4*H*-Pyrrolo[1,2-*a*]thieno[3,2-*f*] and 4*H*-pyrrolo[1,2-*a*]thieno-[2,3-*f*][1,4]diazepines: Synthesis and Pharmacological Evaluation[+)]

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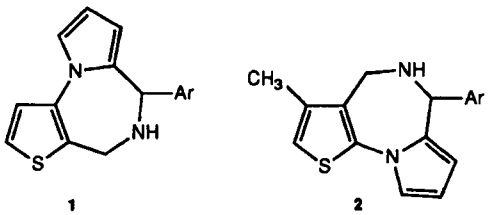
As an extension of a previous work, in which a number of 4*H*-pyrrolo-[1,2-*a*]thieno[2,3-*f*][1,4]diazepines were described, a new series of derivatives of the isomeric pyrrolo[1,2-*a*]thieno[3,2-*f*]diazepines ring system has been synthesized. The products obtained, together with those reported in the previous paper, were tested for acute toxicity and CNS activity in mice.

4*H*-Pyrrolo[1,2-*a*]thieno[3,2-*f*] und 4*H*-Pyrrolo[1,2-*a*]thieno[2,3-*f*]-[1,4]diazepine: Synthese und pharmakologische Untersuchung
Als Ergänzung zu unserer vorangehenden Arbeit, in der eine Reihe von 4*H*-Pyrrolo[1,2-*a*]thieno[2,3-*f*][1,4]diazepinen beschrieben wurde, wurden Derivative des isomeren Pyrrolo[1,2-*a*]thieno[3,2]-*f*] Ringsystems synthetisiert. Die erhaltenen Verbindungen - zusammen mit den in der vorangehenden Publikation beschriebenen Verbindungen - wurden auf ihre akute Toxizität und CNS-Wirkung an Mäusen untersucht.

Since several years, we have been interested in the design and synthesis of new psychotropic agents based on the isosteric replacement of the benzene ring in pharmacologically active drugs. Besides the thiophene moiety[1-5] other heterocyclic rings have been employed[6]. As a consequence of this work a considerable number of new heterocyclic compounds have been decribed and many of them, QM-6008 (bentazepam) or QM-7184 (a thiophene analogue of taclamine) e.g., exhibit important anxiolytic, antidepressive, and antipsychotic activities[7][8].



Scheme 1

Following these studies, we have synthesized 4*H*-pyrrolo[1,2-*a*]thieno[2,3-*f*][1,4]diazepines[9] **1** thiophene analogs of known 1,2-annelated 1,4-benzodiazepines[10]. In this paper we report the synthesis of the isomeric 4*H*-pyrrolo[1,2-*a*]thieno[3,2*f*][1,4]diazepine ring system **2** and the results obtained in its CNS preliminary pharmacological screening. We also describe the central pharmacological properties of compounds **1** which were assayed to record the influence which the change of position of the pyrrol ring may have in the activities of both structures.

Chemistry

The synthesis of the new 4*H*-pyrrolo[1,2-*a*]thieno[3,2-*f*][1,4]diazepines **2** was carried out following Scheme 2. Thus, 2-amino-4-methylthiophene-3-carbonitrile (**3**), readily accessible from mercaptoacetone, malononitrile, and piperidine by the *Gewald* reaction[11], was condensed with 2,5-dimethoxytetrahydrofuran in the presence of glacial acetic acid to give the new 1-[2-(4-methylthienyl)-3-carbonitrile]pyrrole (**4**) in 62% yield. $LiAlH_4$ reduction of **4** in dry ether furnished the expected aminomethyl derivative **5** which was reacted with different substituted benzaldehydes in boiling ethanol to give the benzylideneamino compounds **6a-i**.

Cyclization of these latter compounds by treatment with gaseous HCl in ethanol afforded the desired tricyclic pyrrolo-thieno-diazepines which were isolated as hydrochlorides **7a-i**. They were subsequently converted to the corresponding free bases **2a-i**.

Only compounds **6c**, **6e**, and **6g** are crystalline solids. The rest of the benzylideneamino intermediates are oily products which could not be purified by distillation, crystallization, or column chromatography, and for this reason they were used as such in the cyclization reaction.

Some compounds **2** could be synthesized through the alternative route shown in Scheme 2. It involved acylation of the aminomethyl-thienyl-pyrrole **5** with the appropriately substituted acid chlorides and subsequent cyclization of the acyl derivatives **8** with $POCl_3$ or a mixture of PCl_5 and

[+)] Dedicated to the memory of *Professor Dr. Ramón Madroñero* who died December 1988 and who initiated this investigation.

