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Synthesis and Anticonvulsant Activity of Some 1,2,3,3*a*-Tetrahydropyrrolo[2,1-*b*]-benzothiazol-, -thiazol- or -oxazol-1-ones in Rodents

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Abstract

To identify more potent anticonvulsant agents and to gain insights into the structural properties determining the potency of a new class of anticonvulsants, some 3*a*-substituted tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones (**1a–d**) and the thiazole and oxazole analogues (**2a–c** and **3a–c**, respectively) have been synthesized and tested for anticonvulsant activity against isoniazid-induced seizures in rodents.

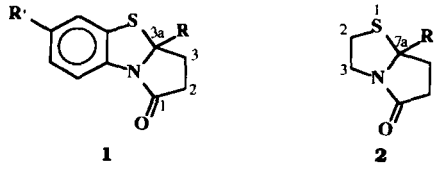
The most active compound, **2a**, with a median effective dose (ED50, i.p.) of 24·3 mg kg^{-1} and 15·9 mg kg^{-1} in mice and in rats, respectively, was more extensively investigated and found to strengthen the effects of diazepam. No clear correlation was observed between the anticonvulsant activity and molecular lipophilicity descriptors of compounds **1–3**. Structural similarity between the antiepileptic drug phenobarbital and compounds **1–3** was evidenced by molecular modelling studies and used to derive preliminary structure-activity relationships.

The results demonstrate that **2a** is an attractive candidate as an anticonvulsant agent worthy of further study and may help the design of other anticonvulsant drugs.

Several lines of evidence have suggested that in the mammalian brain, the γ-aminobutyric $acid_A$ receptor complex ($GABA_A$) is involved in the pharmacology of anticonvulsant drugs and in the pathophysiology of seizures and epilepsy. Drugs that facilitate GABAergic transmission have a potential anticonvulsant action (Lloyd et al 1986; Olsen et al 1986; Concas et al 1992), whereas administration of negative modulators of $GABA_A$ receptors, such as β-carboline derivatives (Braestrup et al 1982, 1984) or the chloride-channel blocker pentylenetetrazole (Squires et al 1984) induces convulsion. It is known that some antiepileptic drugs presently in use are frequently unable to control certain forms of epileptic seizure in a significant number of patients. These agents are, furthermore, also associated with a plethora of side-effects that can seriously detract from their clinical use (Upton 1994). A major aim of research is, therefore, to develop new anticonvulsant drugs with great efficacy, specificity and devoid of unwanted effects.

In a previous paper (Trapani et al 1994) we reported that some 1,2,3,3*a*-tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones (**1**) and thiazole analogues (**2**) (Fig. 1) have weak anticonvulsant activity against bicuculline-induced seizures. Binding studies on some representative compounds using radioligands for the benzodiazepine ([^{3}H]flunitrazepam) and GABA ([^{3}H]muscimol) receptors have, moreover, demonstrated that these compounds have little or no affinity for these sites. It was, therefore, hypothesized that these agents might produce their effects by altering the picrotoxin binding site of the GABA receptor-chloride ionophore complex. In fact, binding studies using [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS), a specific ligand for the picrotoxin site (Squires et al 1983), indicate that high concentrations of pyrrolobenzothiazoles **1a–d** and thiazole analogue **2a** (Table 1) inhibit this binding in unwashed membranes from rat cerebral cortex. No correlation was, however, found between the anticonvulsant activity of the compounds **1a-d**, and **2a** and their ability to reduce [^{35}S]TBPS binding.

To investigate further the possible involvement of a GABAergic mechanism in the action of these compounds, we have, therefore, studied the ability of compounds **1** and **2** to antagonize the pharmacological effects induced by isoniazid, an inhibitor of GABA synthesis (Horton et al 1979). By reducing the amount of GABA at the synaptic cleft, isoniazid characteristically induces tonic-clonic seizures (Horton 1980) and dose-dependently increases [^{35}S]TBPS binding (Serra et al 1989), effects antagonized by the previous administration of positive modulators of GABAergic transmission (Sanna et al 1991; Serra et al 1989, 1992, 1994). Accordingly, as previously shown, isoniazid-treated animals represent a rather unique model in the same animal both biochemically and pharmacologically capable of evaluating the efficacy of putative GABAergic drugs. Because, with the exception of **1d**, all the previously studied compounds, e.g. **1a–c** and **2a**, to some



1 **2**

FIG. 1. Structures of pyrrolobenzothiazoles **1** and pyrrolothiazoles **2**.

