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New thiazolo[3,2-*a*]pyrimidine derivatives, synthesis and structure–activity relationships*

E Jeanneau-Nicolle, M Benoit-Guyod, A Namil, G Leclerc

Groupe de Pharmacochimie Moléculaire, Laboratoire de Chimie Organique, UFR de Pharmacie, Université Joseph Fourier (Grenoble I), BP 138, 38240 Meylan Cedex, France

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Summary — Twenty-six derivatives of 5- and 7-oxo or 5- and 7-aminothiazolo[3,2-*a*]pyrimidines were prepared and evaluated as positive inotropic, anti-inflammatory and antihypertensive agents both *in vitro* and *in vivo*. The structures of the 5- and 7-isomers have been confirmed unambiguously by IR, UV, ^{1}H and ^{13}C NMR and MS. The structure of **1a** was confirmed by the synthesis of compound **1** whose structure had been previously established by X-ray analysis. The 5-aminothiazolo-pyrimidine-6-carbonitrile derivatives **5a** and **5e** exhibited potent inotropic activities. **5e** was more potent than the classical reference amrinone. Three compounds **5e**, **5i** and **5j** displayed moderate antihypertensive activities. The 4-chlorophenyl derivative **5d** exhibited an anti-inflammatory activity 2-fold that of aspirin. This study has demonstrated that thiazolo[3,2-*a*]pyrimidine compounds have interesting biological potentialities, particularly as inotropic agents, a field which had never been explored so far for this series.

thiazolo[3,2-*a*]pyrimidines / cardiotonic / inotropic / antihypertensive / anti-inflammatory activity

Introduction

For several years our laboratory has been interested in immunomodulators and anti-inflammatory substances derived from 2-aminothiazoline [1–3]. More recently we have examined non steroidal cardiotonic agents for the treatment of heart failure [4]. Much research aimed at finding a safe and orally active compound has been carried out [5]. We therefore thought it of interest to explore the potentialities of derivatives of 2-aminothiazoline and more particularly of thiazolo[3,2-*a*]pyrimidines as anti-inflammatory and positive inotropic agents [6].

Thiazolo[3,2-*a*]pyrimidines bear a structural analogy with the potent immunomodulator levamisole and are also associated with the butyrophenone skeleton in 2 antipsychotic drugs, namely ritanserine and setoperone [7, 8] (fig 1).

Although thiazolo[3,2-*a*]pyrimidines have been well studied as immunomodulators [9, 10], anticancer agents [11, 12], analgesics [13, 14], and psychotropes [11, 15], they have not been explored as possible positive inotropic agents.

In this paper we report on the synthesis and pharmacological activities of 26 new thiazolo[3,2-*a*]-pyrimidines, whose structures have been unambiguously assigned by spectroscopic and chemical investigation.

