ammonium hydroxide 30% (10 ml) was stirred at room temperature for 24 h. The precipitate collected by filtration was purified by crystallization from methanol to give pure **2k**. IR ν_{max} 3400, 3300, 1620cm^{-1}; ^{1}H-NMR (DMSO–d_6) (200 MHz) δ: 2.4–2.8 (m, 4H, CH_2), 7.0–7.2 (m, 3H, ArH), 7.4–7.5 (m, 1H, ArH); MS, m/e 234 (M^+, 3), 190 (100). Anal $C_{11}H_{10}N_2O_2S$ (C, H, N).

3a-Cyano-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-1-one ***2l***
Trifluoroacetic anhydride (0.65 ml) was added to a stirred ice-cooled mixture of **2k** (1 g, 4.6 mmol) in anhydrous dioxane (11 ml) and anhydrous pyridine (0.69 ml). The stirring was prolonged overnight. Then the mixture was diluted with ice water and the resulting precipitate was filtered off to give the pure compound **2l**. IR ν_{max} 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) (200 MHz) δ: 2.7–3.1 (m, 4H, CH_2), 7.1–7.2 (m, 3H, ArH), 7.6–7.7 (m, 1H, ArH); MS, m/e 216 (M^+, 28), 161 (100). Anal $C_{11}H_8NO_2S$ (C, H, N).

3a-Methyl-7-chloro-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-1-one **2b**
A solution of 4,4'-dichloro-2,2'-dithiodianiline [19] (1.2 g, 3.79 mmol) and ethyl levulinate (1.09 g, 7.58 mmol) in toluene (40 ml) containing catalytic amounts of *p*-toluenesulfonic acid was refluxed for 19 h. Evaporation of the solvent under reduced pressure gave a residue which was purified by column chromatography (petroleum ether/ethyl acetate 7:3 v/v as eluent) to give 0.9 g **2b**. IR ν_{max} 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.73 (s, 3H, CH_3), 2.3–3.0 (m, 4H, CH_2), 7.0–7.2 (m, 2H, ArH), 7.58 (d, 1H, ArH); MS, m/e 239 (M^+, 30), 224 (100). Anal $C_{11}H_{10}ClNOS$ (C, H, N). The following pyrrolobenzothiazolones were prepared in a similar way. The time of reflux and solvent used are indicated.

3a-Methyl-7-methoxy-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-1-one **2c**
Reflux time in toluene 7 h. IR ν_{max} 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.76 (s, 3H, CH_3), 2.3–2.9 (m, 4H, CH_2), 3.76 (s, 3H, OCH_3), 6.6–6.7 (m, 2H, ArH), 7.53 (d, 1H, ArH); MS, m/e 235 (M^+, 40), 220 (100). Anal $C_{12}H_{13}NO_2S$ (C, H, N).

3a-Phenyl-7-methoxy-1,2,3,3a-tetrahydropyrrolo[2,1-b]benzothiazol-1-one **2g**
Reflux time in xylene 27 h. IR ν_{max} 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.8–3.5 (m, 4H, CH_2), 4.00 (s, 3H, OCH_3), 6.9–7.0 (m, 2H, ArH), 7.4–7.7 (m, 5H, ArH), 8.00 (d, 1H, ArH); MS, m/e 297 (M^+, 100). Anal $C_{17}H_{15}NO_2S$ (C, H, N).

7a-Phenyl-3,4,5,6,7,7a-hexahydro-2H-pyrrolo[2,1-b]thiazol-5-one **3b**
A solution of cysteamine (0.86 g, 11.2 mmol) and 3-benzoylpropionic acid (2.0 g, 11.2 mmol) in *n*-butanol (50 ml) containing catalytic amounts of *p*-toluenesulfonic acid was refluxed for 2 h. Evaporation of the solvent under reduced pressure gave a residue which was purified by column chromatography (petroleum ether/ethyl acetate 8:2 v/v as eluent) to give 1.4 g of **3b**. IR ν_{max} 1700 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.1–3.4 (m, 7H, CH_2 + CH(3)), 4.2–4.5 (m, 1H, CH(3)), 7.2–7.4 (m, 5H, ArH); MS, m/e 219 (M^+, 100). Anal $C_{12}H_{13}NOS$ (C, H, N). The following pyrrolothiazolones were prepared in a similar way. The time of reflux and solvent used are indicated.