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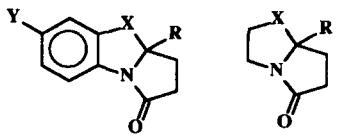
Table 1. Physicochemical and microanalytical data of compounds **1a–d**, **2a–c**, and **3a–c**.



Compound **1** Compound **2, 3**

Cmpd	R	X	Y	Yield (%)	m.p. (°C)	R_M[a]	CLOGP	Formula[c]	Anal.[c]
1a	C_6H_5	S	H	47	98–100	0·63[b]	3·10		
1b	4-OCH_3-C_6H_4	S	H	57	117–118	0·52[b]	3·02		
1c	C_6H_5	S	OCH_3	57	120–122	0·55[b]	3·12		
1d	$COOC_4H_9$	S	H	65	90–92	[b]	2·92		
2a	C_6H_5	S		67	69–70	− 0·017	1·96		
2b	4-OCH_3-C_6H_4	S		46	98–100[c]	− 0·016	1·87	$C_{13}H_{15}NO_2S$	C,H,N
2c	4-Cl-C_6H_4	S		50	88–90[c]	0·160	2·67	$C_{12}H_{12}ClNOS$	C,H,N
3a	C_6H_5	0		65	98[d]	− 0·160	1·59	$C_{12}H_{12}NO_2$	C,H,N
3b	4-OCH_3-C_6H_4	0		18	158–160	− 0·080	1·51	$C_{13}H_{15}NO_3$	C,H,N
3c	4-Cl-C_6H_4	0		78	242–244[d]	0·200	2·31	$C_{12}H_{12}ClNO_2$	C,H,N

[a]RM measured by reversed-phase thin-layer chromatography at 30 ± 1°C. [b]Trapani et al 1994. [c]From 2-propanol. [d]From petroleum ether-ethyl acetate. [e]Reported for new compounds only.

extent reduced [^{35}S]TBPS binding (Trapani et al 1994) when a phenyl ring was present at the angular position (e.g. 3*a*- and 7*a*-positions, respectively), we kept this structural feature constant by changing the ring hetero- atoms (sulphur for oxygen). The main goals of this work were to identify more potent anticonvulsant agents and to gain insights into the structural properties controlling the potency of this new class of anticonvulsant.

Materials and Methods

Chemistry

Melting points were determined in open capillary tubes on a Büchi apparatus and are uncorrected. IR spectra were obtained on a Perkin–Elmer 283 spectrometer (KBr pellets unless otherwise stated). ^{1}H NMR spectra were recorded on a Bruker instrument operating at 300 MHz. Chemical shifts are given as δ values down-field from Me_4Si as internal marker. 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 performed with a Carlo Erba mod. 1106 analyser and results for C, H, N were within ± 0·4% of theoretical values. The tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones **1a–d** (Table 1) and the thiazole analogues **2a–c** were prepared according to a published method (Trapani et al 1994) by reaction of the appropriate 3-acylpropionic acid **4** with 2-aminobenzenethiol **5** or 2-aminoethanethiol **6**, respectively. Similarly, pyrroloxazole compounds **2d–f** were prepared starting from 2-aminoethanol **7** (Fig. 2).

The structures of the new compounds were assigned on the basis of elemental analyses and spectral data (IR, ^{1}H NMR, and mass spectra).

The following reactions were performed under a nitrogen atmosphere.

Preparation of pyrrolo[2,1-b]thiazol-5-one derivatives

7a-(4-methoxyphenyl)-3,4,5,6,7,7a-hexahydro-2H-pyrrolo[2,1-b]thiazol-5-one **2b**. A solution of cysteamine (2·0 g; 26 mmol) and 3-(4-methoxyphenyl)benzoylpropionic acid (5·4 g; 26 mmol) in *n*-butanol (50 mL) containing catalytic amounts of *p*-toluenesulphonic acid was heated under reflux for 2 h. Evaporation of the solvent under reduced pressure gave a residue which was purified by crystallization from 2-propanol to give **2b** (3·0 g). IR ν_{max}: 1700 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ (ppm): 2·25–2·37 (m, 1H, CH(7)); 2·53 (m, 3H, CH(7) + CH_2(6)); 2·92–3·07 (m, 2H, CH_2(2)); 3·15–3·22 (m, 1H, CH(3)); 3·78 (s, 3H, OCH_3); 4·31–4·38 (m, 1H, CH(3)); 6·82–6·87 (m, 2H, ArH); 7·31–7·35 (m, 2H, ArH). MS, m/z 249 (M+, 100). Anal.: $C_{13}H_{15}NO_2S$ (C, H, N).