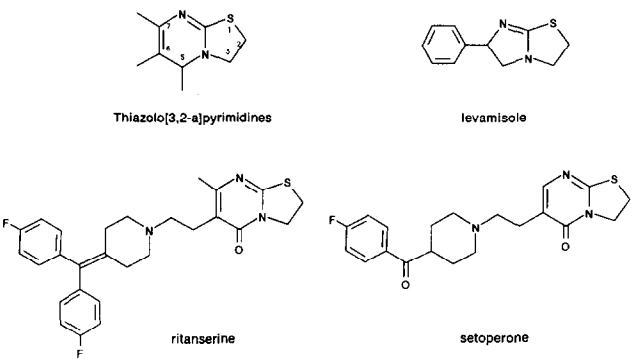
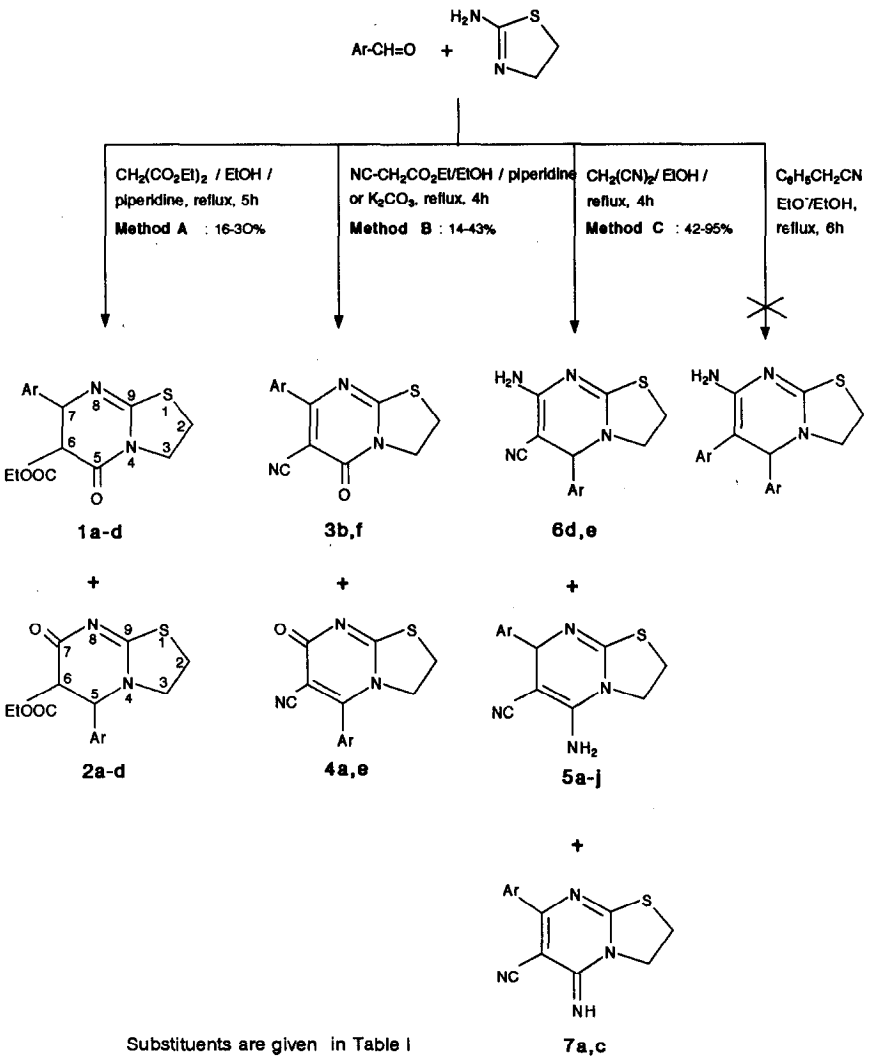


Fig 1.

Chemistry

All the compounds except **1** were prepared according to scheme 1. The one-pot reaction between 2-aminothiazoline, an aromatic aldehyde and a compound bearing an activated methylene group such as diethylmalonate, ethylcyanoacetate or malononitrile, allowed the obtention of 5- and 7-oxo-thiazolo-dihydropyrim-

*Dedicated to Prof A Boucherle who inspired this work, on the occasion of his 67th birthday.



Scheme 1.

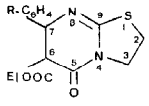
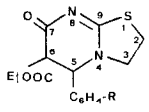
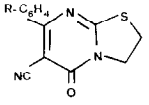
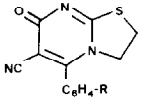
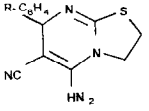
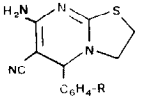
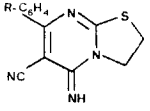
idine-6-ethyl-carboxylates (**1a–d** and **2a–d**), 5- and 7-oxothiazolo-pyrimidine-6-carbonitriles (**3b,f** and **4a, e**), and 5- and 7-aminothiazolo-pyrimidine-6-carbonitriles (**5a–j** and **6d, e**) (table I).