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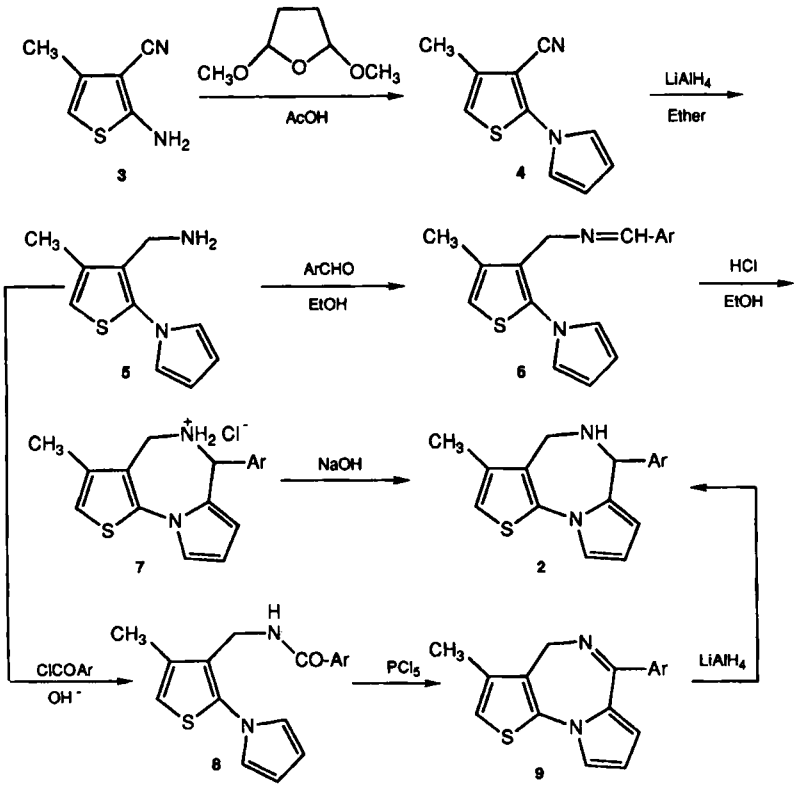


Scheme 2

$AlCl_3$. The 6*H*-pyrrolo[1,2-*a*]thieno[3,2-*f*][1,4]diazepines **9** were reduced with $LiAlH_4$. By this method compounds **2a**, **2d**, and **2e** were also obtained.

Yields, physical, and analytical data of **2a-i** are listed in Table 1. Their main ^{1}H-NMR characteristics are shown in Table 2.

Pharmacology

The benzodiazepine drugs exhibit antianxiety behavioural activity generally accompanied by anticonvulsant, miorelaxant, and sedative-hypnotic effects. Therefore, the new compounds were assayed in several tests in order to explore these properties.

Compounds **1a-i** and **2a-i** were initially submitted for acute toxicity and behavioural studies in mice following the polydimensional scheme of *Irwin*. Antagonism to pentylenetetrazole and strychnine, potentiation of pentobarbital sleeping time, exploratory behaviour, and effects on spontaneous motility, normal body temp. and muscle relaxation were also studied in male mice. Chlordiazepoxide and diazepam were used as reference standards and the results obtained in these studies are outlined in Tables 3, 4, and 5.

The new series showed very low toxicity (LD_{50} > 1500 mg/kg p.o.; 1000 mg/kg i.p.) and, in general, scarce behavioural effects in the *Irwin* test, since only a slight muscular relaxation and motor activity decrease as well as an increase of the passivity of animals was detected at the highest dose assayed (500 mg/kg p.o.).

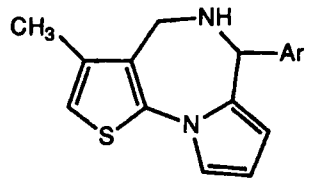
Besides, the majority of the compounds did not modify significantly the spontaneous locomotor activity up to the dose of 500 mg/kg p.o. (Table 3). Only **1g**, **2a**, **2g**, **2h**, and **2i** reduced moderately this activity. Compounds **1e** and **1f**, in contrast, showed a certain stimulating effect during the first h after administration (32 and 44%, respectively), increasing the motor activity with maximal values at the dose of 250 mg/kg which did not reach the statistical significance.

Compounds of series **1** and **2** do not exert any effect of interest on the normal body temp. of animals (Table 3). Only **1b** reduced the body temp. for more than 2°C ($p < 0.01$) for at least four h. Concerning miorelaxant activity (traction and chimney tests) only compounds **2b** and **2h**, at the dose of 250 mg/kg p.o., showed some degree of activity (20 and 40% of fails, respectively) which, notwithstand, was far from that found for the standards (Chlordiazepoxide: 70%; Diazepam: 90% of fails) at the dose of 20 mg/kg p.o.