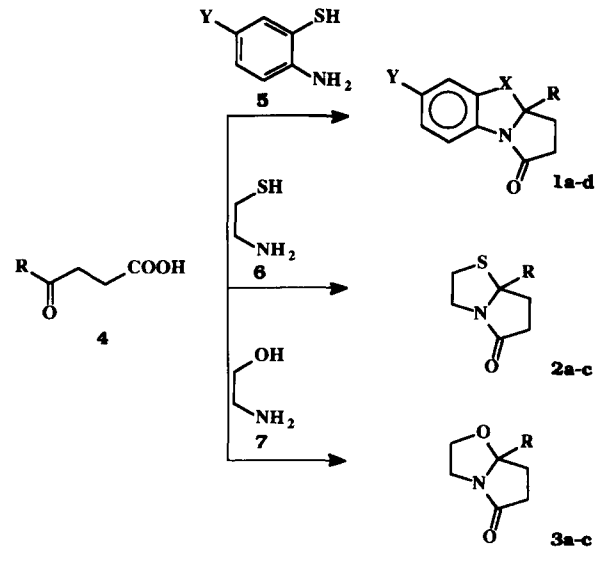


FIG. 2. Synthetic pathways.

7a-(4-Chlorophenyl)-3,4,5,6,7,7a-hexahydro-2H-pyrrolo[2,1-b]thiazol-5-one **2c.** This compound was prepared in a similar manner. After crystallization from 2-propanol **2c** was obtained in 50% yield. *IR* ν_{max} 1720 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2·24–2·33 (m, 1H, CH(7)); 2·60–2·76 (m, 3HCH(7) + CH_2(6)); 2·92–3·04 (m, 2H, CH_2(2)); 3·16–3·22 (m, 1H, CH(3)); 3·25–4·41 (m, 1H, CH(3)); 7·23–7·37 (m, 4H, ArH). MS, m/z 253 (M^+, 100). Anal.: $C_{12}H_{12}ClNOS$ (C, H, N).

Preparation of pyrrolo[2,1-b]oxazol-5-one derivatives

7a-Phenyl-3,4,5,6,7,7a-hexahydro-2H-pyrrolo[2,1-b]oxazol-5-one **3a.** A solution of 2-aminoethanol (1·17 g, 19·2 mmol) and 3-benzoylpropionic acid (3·35 g, 18·8 mmol) in toluene (50 mL) was heated under reflux for 4 h with azeotropic removal of the water. Evaporation of the solvent gave a residue which was purified by column chromatography with petroleum ether-ethyl acetate, 7:3 (v/v) to give 2·5 g of **3a**. IR ν_{max} 1720 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2·15–2·29 (m, 1H, CH(7)); 2·48–2·65 (m, 2H, CH_2(6)); 2·73–2·86 (m, 1H, CH(7)); 2·95–3·05 (m, 1H, CH(3)); 3·64–3·73 (m, 1H, CH(3)); 3·93–4·07 (m, 2H, CH_2(2)); 7·28–7·45 (m, 5H, ArH). MS, m/z 203 (M^+, 99), 172 (base). Anal.: $C_{12}H_{13}NO_2S$ (C, H, N).

The pyrroloxazolones **3b** and **3c** were prepared in similar manner. Spectral data for compounds **3b** and **3c** were:

3b. IR ν_{max} 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2·15–2·27 (m, 1H, CH); 2·46–2·55 (m, 1H, CH); 2·58–2·64 (m, 1H, CH); 2·71–2·84 (m, 1H, CH); 2·95–3·05 (m, 1H, CH); 3·66–3·73 (m, 2H, CH); 3·80 (s, 3H, OCH_3); 3·91–4·05 (m, 2H, CH_2); 6·86–6·90 (m, 2H, ArH); 7·31–7·36 (m, 2H, ArH). MS, m/z 233 (M^+, 29), 202 (base). Anal.: $C_{13}H_{15}NO_3$ (C, H, N).