A possible reaction mechanism of this reaction involves a 1,4-nucleophilic addition of 2-aminothiazoline on an α,β-insaturated intermediate which stems from the reaction between the aromatic aldehyde and the compound bearing the activated methylene group [16]. When the methylene group compound contained only one electron attractive substituent (*eg* benzyl-cyanide) the cyclisation failed as reported for pyrazolo[1,5-*a*]pyrimidine derivatives [17]. The mechanism of cyclisation is in agreement with the formation of 2 types of isomer, either 5-oxo or 5-amino and 7-oxo or 7-amino, whose structures have been established by ^{1}H NMR, ^{13}C NMR, IR, UV, and MS [18].

The preparation of oxothiazolopyrimidines (**1a–d** and **2a–d**) called for an ethanolic medium rendered basic by the addition of piperidine or potassium carbonate, whereas aminothiazolo-pyrimidines (**5a–j** and **6d, e**) were obtained under neutral conditions.

The structure of the oxothiazolopyrimidine **1a**, has, for the first time, been confirmed unambiguously by synthesis of the reference compound **1** *(Method E)* [19]. Indeed, the decarboxylation of **1a** led to **1** *(Method D)* [20, 21] whose structure has been established by X-ray analysis (Debarre *et al*, personal communication) (scheme 2). Comparison of the

Table I. Physicochemical and biological data of thiazolo[3,2-*a*]pyrimidines.

Structure	*No*	*R*	*mp* [a] *(°C)*	*% Yield* [b]	*Formula*	*Anal* [c]	*Cardiovascular activity*		*Anti-inflam activity*
							+ INA [d] *% variation (mg/kg)*	*AHTA* [e] *% decrease (mg/kg)*	*AEA* [f] *% decrease (mg/kg)*
	**1a**	H	112–113	11	$C_{15}H_{16}N_2O_3S$	C, H, N	–20 (100)	1,2,2 (100)	25 (200)
	1b	4-$CH(CH_3)_2$	123–124	4	$C_{18}H_{22}N_2O_3S$	C, H, N, O	NTg		
	1c	4-OCH_3	oil	44	$C_{16}H_{18}N_2O_4S$	–	NTg		
	1d	4-$N(CH_3)_2$	118–119	7	$C_{17}H_{21}N_3O_3S$	C, H, N	–15 (100)	3,3,0 (100)	6 (200)
	**2a**	H	142–143	5	$C_{15}H_{16}N_2O_3S$	–	NTg		
	2b	4-$CH(CH_3)_2$	152–153	12	$C_{18}H_{22}N_2O_3S$	C, H, N	–27 (100)	0,3,3 (100)	15(200)
	2c	4-OCH_3	154–155	26	$C_{16}H_{18}N_2O_4S$	C, H, N	18 (200)	0,0,0 (200)	18 (400)
	2d	4-$N(CH_3)_2$	170–171	14	$C_{17}H_{21}N_3O_3S$	C, H, N	–33 (100)	3,5,1 (100)	10 (100)
	**3b**	4-$CH(CH_3)_2$	160–161	24	$C_{16}H_{15}N_3OS$	C, H, N	0 (200)	5,0,0 (200)	0 (400)
	3f	4-CH_3	230–231	38	$C_{14}H_{11}N_3OS$	C, H, N	0 (150)	0,8,7 (300)	3 (600)
	**4a**	H	179–180	14	$C_{13}H_9N_3OS$	C, H, N, S	0 (50)	3,2,3 (100)	0 (200)
	4e	3-NO_2	191–192	43	$C_{13}H_8N_4O_3S$	C, H, N	0 (200)	1,0,0 (200)	8 (400)
	**5a**	H	196–197	90	$C_{13}H_{12}N_4S$	C, H, N	50 (200)	1,5,5 (200)	25 (400)
	5b	4-$CH(CH_3)_2$	190–191	42	$C_{16}H_{18}N_4S$	C, H, N	16 (100)	3,0,5 (100)	25 (200)
	5c	4-OCH_3	194–195	70	$C_{14}H_{14}N_4S$	C, H, N	0 (100)	0,0,4 (100)	15 (200)
	5d	4-Cl	196–197	40	$C_{13}H_{11}Cl\,N_4S$	C, H, N	8 (200)	0,0,3 (200)	47 (100)
	5e	3-NO_2	180–181	70	$C_{13}H_{11}N_4O_2S$	C, H, N	80 (100)	4,2,2 (100)	15 (200)
	5e-HCl		160–161		$C_{13}H_{12}N_4O_2SCl$	C, H, N Cl	73 (100)	11,11,11 (200)	11 (400)
	5f	4-CH_3	198–199	73	$C_{14}H_{14}N_4S$	C, H, N	20 (200)	6,8,4 (200)	21 (400)
	5g	3,4-diCl	190–191	50	$C_{13}H_{10}Cl_2N_4S$	C, H, N	– 8 (300)	2,4,0 (300)	35 (600)
	5h	2-NO_2	182–183	90	$C_{13}H_{11}N_4O_2S$	C, H, N	NTg	NTg	–
	5i	3-Cl	194–195	45	$C_{13}H_{11}N_4S\,Cl$	C, H, N	0 (300)	11,12,14 (300)	25 (600)
	5j	3-Pyridyl	228–229	85	$C_{12}H_{11}S\,N_5$	C, H, N	0 (200)	13,17,16 (200)	13 (400)
	**6d**	4-Cl	214–215	5	$C_{13}H_9ClN_4S$	C, H, N	NTg	NTg	
	6e	3-NO_2	250–251	5	$C_{13}H_{11}N_4O_2S$	C, H, N	NTg	NTg	
	**7a**	H	202–203	5	$C_{13}H_{10}N_4S$	C, H, N	–24 (100)	0,0,0 (100)	51 (100)
	7c	4-OCH_3	214–215	5	$C_{14}H_{12}N_4S$	C, H, N	– 8 (100)	0,0,0 (100)	25 (200)
Amrinone							48 (100)		
α-methyldopa								15,22,27 (50)	
Aspirin									40 (150)
Phenylbutazone									33 (50)