At variance with the same benzodiazepines, almost all compounds were devoid of anticonvulsant activity in the pentetrazole and strychnine seizures. Exceptions to this rule were **2a** and **2f** which protected the animals in approximately 40% from the lethal effects of pentylenetetrazole.

In the investigation of the possible hypnotic effect most of the components of these series do potentiate the barbital induced sleep of mice (40 mg/kg i.p.), not only increasing the sleeping time of them but reducing their sleeping induction period (Table 4). In order to discard the possible meta-

Tab. 1: 5,6-Dihydro-7-methyl-4*H*-pyrrolo[1,2-*a*]thieno[3,2-*f*][1,4]diazepines[a] **2a-i.**



Compound	Ar	Yield (%)	Free base M.p. (°C) (ethanol)	Hydrochloride M.p. (°C) (ethanol-water)	Formula (m.w.)	Analysis Calcd./Found (%) C	H	N
2a	C_6H_5	80	63-65	235-237	$C_{17}H_{17}ClN_2S$ (316.85)	64.45 64.37	5.37 5.56	8.84 8.89
2b	*o*-Cl-C_6H_4	84	132-135	238-240	$C_{17}H_{16}Cl_2N_2S$ (351.29)	58.11 58.31	4.55 4.78	7.97 8.19
2c	*m*-Cl-C_6H_4	56	100-102	210-212[b]	$C_{17}H_{16}Cl_2N_2S$ (351.29)	58.11 58.20	4.55 4.85	7.97 8.10
2d	*p*-Cl-C_6H_4	74	104-106	228-230[b]	$C_{17}H_{16}Cl_2N_2S$ (351.29)	58.11 58.42	4.55 4.53	7.97 8.03
2e	*p*-NO_2-C_6H_4	73	142-144	231-233[b]	$C_{17}H_{16}ClN_3O_2S$ (361.85)	56.43 56.66	4.42 4.66	11.61 11.76
2f	*m*-OCH_3-C_6H_4	47	95-97	209-211	$C_{18}H_{19}ClN_2OS$ (346.87)	62.33 62.61	5.48 5.80	8.08 8.43
2g	*p*-OCH_3-C_6H_4	65	98-100	220-222	$C_{18}H_{19}ClN_2OS$ (346.87)	62.33 61.98	5.48 5.80	8.08 7.92
2h	$(OCH_3)_2$-C_6H_3 (3,4)	74	139-141	222-223	$C_{19}H_{21}ClN_2O_2S$ (376.90)	60.55 60.83	5.57 5.80	7.43 7.65
2i	OCH_2O-C_6H_3 (3,4)	91	oil	228-230	$C_{18}H_{17}ClN_2O_2S$ (360.85)	59.91 60.06	4.71 5.00	7.76 8.04

[a] Characteristic IR bands of **2** (KBr): 3320 - 3300 cm^{-1} (NH). [b] n-Propanol (recrystallization solvent).

Tab. 2: ^{1}H-NMR[a] data for compounds **2a-i.**

N°	δ 1-H (dd)[b]	δ 2-H (dd)	δ 3-H (ddd)	δ 4-H (s)	δ CH_2 (AB)	δ CH_3 (d)	δ 8-H (c)	δ NH (bs)[c]	δ Ar
2a	7.01	6.07	5.40	4.96	4.03 4.14	2.10	6.55	1.80	7.30-7.42 (m, 2'-H, 3', 4' and 5')
2b	7.03	6.06	5.32	5.34	4.04 4.16	2.10	6.55	1.95	7.50-7.59 (m, 4'-H, 5' and 6') 8.21-8.25 (m, 3'-H)
2c	6.93	6.01	5.36	4.88	3.94 4.04	2.03	6.49	1.85	7.17-7.20 (m, 4'-H, 5' and 6') 8.21-8.25 (m, 2'-H)
2d	7.01	6.08	5.41	4.95	4.00 4.11	2.10	6.56	1.79	7.33 (s, 2'-H, 3', 5' and 6')
2e	6.94	6.02	5.33	5.03	3.93 4.03	2.04	6.50	1.99	7.50 (d, J2'H,3'H=J6'H,5'H=8.9, 2'-H and 6') 8.12 (d, 3'-H and 5')
2f	6.88-6.95 (m)[d]	6.00	5.39	4.87	3.95 4.06	2.03	6.48	1.88	3.72 (s, OCH_3) 6.77 (ddd, J4'H,5'H=8.2, J4'H,6'H=2.5, J4'H,2'H=1.1, 4'-H) 6.88-6.95 (m, 2'-H and 6') 7.19 (t, J5'H,6'H=8.0, 5'-H)
2g	7.01	6.08	5.44	4.92	4.00 4.13	2.10	6.55	2.02	3.82 (s, OCH_3) 6.90 (d, J2'H,3'H=J6'H,5'H=8.7, 2'-H and 6')

Tab. 2: Continued.