3c. IR ν_{max} 1720 cm^{-1}. 1H NMR ($CDCl_3$) δ (ppm): 2·11–2·33 (m, 1H, CH); 2·27–2·36 (m, 1H, CH); 2·50–2·58 (m, 3H, CH + CH_2); 2·62–3·35 (m, 1H, CH); 3·57–3·65 (m, 1H, CH); 4·37–4·66 (m, 1H, CH); 7·33–7·40 (m, 4H, ArH). MS, m/z 237 (M^+, 57), 206 (base). Anal.: $C_{12}H_{12}ClNO_2$ (C, H, N).

Lipophilicity measurements

The relative lipophilicities of compounds **1–3** was measured by reversed-phase thin-layer chromatography. Nano-Sil C_{18}-100 UV_{254} plates (Aldrich) were used as non-polar stationary phase. The mobile phase was a 6:4 (v/v) mixture of acetone and water. The compounds were dissolved in ethanol (2·5 mg mL^{-1}) and 5 μL of the solution was applied to the plate. R_F values are means from four determinations; R_M values were calculated using the formula: $R_M = \log[(1/R_F) - 1)]$ where R_F (retention factor) is the ratio of the distances covered by analyte and solvent.

In-vivo studies

Male CD-1 mice, 25–30 g, and Sprague–Dawley rats, 200–225 g (Charles River, Como, Italy) were maintained under a 12 h light, 12 h dark cycle at a temperature of 23 ● 2°C and 65% humidity. After arrival at the animal facility, animals were acclimatized for a minimum of 7 days, during which time they had free access to food and water. The tested compounds and diazepam (Hoffman-La Roche, Basle, Switzerland) were suspended in distilled water with one drop of Tween 80 per 5 mL of water, sonicated and injected intraperitoneally (10 or 3 mL kg^{-1}). Isoniazid (200 or 350 mg kg^{-1}) or pentylenetetrazole (5 mg kg^{-1}) was dissolved in distilled water and administered subcutaneously or intraperitoneally, respectively. Mice or rats were injected with the drugs at the times indicated in the tables. Animals were observed for the appearance of convulsions for at least 2 h after administration of isoniazid or pentylenetetrazole during which the time of onset of seizure activity, the pattern of seizures and mortality were recorded.

Statistics

Biochemical data were analysed by analysis of variance followed by Scheffé's test. Behavioural data were analysed by Fisher's exact probability test or Student's *t*-test.

Molecular modelling

These studies were performed on an Evans and Sutherland PS 390 graphics workstation networked to a Digital VAX 3100 using SYBYL version 5·41 molecular modelling software (Tripos Associates, St Louis, MO, USA). Molecular models were constructed using standard bond distances and angles within SYBYL and fully optimized by the semi-empirical quantum chemical method AM1 (QCPE 506). The FIT and MVOLUME of SYBYL commands were used for least-squares fitting and volume analysis, respectively. Calculations of ring centroids, inter-atomic distances, and angles were performed using the graphic capability of the software.

Results and Discussion

As shown in Table 2, 25 mg kg^{-1} (i.p.) of **2a** significantly reduced the number of mice that exhibited tonic-clonic seizures or that died, and markedly prolonged the time before onset of convulsions in isoniazid-treated mice (200 mg kg^{-1}, s.c.). In addition, at a dose of 50 mg kg^{-1} (i.p.), this compound completely prevented seizures for at least 2 h after the administration of isoniazid.

Table 3 shows the effect of compounds **1a–d**, **2b**, **2c** and **3a–c** on isoniazid-induced seizures. Compound **1a** (100 mg kg^{-1}, i.p.) markedly reduced both the number of animals with convulsions and the mortality. It also significantly delayed (60 min) the onset of the convulsive episodes elicited by isoniazid. In contrast, compounds **1b–d** (50–100 mg kg^{-1}, i.p.) failed to reduce significantly the number of animals exhibiting convulsions. Compounds **1b** and **1c**, however, delayed the onset of seizures by 35 min. Finally, compounds **2b–c** and **3a–c** (50 mg kg^{-1}, i.p.) failed to antagonize isoniazid-induced seizures in mice.