[a]Recrystallization from 95% EtOH except **3f** (DMSO), **5d**, **7a**, **4e**, **6e** (95% EtOH/DMSO), **5b** (60% EtOH), **5h** (100% EtOH). [b]Yield of pure product. [c]Satisfactory microanalyses obtained: C ± 0.30; H ± 0.29; N ± 0.40 except **1b** (C ± 0.48) and **7a** (N ± 0.43). [d]Positive inotropic activity on guinea pig left atria. [e]Antihypertensive activity: blood pressure measured in unanesthetized, untreated SHR. [f]Antiedema activity measured by inhibition of paw edema in rat. [g]NT = non tested product.

physicochemical and spectroscopic data of compound **1** obtained by *Method E* and by *Method D* allowed to attribute the 5-oxo structure to **1a** and, in turn, to compounds **1b**, **1c** and **1d** which possessed the same characteristics.

In the oxothiazolo[3,2-*a*]dihydropyrimidine series, the coupling constant [18] between the vicinal protons H_5–H_6 (7.3 Hz) and H_6–H_7 (10.6 Hz), and the experiments of nuclear Overhauser effect (NOE) (Jeanneau-Nicolle *et al*, in preparation) indicated a *trans*-diequatorial position of the carboxylate and aryl substituent as represented in figure 2.

Results and discussion

Compounds were evaluated for positive inotropic activity on isolated left guinea pig atria for anti-hypertensive activity in spontaneously hypertensive rat (SHR), and for antiedema activity in rat by inhibition of the paw edema, as described in the *Experimental protocols*. Results are shown in table I. Among 26 compounds examined, two 5-amino-thiazolo-pyrimidine-6-carbonitrile derivatives showed significant effects in cardiovascular tests with amrinone and α-methyl-dopa as standards. We tried to get close to the structure of amrinone and milrinone (fig 3) with **5j** but this compound was inactive as far as positive inotropic test was concerned. However,