5-Oxo-3,4,5,6,7,7a-hexahydro-2H-pyrrolo[2,1-b]thiazol-7a-carboxylic acid **3c**
Reaction time at room temperature in methanol 2 h. IR ν_{max} 1750, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.2–2.8 (m, 4H, CH_2), 2.9–3.3 (m, 3H, CH_2 + CH(3)), 4.0–4.4 (m, 1H, CH(3)). Anal $C_7H_9NO_3S$ (C, H, N).

Methyl-5-oxo-3,4,5,6,7,7a-hexahydro-2H-pyrrolo[2,1-b]thiazol-7a-carboxylate ***3d***
The crude product obtained following the procedure to prepare **3c** was dissolved in methanol (50 ml) containing sulfuric acid (1 ml) and the mixture was refluxed for 3 h. Evaporation of the solvent under reduced pressure gave a residue which was dissolved in 50 ml dilute $NaHCO_3$ and extracted with chloroform (3 x 30 ml). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give the crude **3d** which was purified by crystallization from methanol/petroleum ether. IR ν_{max} 1740, 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.4–2.9 (m, 4H, CH_2), 3.0–3.5 (m, 3H, CH_2 + CH(3)), 3.80 (s, 3H, OCH_3), 4.2–4.5 (m, 1H, CH(3)); MS, m/e 201 (M^+, 0.5), 142 (100). Anal $C_8H_{11}NO_3S$ (C, H, N).

Pharmacology

CD–1 mice (Charles River Lab Calco, Italy) weighing 35–40 g were used for pharmacological studies. The animals were allowed free access to food and water and were housed at room temperature (20–22°C). A freshly prepared solution of bicuculline (Sigma, 3 mg/kg) in citrate buffer (pH 4.0) was injected sc in a volume of 10 ml/kg. The tested compounds dissolved in water/DMSO mixture (80% DMSO and 20% distilled water) were administered ip 1 h before bicuculline injection. The animals were placed singly in Plexiglass cages and observed for 1 h after the bicuculline injection. A clonic convulsion was defined as a single episode of clonic spasms of at least 5 s duration. A tonic seizure was defined as a brief period of hind-limb flexion followed by a prolonged period of hind-limb extension. The failure to show seizures indicated protecting activity. The time to onset of clonic and tonic convulsions was measured. The pharmacological effects were compared with controls and the activity showed by other clinically useful anticonvulsants such as diphenylhydantoin (100 μmol/kg) and valproate (2000 μmol/kg). The statistical significance of the data was checked by using Student's *t*-test and χ^2 test for the latency time and protecting activity, respectively. The LD_{50} values of **2a** and **3a** were determined by probit analysis.

Binding studies
The brains of male Sprague–Dawley CDR rats (200–225 g) were used for the measurements of [^{3}H]flunitrazepam, [^{3}H]muscimol and [^{35}S]TBPS binding.

[^{3}H]Flunitrazepam binding. Cerebral cortices were homogenized in 10 vol ice-cold distilled H_2O with a Polytron PT 10 (setting 5, for 20 sec) and centrifugated at 20 000 *g* for 10 min. The pellet was reconstituted in 50 vol 50 mM Tris-HCl buffer, pH 7.4. Aliquots of 200 μl of tissue homogenate (0.4 mg of protein/ml) were incubated in the presence of [^{3}H]flunitrazepam at a final concentration of 0.5 nM, in a total incubation volume of 1 ml. The drugs were dissolved in ethanol and serial dilutions were made up in ethanol and added in 5 μl aliquots. After 60 min incubation at 4°C, the assay was terminated by rapid filtration through glass-fiber filter strips (Whatman, GF/B). The filters were rinsed with two 4 ml portions of ice-cold 50 mM Tris-HCl buffer with a cell Harvester filtration manifold (Model M–24, Brandel) and transferred in plastic minivials with 3 ml of scintillation fluid (Atomlight, New England Nuclear). Non-specific binding was determined as binding in the presence of 5 μM diazepam.