To clarify the mechanism of action of compound **2a** further, we studied the ability of this compound to enhance the anticonvulsant effect of diazepam, a benzodiazepine receptor agonist. The combination of a low dose (15 mg kg^{-1}, i.p.) of **2a** (which by itself failed to antagonize isoniazid-induced seizures (Table 2)) with ineffective doses (0·2–0·5 mg kg^{-1}, i.p.) of diazepam resulted in a potentiation of the anticonvulsant effect of both drugs (Table 4). The co-administration of **2a** (15 mg kg^{-1}, i.p.) with diazepam (0·4 mg kg^{-1}, i.p.) significantly reduced the number of mice which presented tonic-clonic seizures (− 60%) or died (0%) and markedly delayed both the onset of convulsions or death. The combination of 15 mg kg^{-1} (i.p.) **2a** with 0·5 mg kg^{-1} (i.p.) diazepam completely abolished the convulsant action of isoniazid.

Table 5 shows the effect of compounds **2a–c** and **3a–c** in

Table 2. Effect of **2a** on isoniazid-induced tonic-clonic seizures in mice.

Experimental group	Dose (mg kg^{-1})	Convulsions			Mortality	
		Onset (min)	No. of animals	%	Onset (min)	No. of animals
Isoniazid	200	63 ± 2·0	9/9	100	69 ± 6	9/9
Isoniazid + **2a**	15	72 ± 5·2	10/10	100	75 ± 2	8/10
Isoniazid + **2a**	25	111 ± 3·0[a]	4/10[b]	40	79	2/10
Isoniazid + **2a**	50	–	0/10[b]	0	–	0/10

Mice were injected simultaneously with isoniazid (s.c.) and **2a** (i.p.) and were observed for the next 2 h during which time the latency of tonic-clonic seizures was recorded. [a]$P < 0·05$ compared with isoniazid-treated mice (Student's *t*-test) [b]$P < 0·05$ compared with isoniazid-treated mice (Fisher's exact probability test).

Table 3. Effect of **1a–c**, **2b**, **2c** and **3a–c** on isoniazid-induced tonic-clonic seizures in mice.

Experimental group	Dose (mg kg^{-1})	Convulsions			Mortality	
		Onset (min)	No. of animals	%	Onset (min)	No. of animals
Isoniazid	200	58 ± 7	27/27	100	73 ± 6	25/27
Isoniazid + **1a**	100	121[a]	1/5[b]	20	–	0/5
Isoniazid + **1b**	50	100 ± 6[a]	3/5	60	–	0/5
Isoniazid + **1c**	100	99 ± 8[a]	3/5	60	140[a]	1/5[b]
Isoniazid + **1d**	100	71 ± 1·5	3/5	60	87 ± 16	3/5
Isoniazid + **2b**	50	69 ± 6	5/5	100	102 ± 9[a]	2/5[b]
Isoniazid + **2c**	50	51 ± 7	5/5	100	93 ± 2	4/5
Isoniazid + **3a**	50	66 ± 3	5/5	100	93 ± 8	2/5[b]
Isoniazid + **3b**	50	54 ± 4	5/5	100	81 ± 3	4/5
Isoniazid + **3c**	50	48 ± 2	5/5	100	85 ± 8	5/5

Mice were injected simultaneously with isoniazid (s.c.) and the tested compounds (i.p.) and were observed for the next 2 h during which time the latency of tonic-clonic seizures was recorded. [a]$P < 0·05$ compared with isoniazid-treated mice (Student's *t*-test) [b]$P < 0·05$ compared with isoniazid-treated mice (Fisher's exact probability test).

rats treated with isoniazid (350 mg kg^{-1}, s.c.). A dose of **2a** as low as 15 mg kg^{-1} (i.p.), ineffective in mice (Table 2), significantly delayed the onset of seizures (23 min), and slightly reduced the percentage (− 43%) of convulsant rats; **2a** (50 mg kg^{-1}, i.p.) also completely antagonized seizures elicited by isoniazid. Finally, like **2a**, 50 mg kg^{-1} **2c** completely antagonized isoniazid-induced convulsions and 25 mg kg^{-1} **3c** significantly reduced the number of rats which presented convulsions (Table 5). These results indicate that these compounds have greater efficacy in antagonizing the effect of isoniazid in rats (ED50 value for **2a** 15·9 mg kg^{-1}) than in mice (ED50 value for **2a** 24·3 mg kg^{-1}). To investigate the anticonvulsant action of compound **2a** further the efficacy of the compound was tested in mice treated with pentylenetetrazole (60 mg kg^{-1}, i.p.), a convulsant drug that blocks the GABA-gated chloride channel (Squires et al 1984).