									7.32 (d, 3'-H and 5')
2h	7.03	6.11	5.51	4.95	4.02 4.15	2.12	6.57	2.06	3.88 (s, OCH_3) 3.91 (s, OCH_3) 6.87 (d, J5'H,6'H=8.2, 5'-H) 6.93 (dd, J2'H,6'H=1.9, 2'-H) 6.96-7.00 (m, 6'-H)
2i	6.93	6.03	5.42	4.81	3.92 4.04	2.03	6.47	1.86	5.87 and 5.89 (AB, JAB=1.5, OCH_2O) 6.70 (d, J5'H,6'H=8.0, 5'-H) 6.77 (dd, J6'H,2'H=1.6, 6'-H 6.85 (d, 2'-H)

[a] In $CDCl_3$ and 200 MHz. [b] Multiplicity. [c] Exchange with D_2O. [d] Overlapped by protons signals of Ar.
Coupling constants: J_{AB} = 16.7, $J_{1H,2H}$ = 3.0, $J_{1H,3H}$ = 1.7, $J_{2H,3H}$ = 3.5, $J_{3H,4H}$ = 1.1, $J_{CH3,8H}$ = 1.1.

Tab. 3: Spontaneous motor activity and variation of body temperature of **1** and **2**.

	Spontaneous motor activity Oral ED_{50} values (95% confidence interval)		Variation of body temperature			
				Mean decrease in rectal temperature (°C)		
Compound	1 h	2 h	Dose (mg/kg p.o.)	1 h	2 h	4 h
Control			—	-0.19 ± 0.23	-0.50 ± 0.24	0.67 ± 0.21
1a	> 500	> 500	250	1.14 ± 0.24*	1.86 ± 0.40*	1.58 ± 0.20
1b	> 500	> 500	250	2.14 ± 0.46**	2.06 ± 0.29**	2.28 ± 0.29**
1c	> 500	403.6 (331-557)	250	0.70 ± 0.40	-0.30 ± 0.12	-0.25 ± 0.18
1d	> 500	355.8 (312.3-450.1)	250	1.14 ± 0.31*	1.22 ± 0.49**	1.72 ± 0.36*
1e	31.76 ± 3.13[a]	437.5 (368.7-581.8)	250	0.15 ± 0.08	0.65 ± 0.63	1.27 ± 0.71
1f	43.61 ± 7.01[a]	> 500	250	0.77 ± 0.33	-0.07 ± 0.26	1.27 ± 0.41
1g	318.5 (230-463.5)	427.0 (375.4-549.8)	250	0.50 ± 0.38	0.20 ± 0.35	0.64 ± 0.24
1h	> 500	> 500	250	0.00 ± 0.28	-0.14 ± 0.24	0.26 ± 0.52
1i	≅ 500	> 500	250	0.76 ± 0.18	0.60 ± 0.38*	1.22 ± 0.53
Chlordiazepoxide	31.0 (19.3-46.8)	9.5 (3.9-20.4)	20	1.21 ± 0.23**	1.60 ± 0.49**	2.48 ± 0.47**
Control			—	-0.17 ± 0.15	0.26 ± 0.29	1.18 ± 0.16
2a	243.8 (187.9-305.2)	255.6 (201.5-319.7)	250	-0.33 ± 0.56	-0.30 ± 0.88	1.25 ± 0.39**
2b	> 500	368.1 (315.2-440)	250	-0.08 ± 0.34	0.20 ± 0.53	1.88 ± 0.42
2c	≅ 500	≅ 500	250	0.38 ± 0.42	0.54 ± 0.74	1.64 ± 0.45
2d	> 500	> 500	250	-0.26 ± 0.29	-0.30 ± 0.22	0.80 ± 0.28
2e	5.35 ± 4.86[a]	> 500	250	1.10 ± 1.05	0.60 ± 0.76	1.56 ± 0.19
2f	16.52 ± 0.51[a]	> 500	250	-0.44 ± 0.21	-0.48 ± 0.28	0.72 ± 0.20
2g	326.7 (275-431.8)	276.9 (225-329.5)	250	-0.26 ± 0.39	-0.16 ± 0.27	0.44 ± 0.15*
2h	427.3 (341-539)	288.4 (230.5-332)	250	0.80 ± 0.25	0.36 ± 0.24	0.54 ± 0.17*
2i	205.8 (137.5-293.9)	37.0 (17.9-86.5)	250	0.82 ± 0.38**	0.68 ± 0.23	0.54 ± 0.16*
Diazepam	8.9 (3.3-15.5)	7.7 (4.2-16.9)	20	2.27 ± 0.24**	4.62 ± 0.85**	2.15 ± 0.45*