[^{3}H]Muscimol binding. Fresh cerebral cortices were homogenized with a Polytron PT 10 (setting 5, for 30 sec) in 10 vol ice-cold water and centrifugated 10 min at 48 000 *g* at 0°C. The pellet was washed once by resuspension and recentrifugation in 10 vol 10 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.4) containing 10 mM KCl. The membranes were stored at –20°C until use 1–15 d later. On the day of the assay, the membranes were thawed and centrifugated. The pellet was washed 3 additional times by resuspension and recentrifugation in ice-cold phosphate buffer. The tissue was resuspended in 50 vol of the same buffer and 350 μl of membrane suspension (250–300 μg of protein) was added to plastic minivials. Drugs were dissolved as described above and were added in 5 μl aliquots. The total incubation volume was 1 ml. Non-specific binding was defined as binding in the presence of 1 mM GABA. The incubation (30 min at 4°C) was started by the addition of 10 nM [^{3}H]muscimol and was stopped by centrifugation of the incubation mixture at 48 000 *g* for 10 min. The supernatant was discarded and the resultant pellet was gently washed twice with 4 ml ice-cold distilled water and was then resuspended in 500 μl distilled water. Then 3.5 ml of scintillation fluid was added.

[^{35}S]TBPS binding. Cerebral cortices were homogenized with a Polytron PT 10 (setting 5, for 20 s) in 50 vol ice-cold 50 mM Tris-citrate buffer (pH 7.4 at 25°C) containing 100 mM NaCl. The homogenate was centrifuged at 20 000 *g* for 20 min and reconstituted in 50 vol 50 mM Tris-citrate buffer without NaCl. [^{35}S]TBPS binding was determined in a final volume of 1 ml, consisting of 200 μl tissue homogenate (200–300 μg of protein), 100 μl 2 nM [^{35}S]TBPS, 100 μl 0.2 M NaCl, 5 μl drugs, dissolved as described above, or solvent (total and non-specific samples). The incubation (25°C) were started by the addition of tissue homogenate and were terminated 90 min later by rapid filtration through glass-filter strips (Whatman GF/B) with a filtration manifold (Model M–24, Brandel). The filters were rinsed with two 4 ml portions of ice-cold Tris-citrate buffer. Non-specific binding was defined as binding in the presence of 100 μM picrotoxin. Filter-bound radioactivity was quantified by liquid scintillation spectrometry. Protein concentration was assayed by the method of Lowry *et al* [20] with bovine serum albumin as standard. Biochemical data were analyzed using Student's *t* test.

Lipophilicity measurements
The relative lipophilicity of compounds **2a–l** and **3a**, **b**, **d** was measured by reversed-phase thin-layer chromatography [21]. Nano-Sil C_{18}-100 UV_{254} plates (from Aldrich) were used as the non-polar stationary phase. The mobile phase was a 6:4 v/v mixture of acetone and water. The compounds dissolved in ethanol (2.5 mg/ml) and 5 μl of the solution was applied to the plate. The R_f values used are the mean of 4 determinations. The R_M values were calculated by the following formula $R_M = \log[(1/R_f) - 1)]$.

Stability study of ***2a***
Stability experiments were carried out at 4 ± 0.5°C and 35 ± 0.5°C. Male rats (180–200 g) were used to obtain brain and plasma. To a suspension of 1.1 mg of brain membranes in Tris-buffer (pH 7.4), was added a solution of 50% ethanol/Tris-HCl buffer of **2a** (5 ml final volume) to give a concentration of 300 μM. At appropriate intervals, aliquots of suspension were withdrawn and centrifugated at 12 000 *g* for 5 min. The supernatants were subjected to HPLC analysis.

A solution of **2a** was added to the rat plasma (80%) to give a final concentration of 300 μM. At appropriate time intervals aliquots of solution were withdrawn and acetonitrile was added to precipitate the plasma proteins. The supernatants, obtained after centrifugation, were subjected to HPLC analysis.

The stationary phase used was μBondapak C18 (150 x 4.6 mm id). Methanol/water/acetic acid mixtures 60:40:0.25 v/v pH 5.2 were used as mobile phase with a flow rate of 1.0 ml/min. The samples were monitored at 254 nm.

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