As shown in Table 6, compound **2a** showed low efficacy and potency in antagonizing pentylenetetrazole-induced seizures; **2a** (25–50 mg kg^{-1}, i.p.) gave only partial protection (− 44% and − 56%, respectively). The combination of **2a** (25 mg kg^{-1}) and diazepam (0·2 mg kg^{-1}) at doses unable alone to antagonize convulsions completely, failed to antagonize the effect of pentylenetetrazole. In contrast, the co-administration of 50 mg kg^{-1} of **2a** and 0·2 mg kg^{-1} of diazepam resulted in complete antagonism.

Despite the low number of compounds examined, some trends of structure-activity relationships (SAR) can be observed. Analysis is limited to activity expressed as a percentage of protection from the onset of convulsions. To help the discussion, those data have been transformed to an arbitrary scale (from 0 to 4) in which the higher the number, the greater the activity. Comparison of anticonvulsant activity in mice of **2a–c** with that of **1a–c** suggests that the fused benzene ring is not an essential feature for biological activity. Within the set of *7a*-aryl-substituted compounds **2a–c** the anticonvulsant activity in rats seems to decrease slightly on introduction of a methoxy group in the *para* position. Substitution of the ring heteroatom (oxygen for sulphur) resulted in reduced potency both in mice (compare **2a** with **3a**) (Tables 2 and 3) and in rats (compare **2a–c** with **3a–c**) (Table 4).

In an effort to elucidate the physicochemical features affecting pharmacological activity, we attempted to find a relationship between anticonvulsant activity and molecular lipophilicity descriptors. No clear correlation was observed with the molecular lipophilicity as measured by the chromatographic parameter R_M. For instance, compound **2b**, which has an R_M value comparable with that of the most active compound **2a** (Table 1), showed no anticonvulsant activity in mice and was less potent than **2a** in rats (Tables 2 and 3). Similarly, no definitive correlation was found even with the octanol-water partition coefficient estimated by use of the CLOGP algorithm (MacLogP, version 1·0·1, BioByte Corp., Claremont, CA 91711, USA). These observations suggest that physicochemical parameters other than lipophilicity should be

Table 4. Effect of co-administration of low doses of **2a** and diazepam on isoniazid-induced convulsions in mice.

Experimental group and dose (mg kg^{-1})	Convulsions			Mortality	
	Onset (min)	No. of animals	%	Onset (min)	No. of animals
Isoniazid (200)	49 ± 2	18/19	95	75 ± 3	15/19
Isoniazid + **2a** (15)	51 ± 2	19/19	100	71 ± 2	15/19
Isoniazid + diazepam (0·2)	52 ± 3	15/15	100	71 ± 4	12/15
Isoniazid + diazepam (0·4)	61 ± 1	15/15	100	97 ± 4[a]	12/15
Isoniazid + diazepam (0·5)	69 ± 3[a]	10/10	100	77 ± 2	6/15[d]
Isoniazid + **2a** (15) + Diaz (0·2)	68 ± 3[b]	12/15	80	97 ± 3[b]	6/15[e]
Isoniazid + **2a** (15) + Diaz (0·4)	91 ± 8[b]	8/20[c]	40	–	0/20
Isoniazid + **2a** (15) + Diaz (0·5)	88[b]	1/15[c]	6	–	0/15

Mice were injected with **2a** (15–25 mg kg^{-1}, i.p.) or diazepam (0·2–0·5 mg kg^{-1}, i.p.) 20 and 25 min, respectively, after administration of isoniazid (200 mg kg^{-1}, s.c.). Mice were observed for the next 2 h during which time the latency of tonic-clonic seizures was recorded. [a]$P < 0·01$ compared with isoniazid-treated mice; [b]$P < 0·01$ compared with mice treated with isoniazid + diazepam (Student's *t*-test). [c]$P < 0·005$ compared with mice treated with isoniazid + diazepam; [d]$P < 0·005$ compared with isoniazid-treated mice; [e]$P < 0·05$ compared with mice treated with isoniazid + diazepam (Fisher's exact probability test). Each value of the co-administrations is compared with the respective dose of isoniazid + diazepam alone.