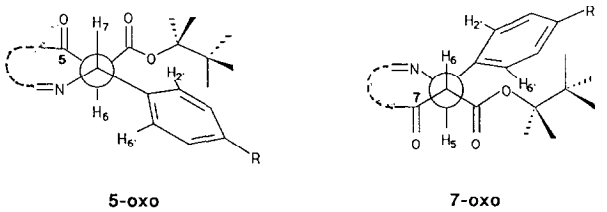


Fig 2.

phenyl substituted, and more especially 3-nitrophenyl substituted derivatives **5a** and **5e** exhibited potent inotropic activities. **5e** was more potent than amrinone. This activity was not altered by the addition of propranolol, indicating that the cardiotonic effect was not likely to be due to a β_1-adrenergic agonist mechanism (see *Experimental protocols*).

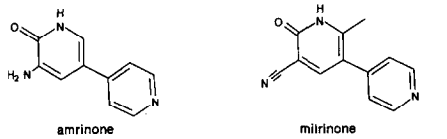
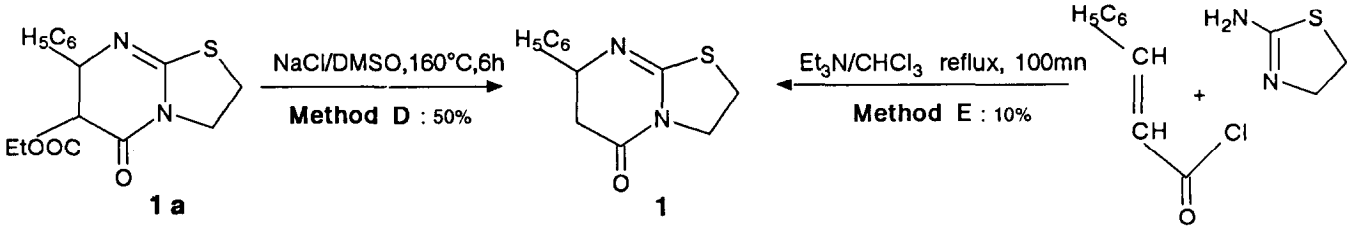


Fig 3.

As far as antihypertensive activity was concerned, the best compounds **5e**, **5i** and **5j** displayed activities *ca* one eighth that of α-methyl-dopa. We note that these compounds bear electron attractive groups nitro, chloro and pyridino respectively in the 3-position of the aromatic nucleus.

The 4-chlorophenyl compound **5d** exhibited anti-inflammatory activity about 2-fold higher than that of aspirin, whereas **5a**, **5b**, **5f** and **5g** were 2–5 times less potent than aspirin. Thus a 4-chlorine atom appeared to be beneficial to this activity when compared to 4-electron donating groups. However, the introduction of a second chlorine atom in 3-position was detrimental. Similarly the 4-phenyl and 4-methoxyphenyl substituted 5-imino derivatives **7a** and **7c** showed anti-inflammatory properties.

In addition, the 7-oxo derivative **2d** displayed anti-dopaminergic activity in mice (at 25 mg/kg ip) comparable to that of sulpiride in attenuating apomorphine-induced climbing behavior (see *Experimental protocols*). At a 4-fold higher dose in rats, no activity to attenuate apomorphine induced stereotypic be-



Scheme 2.

havior was found. This may constitute an interesting separation of activities in relation to current theories about pre-synaptic and post-synaptic sites of action and side-effects of neuroleptic agents.

The antipsychotic activity of **2d** could be related to drugs such as ritanserine and setoperone containing an oxothiazolopyrimidine structure associated to a butyrophenone moiety (fig 1).

Complementary investigations in dogs were carried out with **5a** and **5e**. Compound **5a** was found to be a moderate hypotensive drug (20% of BP fall). The *in vitro* chronotopic effect (+20%) was not blocked by propranolol, indicating a pure cardiotonic action. Compound **5e** was found to be a potent vasodilatator comparable to naftidrofuryl with no action on the heart. Most of the compounds were devoid of toxicity up to 600 mg/kg *po*, and 400 mg/kg ip, except the imine structures **7a** and **7c**.

