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Short communication

Synthesis and anticonvulsant activity of some 2-(*N*-substituted glycylamino)-4-methyl thiazoles

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Summary – During the search for new biologically active agents in the thiazole field, some 2-(*N*-substituted glycylamino)-4-methyl thiazoles were synthesized. Preliminary pharmacological evaluation showed that these compounds exhibited anticonvulsant activity.

Résumé – **Synthèse et activité anticonvulsivante de quelques dérivés du 2-amino-4-méthylthiazole acylé par des glycines *N*-substituées.** *Au cours de la recherche de nouveaux agents biologiquement actifs dans la série du thiazole, quelques dérivés du 2-amino-4-méthylthiazole ont été synthétisés. Une étude pharmacologique préliminaire montre que ces composés ont une activité anticonvulsivante.*

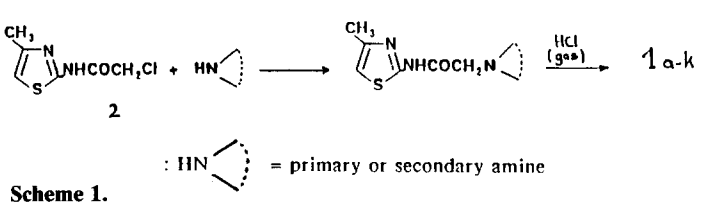
substituted thiazoles / anticonvulsant activity

Introduction

Interest has been shown in thiazole derivatives due to their effects on the central nervous system (CNS) and their α-adrenoreceptor blocking activities [1, 2]. The majority of conventional anticonvulsant drugs have a dicarboximide (–CONHCO–) or an ureide (–NHCONH–) function, as in hydantoins, barbiturates, succinimides and oxazolidinediones. The dicarboximide function contributes to the hypnotic and sedative activity of barbiturates and related compounds. Its absence may therefore be expected to reduce the toxic side-effects in potential thiazole anticonvulsants. In order to explore this hypothesis, new 2-(*N*-substituted glycylamino)-4-methyl thiazoles **1a–k** have been prepared and their anticonvulsant activity has been investigated.

Chemistry

The 2-(*N*-substituted glycylamino)-4-methyl thiazoles **1a–k** were prepared as shown in Scheme 1.



Scheme 1.

The reaction of the 2-chloroacetamido-4-methyl thiazole **2** [3] with an excess of primary or secondary amines gave the desired acetamides **1a–k,** described as their hydrochloride.

The structure of derivatives **1a–k** was confirmed by elemental analysis and spectral data. The IR and NMR spectra were in agreement with the proposed structures.

Pharmacological results and Discussion

Pharmacological properties of the synthesized compounds are summarized in Tables I–III. All the molecules have an LD_{50} value higher than 1 $g \cdot kg^{-1}$ *(p.o.)*, so their toxic potential is not very important. The majority of the compounds possessed significant anticonvulsant activity blocking both chemically and electrically induced seizures, with a weak level of neurotoxicity. The most potent molecules occurred in the piperazine derivatives. It was found that the *m*-trifluoromethylphenyl piperazine analogue **1g** possessed higher anticonvulsant activity and lesser neurotoxic effects than the other thiazole derivatives. The pharmacological study of the **1g** compound was therefore carried out. The anticonvulsant properties of **1g** were confirmed on 3-mercaptopropionic acid (3-MPA) and bicuculline induced seizures. However, **1g** lacked any effects on strychnine-induced convulsions. A weak anticholinergic activity was shown against oxotremorine-induced tremors at 200 $mg \cdot kg^{-1}$. At this same dose, **1g** compound was found to have a small anxiolytic effect. However, **1g**

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had no sedative activity on the traction test in mice. It can be concluded that the absence of the dicarboximide function in thiazole derivatives, contributes to the separation of the anticonvulsant activity from the sedative action of such compounds. Nevertheless, the small number of compounds investigated and the low anticonvulsant activity have encouraged us to continue with our chemical pharmacomodulation study.

Table I. Effective dose (ED_{50}), lethal dose (LD_{50}) and toxic dose (TD_{50}) values for thiazole compounds and reference substances.

Products	LD_{50} ($mg \cdot kg^{-1}$)	ED_{50} ($mg \cdot kg^{-1}$)		TD_{50} ($mg \cdot kg^{-1}$)
		PTZ	MES	
1a	> 1000	> 400	> 400	> 1000
1b	> 1000	> 400	> 400	> 1000
1c	1000	200	> 400	> 1000
1d	> 1000	132	227	> 1000
1e	> 1000	169	355	712
1f	> 1000	238	> 400	> 1000
1g	> 1000	115	109	> 2000
1h	> 1000	232	365	1000
1i	> 1000	150	302	> 2000
1j	> 1000	> 400	> 400	> 2000
1k	> 1000	280	> 400	> 2000
Valpr.	977	244	242	668
Phenobar.	214	11	8	114

Valpr.: sodium valproate.
Phenobarb.: phenobarbital.