Table 5. Effect of compounds **2a–c** and **3a–c** on isoniazid-induced tonic-clonic seizures in rats.

Experimental group	Dose (mg kg^{-1})	Convulsions		
		Onset (min)	No. of animals	%
Isoniazid	350	55 ± 5	19/22	86
Isoniazid + **2a**	10	63 ± 5	7/7	100
Isoniazid + **2a**	15	78 ± 4[a]	8/14	57
Isoniazid + **2a**	25	77 ± 24[a]	2/12[b]	16
Isoniazid + **2a**	50	–	0/13[b]	0
Isoniazid + **2b**	25	64 ± 2	3/5	60
Isoniazid + **2b**	50	68	1/6[b]	16
Isoniazid + **2c**	25	48 ± 9	2/5[b]	40
Isoniazid + **2c**	50	–	0/6[b]	0
Isoniazid + **3a**	50	72 ± 8	2/6[b]	33
Isoniazid + **3b**	50	54 ± 3	4/6	66
Isoniazid + **3c**	15	62 ± 1	4/7	57
Isoniazid + **3c**	25	59 ± 6	3/12[b]	25
Isoniazid + **3c**	50	87[a]	1/6[b]	16

Rats were injected with isoniazid (s.c.) and 30 min later with the tested compounds (i.p.) They were then observed for 2 h during which time the latency of tonic-clonic seizures was recorded. [a]$P < 0·05$ compared with isoniazid-treated rats (Student's *t*-test). [b]$P < 0·05$ compared with isoniazid-treated rats (Fisher's exact probability test).

considered to explain the structure-activity relationships observed.

It is interesting to note that our compounds possess structural elements which appear essential for anticonvulsant activity, namely a nitrogen heterocyclic system bearing one or two phenyl rings and at least one carbonyl group (Moreau et al 1994). These structural features also occur in antiepileptic drugs in clinical use, e.g. phenytoin, phenobarbital and diazepam. Taking into account that, similarly to our compounds, phenobarbital effectively antagonizes isoniazid-induced convulsions (Loscher & Frey 1977), a molecular modelling study was therefore conducted to determine the structural analogy between this molecule and ours and to derive possible structure-activity relationships.

Molecular models of compounds **1–3** were built using the SYBYL fragment library and minimized. Conformational analyses were performed by means of the SYSTEMATIC SEARCH option of SYBYL. The conformers were generated by screening all the torsion angles with a size step of 30°, and optimized by molecular mechanics (MAXIMIN, Tripos Force Field). The resulting minimum energy conformers were identified and optimized again by the semi-empirical quantum mechanical method AM1. Although good agreement was obtained between the structures of the minima detected by us for phenobarbital and those determined by others (Wong et al 1986), the geometry of the minimum energy conformer was slightly modified to obtain a better fit of the ethyl group with the pyrrolidinone moiety of compounds **1–3**. No significant energy increase was observed. The phenobarbital thus modified was used as the template for the overlay of the different molecules which was performed by the FIT option of SYBYL.

Four pharmacophoric elements were used for the molecular

Table 6. Effect of co-administration of **2a** and diazepam on pentylenetetrazole-induced seizures in mice.

Experimental group and dose (mg kg^{-1})	Convulsions		
	Onset (min)	No. of animals	%
Pentylenetetrazole (60)	2·4 ± 0·4	16/19	84
Pentylenetetrazole + **2a** (25)	4·3 ± 0·8	13/23	56
Pentylenetetrazole + **2a** (50)	4·2 ± 1·2	8/18[a]	44
Pentylenetetrazole + diazepam (0·2)	3·7 ± 0·7	3/5	60
Pentylenetetrazole + **2a** (25) + diazepam (0·2)	4·5 ± 0·5	2/5[a]	40
Pentylenetetrazole + **2a** (50) + diazepam (0·2)	–	–	–

Animals were injected with pentylenetetrazole (60 mg kg^{-1}, i.p.) 25 and 30 min after diazepam (0·2 mg kg^{-1}, i.p.) or **2a** (25 or 50 mg kg^{-1}, i.p.) or after diazepam (0·2 mg kg^{-1}, i.p.) and **2a** (25 or 50 mg kg^{-1}, i.p.) and were observed for the next 2 h during which time the latency and pattern of tonic-clonic seizures were recorded. [a]$P < 0·025$ compared with pentylenetetrazole-treated mice.