[a] Maximal effect of hyperactivity reached at the dose of 250 mg/kg.
* $p < 0.05$. ** $p < 0.01$.

bolic implications of this effect, the assay was carried out using subhypnotic doses of sodium pentobarbital (20 mg/kg i.p.). This permitted to confirm the sedative activity found for compounds **1a**, **1b**, and **1d**, which induced sleep in 50% of animals, and, in a lesser extent, for compounds **1h** and **2i** which attained the sleep induction in 37% of the animals employed (Table 4).

The potential anxiolytic action of the products was assessed in the plus-maze test, which utilizes exploratory activity in an elevated plus-maze in the mouse as a measure of anxiety.

In this test, which has found behavioural, physiological and pharmacological validation[18,19], exposure of animals to the open and closed arms of an elevated maze produces an approaching-avoidance conflict, which results in an inhibition of their exploratory behaviour in the open arms. Anxiolytic drugs elevate both the % of entries into and the time spent on the open arms of the maze, while anxiogenic compounds diminish the two measures. Inmediately before the plus-maze test, the animals were exposed for five min to a holeboard apparatus in order to increase maze exploration. The holeboard test, as is known, measures exploratory behaviour (head-dipping) independently of locomotor activity.

Our compounds exert, as in the case of the reference benzodiazepines, a moderate inhibitory effect of this activity (Table 5).

Respect to their possible anxiolytic activity, the results obtained indicated that, compared with the respective control lots, **1b**, **1g**, and **1i**, together with **2c**. **2d**, **2f**, **2g**, and **2h** increase both the % of entries and % of time on the open arms of the plus-maze and therefore they can be considered as anxiolytic compounds, although with less marked effects than the standard benzodiazepines used in the test.

Tab. 4: Potentiation of pentobarbital sleeping time of **1** and **2**

Compound	Dose (mg/kg p.o.)	Sodium pentobarbital (40 mg/kg p.o.) t_1 x ± e	Sodium pentobarbital (40 mg/kg p.o.) t_2 x ± e	N_s/N_t	Sodium pentobarbital (20 mg/kg p.o.) t_1 x ± e	Sodium pentobarbital (20 mg/kg p.o.) t_2 x ± e
Control	—	9.41 ± 1.03	26.58 ± 2.93	0/16	—	—
1a	250	5.17 ± 0.51*	64.56 ± 12.75**	4/8	11.50 ± 1.8	26.47 ± 1.78
1b	250	5.62 ± 0.73*	50.68 ± 3.58**	4/8	13.60 ± 1.51	28.58 ± 4.78
1c	250	5.40 ± 0.41*	33.27 ± 3.70	0/8	—	—
1d	250	8.25 ± 2.45	45.73 ± 4.90**	4/8	10.98 ± 0.75	30.17 ± 9.17
1e	250	4.58 ± 0.19*	28.87 ± 3.45	0/8	—	—
1f	250	6.39 ± 0.41	48.49 ± 6.59**	1/8	14.80 ± 0.00	70.31 ± 0.00
1g	250	7.01 ± 0.67	25.33 ± 4.68	0/8	—	—
1h	250	6.39 ± 1.05	52.54 ± 8.49**	3/8	14.87 ± 1.64	23.76 ± 6.22
1l	250	7.40 ± 1.09	61.17 ± 9.57	2/8	26.59 ± 1.14	54.98 ± 0.80
Chlordiazepoxide	15	3.58 ± 0.26**	42.30 ± 6.85*	13/16	9.39 ± 0.37	41.97 ± 4.29
Control	—	6.15 ± 0.53	24.81 ± 2.38	0/8	—	—
2a	250	7.13 ± 1.02	46.08 ± 10.07**	0/8	—	—
2b	250	5.26 ± 0.30	47.17 ± 5.50**	1/8	38.15 ± 0.00	12.40 ± 0.00
2c	250	5.60 ± 0.29	36.50 ± 5.80**	1/8	19.10 ± 0.00	9.74 ± 0.00
2d	250	6.57 ± 0.72	67.99 ± 16.65**	0/8	—	—
2e	250	4.95 ± 0.17	23.61 ± 2.56	2/8	8.60 ± 2.63	25.08 ± 1.67
2f	250	6.15 ± 0.53	53.40 ± 8.77**	2/8	11.88 ± 1.94	11.63 ± 8.67
2g	250	4.41 ± 0.22	26.54 ± 2.69	0/8	—	—
2h	250	8.15 ± 2.50	32.95 ± 7.59	1/8	13.45 ± 0.00	9.50 ± 0.90
2l	250	5.02 ± 0.19	107.43 ± 7.92**	3/8	12.67 ± 2.54	17.76 ± 10.39
Diazepam	15	4.04 ± 0.51*	82.71 ± 6.81**	8/8	5.31 ± 0.54	97.46 ± 4.11