Table II. Therapeutic (TI: LD_{50}/ED_{50}) and protective index (PI = TD_{50}/ED_{50}) values for thiazole compounds and reference substance.

Products	TI		PI	
	PTZ	MES	PTZ	MES
1d	> 7.5	> 4.4	7.6	4.4
1e	> 5.9	> 2.8	4.2	2.0
1f	> 4.2	–	4.2	–
1g	> 8.7	> 9.2	> 17.3	> 18.3
1h	> 4.3	> 2.7	4.3	2.7
1i	> 6.6	> 3.3	> 13.3	> 6.6
Valpr.	4	4	3	3
Phenobarb.	20	27.8	11	15

PTZ: maximal pentetrazole seizure test.
MES: maximal electroshock seizure test.

Table III. Extensive anticonvulsivant studies with compound **1g** and reference substances. (The results are expressed by ED_{50} in $mg \cdot kg^{-1}$, *p.o.*).

	Chemically-induced seizures		
	3-MPA	Bicuc.	Strych.
1g	81	100	> 1000
Valpr.	330	654	347
Phenobarb.	39	15	39

3-MPA: 3-mercaptopropionic acid.
Bicuc.: bicuculline.
Strych.: strychnine.

Table IV. CNS studies with compound **1g** (the results are expressed by percent activity at the dose in brackets).

	Oxotrem.	Traction	4 plates
1g	−32% (200)	No effect (1000)	+ 80% (200)
References	Atropine	Meprobamate	Meprobamate
	(5) −79%	(200) −40%	(100) + 100%

Oxtrem.: oxotremorine.

Experimental protocols

Chemistry

Melting points were taken on a Kofler apparatus and are uncorrected. ^1H NMR spectra were recorded on a Perkin–Elmer R 12B (60 MHz) spectrometer using tetramethylsilane as the internal standard. IR spectra were recorded on a Perkin–Elmer 397 spectrophotometer. Microanalyses were performed by ATXSA (Nanterre, France) and values are within ± 0.4% of the theoretical values.

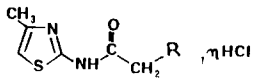
2-[(4-(3-Trifluoromethylphenyl) piperazin-1-yl) acetamido]-4-methyl thiazole

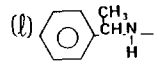
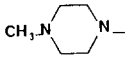
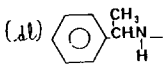
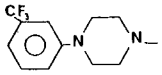
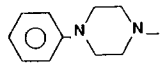
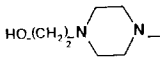
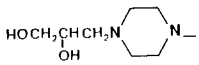
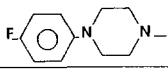
To a stirred suspension of 0.1 mol (19.1 g) of 2-chloroacetamido-4-methyl thiazole [3] in 190 ml of toluene, 0.205 mol (47.2 g) of *N*-3-trifluoromethylphenyl piperazine were added dropwise. The reaction mixture was stirred and refluxed for 2 h, allowed to cool, poured into water and extracted with toluene. The organic phase was washed with brine and then dried (Na_2SO_4) and evaporated to give a brown oil which crystallized in petroleum ether. Recrystallization from toluene–hexane (v/v) gave 29.25 g (76%) of white crystals; mp: 100–102°C.

2-[(4-(3-Trifluoromethylphenyl) piperazin-1-yl) acetamido]-4-methyl thiazole, hydrochloride ***1g***

0.1 mol (38.44 g) of base were transformed into hydrochloride by treatment with dry hydrogen chloride in 775 ml of anhydrous ether. Crystals were filtered, washed with ether and dried. Recrystallization from ethanol gave 30.81 g (73%) of white crystals; mp: 228–229°C.
The other acetamide derivatives **1a–k** were obtained from the suitable amines by the same procedure. Yields, melting points, recrystallization solvents and elemental analyses are listed in Table V.
IR and NMR spectra are reported in Table VI.

Table V. *N*-(4-Methyl-2-thiazolyl) acetamides.