In conclusion, this study has revealed interesting potentialities of aminothiazolo-pyrimidine derivatives not only as immuno-modulators or anti-inflammatories, which is well documented, but also as cardiotonics as exemplified by compound **5e** which showed higher potency than the reference amrinone. To the best of our knowledge positive inotropic activities had never been reported previously in this series.

Experimental protocols

Chemistry

Material and methods

Melting points were determined on a Kofler bench and were uncorrected.

Method A

*5-Oxo-2,3,6,7-tetrahydro-5H-thiazolo[3,2-a]pyrimidine-6-ethyl carboxylates **1a–d***
*7-Oxo-2,3,5,6-tetrahydro-7H-thiazolo[3,2-a]pyrimidine-6-ethyl carboxylates **2a–d***
To a stirred solution of 2-aminothiazoline (10.2 g, 100 mmol), diethylmalonate (16 g, 100 mmol), and aromatic aldehyde (100 mmol) in absolute ethanol (300 ml), were added 5 drops of piperidine. The reaction mixture was heated under reflux for 5 h, and monitored by TLC on silica plates (eluent: toluene/methanol (80:20) or EtOAc/hexane (50:50)).

Generally 7-oxo products crystallized from the solution concentrated to 20% of its initial volume. 5-Oxo products crystallize from filtrates of corresponding 7-oxo products. 7- and 5-oxo compounds could also be separated by column chromatography. **1c** and **2a** could only be purified, after complete evaporation of ethanol, by column chromatography on silica gel (**1c**: toluene/methanol (98:2); **2a**: EtOAc/MeOH (90:10)). Yields were based on pure products obtained after repeated recrystallization from 95% EtOH.

Method B

*5-Oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carbonitriles **3b,f***
*7-Oxo-2,3-dihydro-7H-thiazolo[3,2-a]pyrimidine-6-carbonitriles **4a, e***
2-Aminothiazoline (10.2 g, 100 mmol), ethylcyanoacetate (11.3 g, 100 mmol) and aromatic aldehyde (100 mmol) were dissolved in ethanol (200 ml) and refluxed for 4 h. Products **4a** and **3f** were obtained with K_2CO_3 (100 mmol) and **4e** and **3b** with 5 drops of piperidine. The reaction mixture was concentrated to 50%. The solid product was collected by filtration and recrystallized from 95% EtOH (**4a**, **3b**), DMSO (**3f**), or a mixture of EtOH/DMSO (95:5) (**4e**).

Method C

*5-Amino-2,3-dihydro-7H-thiazolo[3,2-a]pyrimidine-6-carbonitriles **5a–j***
2-Aminothiazoline (10.3 g, 100 mmol), malononitrile (6.6 g, 100 mmol) and aromatic aldehyde (100 mmol) in ethanol (300 ml) were stirred under reflux for 4 h. For **5h** instead, an ice-cooled bath was used. **5e** required a dropwise addition of malonitrile in ethanol. The solid isolated by filtration, was generally recrystallized from 95% EtOH; except **5a** and **5i** [95% EtOH/DMSO (95:5)], **5b** (60% EtOH) and **5h** (absolute EtOH).

*7-Amino-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carbonitriles **6d, e***
The filtrates arising from the filtration of **5d** (for **6d**), and **5e** (for **6e**) *(cf Method C)* were concentrated under pressure to 20% of its initial volume. The precipitate obtained was recrystallized from 95% EtOH, followed by a second recrystallization from DMSO (for **6e** only).

*5-Imino-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carbonitriles **7a, c***
The filtrate arising from the filtration of **5a**, evaporated *in vacuo* to 20%, gave a precipitate of **7a**, recrystallized from 95% EtOH–DMSO (95:5). In the same manner, filtrate of **5c** afforded **7c** (recrystallized from 95% EtOH).