* $p < 0.05$. ** $p < 0.01$. N_t = total number of mice. N_s = number of sleeping mice. t_1 = sleep induction time (min). t_2 = sleeping time (min).

Tab. 5: Plus-Maze and Holeboard tests of compounds **1** and **2**

Compound	Dose (mg/kg p.o.)	Activity: Plus - Maze % O.A.	Plus - Maze % t.O.A.	Plus - Maze T.N.E.	Holeboard (% variation)
Control	—	26.05 ± 3.1	11.33 ± 1.6	9.82 ± 1.2	—
1a	250	22.22 ± 3.7	7.48 ± 3.3	6.75 ± 2.1	-43.30 ± 6.1**
1b	250	27.52 ± 4.1	21.27 ± 2.1**	11.12 ± 1.4	-44.00 ± 5.3**
1c	250	27.73 ± 4.8	14.04 ± 3.2	9.25 ± 3.0	-16.50 ± 4.8
1d	250	25.70 ± 3.9	7.78 ± 2.5	8.75 ± 2.2	18.75 ± 6.2
1e	250	32.00 ± 4.3	15.96 ± 3.6	12.50 ± 2.5	-14.64 ± 5.8
1f	250	9.09 ± 3.3**	1.58 ± 3.1**	11.00 ± 3.3	-25.00 ± 4.2
1g	250	34.28 ± 2.9*	25.07 ± 2.9**	17.50 ± 2.0*	-46.09 ± 5.3
1h	250	22.19 ± 5.1	8.08 ± 3.0	7.62 ± 2.6	-40.43 ± 4.8**
1l	250	38.22 ± 3.0*	20.34 ± 3.2*	10.75 ± 1.9	-27.66 ± 3.9*
Chlordiazepoxide	5	44.05 ± 4.2**	32.70 ± 4.7**	14.00 ± 1.3	-48.48 ± 5.4**
Control	—	28.18 ± 3.0	18.74 ± 1.5	9.43 ± 1.3	—
2a	250	28.57 ± 4.3	22.52 ± 2.8	7.00 ± 2.1	-34.57 ± 4.1**
2b	250	33.33 ± 3.5	16.49. ± 1.9	7.50 ± 1.9	-14.82 ± 5.6
2c	250	39.13 ± 2.1*	23.25 ± 2.7	11.50 ± 2.3	14.63 ± 3.9
2d	250	40.00 ± 2.9*	19.02 ± 3.0	8.75 ± 2.0	-40.25 ± 4.8**
2e	250	19.44 ± 2.2*	11.75 ± 1.6*	9.00 ± 1.8	-22.89 ± 3.8*
2f	250	40.42 ± 3.1*	29.77 ± 1.5*	11.75 ± 2.2	18.07 ± 4.2*
2g	250	46.51 ± 2.6**	40.58 ± 2.3**	10.75 ± 1.5	-33.34 ± 5.1**
2h	250	31.11 ± 4.2	30.69 ± 2.2*	11.25 ± 3.1	-21.22 ± 3.7*
2l	250	26.66 ± 3.8	23.96 ± 3.1	11.25 ± 2.7	-39.40 ± 5.5**
Diazepam	5	53.57 ± 4.8**	56.65 ± 5.3**	8.62 ± 1.1	-46.58 ± 5.8**

* $p < 0.05$. ** $p < 0.01$

% O.A.: Percentage of entries into open arms.

% t.O.A.: Percentage of time spent on open arms.

T.N.E.: Total number of arm entries.

In view of these results, the ability of the reported compounds (dehydro derivatives **9** were also included) to interact *in vitro* with central benzodiazepine receptors ($GABA_A$-BZD - Cl^- ion channel complex) was also investigated. None of these compounds displaced the specific binding of [^{3}H]Flunitrazepam (FNZ) to receptors from bovine cerebral

cortex membranes[20] in a concentration-dependent form. This non apparent correlation between binding data and pharmacological effects can be explained in terms of a participation in these latter of other types of benzodiazepine receptors, different from those of the cortex which were used in these experiments.