Compound	R	Formula[a]	Yield %	Mp °C	Recryst. solv.
1a [4]		$C_{11}H_{17}N_3OS$, 2 HCl	44.7	140 - 142	iso PrOH/AcOEt
1b		$C_{10}H_{15}N_3O_2S$, 2 HCl	45	184 - 186	EtOH
1c		$C_{14}H_{17}N_3OS$, 2 HCl	24	190 - 192	EtOH
1d		$C_{11}H_{18}N_4OS$, 3 HCl	50	220 - 222	EtOH
1e		$C_9H_{15}N_3OS$, 2 HCl	26.5	239 - 241	EtOH
1f		$C_{14}H_{17}N_3OS$, 2 HCl	26	195 - 196	EtOH
1g		$C_{17}H_{19}F_3N_4OS$, HCl	56	228 - 229	EtOH
1h		$C_{16}H_{20}N_4OS$, 2 HCl	40	206 - 207	EtOH
1i		$C_{12}H_{20}N_4O_2S$, 2 HCl	55	212 - 213	EtOH
1j		$C_{13}H_{22}N_4O_3S$, 3 HCl	17	220 - 222	Toluène/MeOH
1k		$C_{16}H_{19}FN_4OS$, 2 HCl	49	210 - 212	EtOH/AcOEt

[a]Satisfactory microanalyses obtained: C, H, Cl, N values are within ± 0.4% of the theoretical ones.

Pharmacology

General procedures

Male mice (OF_1, Iffa Credo origin) in the weight range 18–22 g were used. The compounds were administered *per os (p.o.)* in a vol of 0.01 ml·g^{-1} body weight, generally in a 5% mixture of Tween 80 in distilled water. This vehicle was tested for anticonvulsant and toxic effects and was found to be inactive in all the test procedures.

All the thiazole derivatives were screened in 2 seizures models in the mouse and the central nervous system (CNS) toxicity was evaluated in the rotarod ataxia test as previously described [4]. The most interesting molecule was subjected to an extensive study.

Acute oral toxicity

LD_{50} values were determined by *p.o.* administration and the computerized probit analysis method. Mortality counts were taken 7 days after injection.

Maximal pentetrazole seizure test (PTZ)

125 mg·kg^{-1} of pentylenetetrazol (pentetrazol) were injected as a 0.5% solution intraperitoneally, 30 min after injection of the test compound. Failure to observe the hind limb tonic extensor was defined as protection. The dose required to produce a protection in 50% of the animals (ED_{50}) was obtained graphically by using the method of probit analysis.

Maximal electroshock seizure test (MES)

Maximal seizures were induced by application of an electric current across the brain *via* corneal electrodes. The stimulus parameters for mice were pulses of 30 Hz and 50 V for 1 s. A drop of 0.9% saline was placed in the eye prior to the application of electrodes in order to prevent the death of the mice.

As in the PTZ test, the abolition of the hind limb tonic extensor component of maximal seizures was defined as protection.

Table VI. Spectral data compounds **1a–k**.

Compound	IR (KBr) ν(cm^{-1})		^{1}H NMR (solvent/TMS)
1a	1705, 1625, 1570	TFA:	1.60 (br, 6H, pip.); 2.20 (s, 3H, CH_3 th.); 3.25 (m, 4H, pip.); 4.30 (s, 2H, CH_2); 6.70 (s, 1H, th.).
1b	1710, 1620, 1575	DMSO:	2.28 (s, 3H, CH_3 th.); 3.45 (br, 4H, mor.); 3.90 (br., 4H, mor.); 4.32 (s, 2H, CH_2); 6.75 (s, 1H, th.); 7.90 (br, 3H, $^+$NH + NH).
1c	3100, 1700, 1620, 1560	DMSO:	1.70 (d, 3H, CH_3–CH–); 2.30 (s, 3H, CH_3 th.); 3.75 (s, 2H, CH_2); 6.85 (s, 1H, th.); 7.50 (m, 5H, arom.); 8.60 (br, S, 4H, $^+$NH + NH).
1d	3100, 1710, 1620, 1560	DMSO:	2.26 (s, 3H, CH_3 th.); 2.70 (s, 3H, CH_3); 3.62 (s, 8H pipz.); 4.33 (s, 2H, CH_2); 6.85 (s, 1H, th.); 8.60 (br, 4H, $^+$NH + NH).
1e	3110, 1710, 1600, 1560	DMSO:	1.40 (d, 6H, $(CH_3)_2$–CH); 2.38 (s, 3H, CH_3 th.); 3.35 (br, 1H, CH); 4.05 (s, 2H,CH_2); 6.85 (s, 1H, th.); 9.50 (br, 3H, $^+$NH + NH).
1f	3100, 1700, 1600, 1550	DMSO:	1.68 (d, 3H, CH_3–CH–); 2.28 (s, 3H, CH_3 th.); 3.80 (br, s, 2H, CH_2); 4.45 (s, 1H, CH); 6.82 (s, 1H th.); 7.50 (br, 5H, arom); 8.80 (s, 4H, $^+$NH + NH).
1g	1695, 1605, 1550	DMSO:	2.25 (s, 3H, CH_3 th.); 3.55 (s, 8H, pip.); 4.36 (s, 2H, CH_2); 6.83 (s, 1H, th.); 7.30 (br, 4H, arom.).
1h	3120, 1720, 1620, 1560	DMSO:	2.30 (s, 3H, CH_3 th.); 3.55 (s, 8H pipz.); 4.40 (s, 2H, CH_2); 6.85 (s,1H, th.); 7.20 (br, 5H, arom.).
1i	3100, 1710, 1620, 1560	DMSO:	2.35 (s, 3H, CH_3 th.); 3.40 (br, 4H, $(CH_2)_2$; 3.75 (br, 8H, pipz.); 4.30 (s, 2H, CH_2); 6.80 (s, 1H, th.); 8.65 (br, 3H, $^+$NH + NH).
1j	1705, 1615, 1560	DMSO:	2.28 (s, 3H, CH_3 th.); 3.35 (br, 4H, diol-chain); 3.70 (br, 8H, pipz.); 4.00 (br. 1H, CH); 4.32 s, 2H, CH_2); 6.85 (s, 1H, th.); 7.20 (br, 6H, exchangeable protons).
1k	3110, 1690, 1590, 1550	DMSO:	2.40 (s, 3H, CH_3 th.); 3.70 (s, 8H, pipz.); 4.60 (s, 2H, CH_2); 7.22 (s, 1H, th.); 7.40 (m, 4H, arom.); 9.30 (br, 3H, $^+$NH + NH).