Method D

*5-Oxo-7-phenyl-2,3,6,7-tetrahydro-5H-thiazolo[3,2-a]pyrimidine (**1**)*
A solution of compound **1a** (0.9 g, 3 mmol) and anhydrous NaCl (1.9 g, 3.3 mmol) in anhydrous DMSO (3 ml) was refluxed under nitrogen for 6 h at 160°C (oil bath). After cooling, distilled water (20 ml) was added, and the mixture was extracted with $CHCl_3$ (2 x 30 ml). The organic phases were washed with distilled water (20 ml), dried (Na_2SO_4) and the solvent evaporated. The residue was purified by column chromatography on silica gel with EtOAc/hexane (50:50) as eluent. Mp = 115–116°C. Yield 50%.

Method E

*5-Oxo-7-phenyl-2,3,6,7-tetrahydro-5H-thiazolo[3,2-a]pyrimidine (**1**)*
To a mixture of 2-aminothiazoline (4.1 g, 40 mmol) and triethylamine (4.04 g, 40 mmol) in $CHCl_3$ (14.4 ml), was added dropwise a solution of cinnamoyl chloride (6.66 g, 40 mmol) in $CHCl_3$ (3.4 ml). The reaction mixture was refluxed for

100 min, and then cooled in an ice-bath. The precipitate obtained by filtration was washed by $CHCl_3$ (2 x 5 ml). Organic phases were washed with distilled water (5 ml), dried (Na_2SO_4), concentrated and purified by column chromatography on alumina with cyclohexane/$CHCl_3$ (60:40). The final product was recrystallized from 95% EtOH and then from isopropanol. Mp = 115°C (litt [19]: 114°C). Yield 10%.

Pharmacology: screening tests

Positive inotropic activity (+ INA)
This was shown *in vitro* by increasing the contractile force of electrically stimulated guinea pig left atria by more than 40%, according to Horii [22]. Response obtained for amrinone, the reference product, at the dose of 100 mg was an increase of 48%. When the positive inotropic effect observed was blocked by propranolol (0.1 mg/ml), by more than 80%, a β_1-adrenergic agonist activity was indicated.

Antihypertensive activity (AHTA)
Blood pressure was measured indirectly (tail cuff) in unanesthetized, untreated spontaneously hypertensive rats before 2, 4, and 6 h after administration (200 mg/kg, *po*). Reduction in mean pressure by more than 10% at any 2 consecutive measurement times after administration indicates hypertensive activity. Response obtained for α-methyl-dopa, the reference product, at the dose of 50 mg/kg, was 15, 22 and 27% of reduction.

Antiedema activity (AEA)
Rats were dosed *po* 1 h before intraplantar injection of carrageenin (0.1 ml, 1% suspension). Inhibition of paw edema by more than 30%, 4 h after carrageenin injection, indicates acute antiedema activity. Details are given in reference [23]. Two reference products could be used in this test: aspirin or phenylbutazone. Aspirin (150 mg/kg) showed 40% inhibition, and phenylbutazone (50 mg/kg) showed 33% inhibition.

Dopamin antagonist activity
Preselected non-climbing mice placed in specially constructed cages were given apomorphine (1.5 mg/kg sc) to induce climbing behavior consistently resulting in scores of 5–6 (maximum). Reduction of climbing behavior by more than 50% by the tested compounds (ip) *versus* apomorphine treated controls showed a neuroleptic effect as described previously [24]. This test used sulpiride as a reference product giving a score of 80 for a dose of 25 mg/kg (ip).

*Investigations in dogs: (**5a** and **5e** hydrochlorides)*
Six female Beagle dogs with a tracheal intubation, with a weight range 9.5–12.5 kg, were anesthetized with pentotal sodium salt (30 mg/kg iv). Isolation and vena saphena catheterism allowed product **5a**–HCl injections at the dose of 35, 100 µg/kg and 1, 2, 10 mg/kg and heparin injections (5 mg/kg). For **5e** the doses used were 0.5–1 mg/kg. Isolation of femoral vena permitted blood pressure (BP) recording. Physiological parameters recorded were BP, ECG, respiratory mechanism (by thermistance), and urinary flow (electromagnetic dropper). Further details are given in reference [25].

Acknowledgment

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