Since, at difference with the reference benzodiazepines, these compounds present, in general, very low or none CNS depressant activity and exert practically no miorelaxant, anticonvulsant, and hypnotic effect on mice. The thienopyrrolo-diazepines are endowed with a noticeable anxiolytic profile without the side effects of the classical benzodiazepines. Compound **1f**, and to a lesser extent **2e**, seem to have an anxiogenic profile since they reduced both measures.

Within the moderate results of this study, compounds **2**, bearing a methyl group at C-3 of the thiophene ring, were, in general, more active than compounds **1**, although these are less lipophilic (similar to that of chlordiazepoxide and diazepam), as measured by their respective capacity factors (K') in five different elution conditions[21]. However, except in the case of the two *p*-methoxy derivatives **1g** and **2g**, no clearcut correlation between the different substituents employed in both series and their respective anxiolytic activities could be discerned.

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Experimental Part

Chemistry

Melting points: Gallenkamp capillary apparatus, uncorrected.- IR spectra: Shimadzu IR-435 spectrophotometer.- ^{1}H-NMR spectra (TMS as internal standard): Varian XL-300 and Bruker AM-200 spectrometers (Chemical shifts in δ units (ppm)).

1-(2-Cyano-3-methyl-thien-2yl)pyrrole (**4**)

A mixture of 2-amino-4-methylthiophene-3-carbonitrile[11] (**3**) (138 g, 1.0 mole), 2,5-dimethoxytetrahydrofuran (132 g, 1.0 mole) and glacial acetic acid (1000 ml) was heated under reflux for 30 min. The mixture was concentrated *in vacuo* and the oily residue was distilled at 0.1 mm Hg; a fraction (116 g, 62%) of b.p. 125-130°C crystallized on cooling to give compound **4**. M.p. 55-57°C (ethanol).- $C_9H_8N_2S$ (176.2) Calcd. C 63.8 H 4.25 N 14.9 S 17.0 Found C 64.1 H 4.20 N 15.0 S 16.8.- IR (KBr): 2220 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 7.18 (t, J = 2.2 Hz, 2H, pyrrole 2-H, 5-H), 6.58 (q, J = 1.2 Hz, 1H, thiophene 5-H), 6.35 (t, 2H, pyrrole 3-H, 4-H), 2.33 (s, 3H, CH_3).

1-[2-(3-Aminomethyl-4-methyl)thien-2-yl]pyrrole (**5**)

To a stirred suspension of $LiAlH_4$ (24 g, 0.6 mole) in dry ether (75 ml) was slowly added a solution of **4** (94 g, 0.5 mole) in dry ether (750 ml). The mixture was stirred and heated under reflux for 24 h, then cooled and decomposed with water (500 ml). After filtration, the org. layer was separated, washed with water, dried ($MgSO_4$) and evaporated. Distillation of the oily residue at 0.1 mm Hg gave the aminomethyl derivative **5** of b.p. 110°C (87.4 g, 91%).- $C_{10}H_{12}N_2S$ (192.3) Calcd. C 62.5 H 6.25 N 14.6 S 16.7 Found C 62.4 H 6.29 N 14.3 S 16.9.- IR (film): 3380; 3300; 1580 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 6.84 (t, J = 2.1 Hz, 2H, pyrrole 2-H, 5-H), 6.69 (d, J = 1.1 Hz, 1H, thiophene 5-H), 6.27 (t, 2H, pyrrole 3-H, 4-H), 3.61 (s, 2H, CH_2), 1.29 (br. s, 2H, exchangeable with D_2O, NH_2).- ^{13}C-NMR ($CDCl_3$): δ = 139.0 (thiophene C-4), 135.7 (thiophene C-2), 135.3 (thiophene C-3), 123.4 (^{1}J = 188.0 Hz, pyrrole C-2, C-5), 116.7 (^{1}J = 184.7 Hz, thiophene C-5), 109.6 (^{1}J = 171.7 Hz, pyrrole C-3, C-4), 36.9 (^{1}J = 136.1 Hz, CH_2), 14.9 (^{1}J = 130.6 Hz, CH_3).- MS: m/z = 192 (M$^{+\cdot}$, 100%).