Table 7. Molecular volumes of pyrrolobenzothiazoles **1a–d**, pyrrolothiazoles **2a–c**, and pyrroloxazoles **3a–c**.

Cmpd	Molecular volumes[a]				Activity	
	Molecular volume (Å^3)	Union volume (Å^3)	Intersection volume (Å^3)	Relative intersection volume (Å^3)	Mice	Rats
1a	215·3	255·4	144·9	0·57	3	
1b	238·7	278·8	144·7	0·52	2	
1c	238·4	280·0	143·4	0·51	1	
1d	237·2	284·9	136·6	0·43	1	
2a	181·5	227·8	138·8	0·61	4	4
2b	204·6	249·6	140·0	0·56	0	3
2c	193·0	238·7	139·3	0·58	0	4
3a	172·8	226·3	131·6	0·58	0	2
3b	196·3	249·2	132·3	0·53	0	1
3c	184·5	236·8	132·7	0·56	0	3

[a]The molecular volume of phenobarbital (template) is 185 Å^3.

fitting, namely the centroid of the phenyl ring, the bridgehead carbon atom and the carbon atom α to it, and the lone pair of the lactam carbonyl pointing toward the nitrogen atom. The last element was chosen because it gave a better fit.

Because compound **1d** does not possess a phenyl substituent, two other points, the carbon atom of the ester carbonyl and that of the second methylene group of the aliphatic chain, were used for superimposition on the first aromatic carbon and on the phenyl centroid of the template, respectively.

The molecular volumes were calculated by the VOL option whereas the inclusive (union) volumes, and the intersection volumes of each lowest energy conformer of compounds **1–3** fitted on phenobarbital, were determined by the MVOL option of SYBYL. In addition, the relative intersection volume (RIV), i.e. the ratio between the intersection and union volume, was calculated for each compound as a possible rough measure of the shape similarity with phenobarbital.

Table 7 reports the volume map data. As can be seen, **2a** has a relative intersection volume value of 0·61, significantly higher than the average value for compounds **1a–d**, **2b**, **2c** and **3a–c** (0·545 ± 0·051). It seems, therefore, that the highest value of the relative intersection volume observed for **2a**, could be related to a greater degree of similarity to phenobarbital and this could explain, at least partially, why **2a** has the highest anticonvulsant activity both in mice and in rats. With the exception of **2a**, only compounds with high lipophilicity ($R_M > 0$, log $P > 2·7$) have appreciable anticonvulsant activity in mice and, interestingly, such activity seems to be linearly related to RIV. As far as anticonvulsant activity in rat is concerned, the even lower number of data precludes any safe discussion of structure-activity relationships. It is worth noting, however, that the highest activity is observed for compounds **2a** and **2c**, which again have a high RIV value, and that low activity is associated with less lipophilic compounds, e.g. **3a** and **3b**.

These findings and the preliminary SAR just discussed might facilitate the planning of the synthesis of new compounds which can be used to gain more certain insight into the SAR, and thereafter for the preparation of more potent and efficient anticonvulsants.

In conclusion, of the 7*a*-aryl-substituted pyrrolothiazol- or pyrroloxazol-5-ones prepared, the 7*a*-phenyl-pyrrolothiazol-5-one **2a** showed potent anticonvulsant activity against isoniazid-induced seizures in rodents. As results from previous biochemical studies have shown that there is no direct interaction of this compound with the $GABA_A$ receptor complex (Trapani

et al 1994), the present results could support the idea that compound **2a** exerts its anticonvulsant action by indirect modulation of $GABA_A$ receptor function, a mechanism reported for other positive modulators of GABAergic transmission such as valproate (Concas et al 1991).

Finally, the results reported herein demonstrate that **2a** is an attractive candidate as an anticonvulsant agent worthy of further study in other seizure models and may aid the design of other anticonvulsant drugs.

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