th. = thiazole; pip. = piperidine; mor. = morpholine; pipz. = piperazine.

Rota-rod ataxia test
To evaluate neurotoxicity, the mouse was placed on a wooden 3-cm diameter rod rotating at 15 rpm. Normal trained mice remained on this rotarod for several minutes. Trained animal were dosed with the test compound or drug vehicle and they were tested to measure the effects of the drug on motor performance 30 min after administration. Neurological toxicity was defined as the failure of the animal to remain on the rod for 2 min. The dose at which 50% of the animals fell off the rotarod (TD_{50} value) was graphically determined.

The ratio LD_{50}/ED_{50} defined the therapeutic index (TI) value which is a measure of the margin of safety. The ratio TD_{50}/ED_{50} defined the protective index (PI) value, which assessed the margin between anticonvulsant and neurotoxic effects.

Extensive studies
Antagonism of chemically induced seizures in mice. The ability of the test compound to protect against seizures was measured 30 min after administration (*p.o.*). The seizures were induced by bicuculline (3 $mg \cdot kg^{-1}$, s.c.), 3 mercapto-propionic acid (3 MPA 65 $mg \cdot kg^{-1}$, i.p.) and strychnine (2 $mg \cdot kg^{-1}$, s.c.). Bicuculline and 3 MPA induce convulsions *via* a GABA antagonist effect and strychnine blocks postsynaptic inhibition mediated by glycine. The results were expressed by ED_{50} ($mg \cdot kg^{-1}$) as for PTZ induced seizures.

Effect on tremor-induced by oxotremorine in mice. The test compound was given orally to mice 30 min before administration of oxotremorine (0.4 $mg \cdot kg^{-1}$, i.p.). Half an hour after the injection of oxotremorine each mouse was observed to determine the tremor severity according to a rating scale with scores of 1–10; average suppression of the tremor by each dose of test compound was expressed as percent inhibition of control.

Traction test
Muscular relaxation was evaluated according to the method described by Boissier and Simon [5]. Mice were hung by their forepaws from a metal rod 2.5 mm in diameter and 30 cm in length fixed horizontally 15 cm above a platform. Normal animals climb on the rod within 5 s, hanging by all four paws, whereas mice with impairment of muscular tone fall from the rod or continued to hang by the forepaws only. 30 min after dosing, the mice were suspended by means of their forepaws to the rod and the percentage of them falling from it was recorded.

Four plates electrified box test
According to the procedure described by Boissier *et al.* [6], the mice were put individually in a box with a transparent cover. The floor was divided into four plates of stainless steel and connected electrically and diagonally 2 by 2. The box was connected to an electric shock generator. The reactions of each mouse could be observed as it crossed from one plate to another of opposite polarity.

An anxiolytic compound induces an increase of passages punished by electric shock. The anxiolytic effect of each dose of test compound was expressed as percent increase of control.

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