1-[2-(3-Benzylideneaminomethyl-4-methyl)thien-2-yl]pyrroles **6**

General method

A mixture of **5** (0.02 mole), the appropriate benzaldehyde (0.02 mole) and ethanol (40-50 ml) was heated under reflux for 15 min. The solvent was evaporated *in vacuo* to give the corresponding *Schiff*'s bases **6** which, in general, could not be purified by distillation or crystallization from the common solvents and for this reason they were used as such in the next reaction. **6c**, **6e**, and **6g** precipitated on cooling the reaction mixture, so that they were isolated and identified:

1-[2-(3-m-Chloro-benzylideneaminomethyl-4-methyl)thien-2-yl]pyrrole (**6c**)

68% yield from **m**-chlorobenzaldehyde, white crystals, m.p. 78-79°C (ethanol).- $C_{17}H_{15}N_2SCl$ (314.8) Calcd. C 64.9 H 4.76 N 8.9 S 10.7 Found C 65.0 H 5.01 N 9.1 S 10.4.- IR (KBr): 1640 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 8.03 (s, 1H, CH), 7.66 (s, 1H, benzene), 7.50-7.20 (m, 3H, benzene), 6.82 (t, J = 2.0 Hz, 2H, pyrrole 2-H, 5-H), 6.69 (s, 1H, thiophene 5-H), 6.26 (t, 2H, pyrrole 3-H, 4-H), 4.52 (d, J = 1.4 Hz, CH_2), 2.23 (s, 3H, CH_3).- MS: m/z = 314 (M$^{+\cdot}$, 60%), 176 (100).

1-[2-(3-p-Nitro-benzylideneaminomethyl-4-methyl)thien-2-yl]pyrrole (**6e**)

90% yield from *p*-nitrobenzaldehyde, yellow prisms, m.p. 104-106°C (ethanol).- $C_{17}H_{15}N_3O_2S$ (325.4) Calcd. C 62.8 H 4.61 N 12.9 S 9.8 Found C 62.8 H 4.80 N 13.1 S 9.6.- IR (KBr): 1640 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 8.23 (d, J = 9.0 Hz, 2H, benzene), 8.13 (s, 1H, CH), 7.82 (d, 2H, benzene), 6.82 (t, J = 2.2 Hz, 2H, pyrrole 2-H, 5-H), 6.75 (s, 1H, thiophene 5-H), 6.26 (t, 2H, pyrrole 3-H, 4-H), 4.62 (d, J = 1.4 Hz, CH_2), 2.26 (s, 3H, CH_3).- MS: m/z = 325 (M$^{+\cdot}$, 53%), 176 (100).

1-[2-(3-p-Methoxy-benzylideneaminomethyl-4-methyl)thien-2-yl]pyrrole (**6g**)

77% yield, yellow prisms, m.p. 80-82°C (ethanol).- $C_{18}H_{18}N_2OS$ (310.4) Calcd. C 69.7 H 5.80 N 9.0 S 10.3 Found C 69.8 H 6.00 N 9.3 S 9.9.- IR (KBr): 1630, 1610 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 8.03 (s, 1H, CH), 7.59 (d, J = 8.9 Hz, 2H, benzene), 6.95-6.75 (m, 4H, pyrrole 2-H, 5-H and benzene), 6.66 (s, 1H, thiophene 5-H), 6.23 (t, J = 2.2 Hz, 2H, pyrrole 3-H, 4-H), 4.46 (d, J = 1.5 Hz, CH_2), 3.72 (s, 3H, OCH_3), 2.19 (s, 3H, CH_3).- MS: m/z = 310 (M$^{+\cdot}$, 100%).

1-[2-(3-Benzamidomethyl-4-methyl)thien-2-yl]pyrroles **8**

Method **A**

A stirred suspension of **5** (0.02 mole) in 20% NaOH (20 ml) was cooled to 0°C, treated dropwise with the corresponding benzoyl chloride (0.02 mole) and stirred at room temp. for 1 h. The precipitate formed was filtered, washed with water and dried to give compounds **8** which were recrystallized from the appropriate solvent.

Method **B**

A solution of **5** (0.02 mole) and triethylamine (0.03 mole) in methylene chloride (20 ml) was cooled to 0°C and then treated dropwise with the corresponding benzoyl chloride (0.02 mole). The mixture was stirred at room

temp. for 1 h and the precipitate formed was filtered, washed with methylene chloride, dried and recrystallized.

1-[2-(3-Benzamidomethyl-4-methyl)thien-2-yl]pyrrole (**8a**)

Method **A**, 78% yield from **5** and benzoyl chloride, white solid, m.p. 146-148°C (toluene).- $C_{17}H_{16}N_2OS$ (296.4) Calcd. C 68.9 H 5.40 N 9.5 S 10.8 Found C 68.8 H 5.48 N 9.5 S 10.8.- IR (KBr): 3320; 1680 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 7.66-7.49 (m, 2H, benzene), 7.29-7.23 (m, 3H, benzene), 6.79 (t, J = 2.1 Hz, 2H, pyrrole 2-H, 5-H), 6.69 (s, 1H, thiophene 5-H), 6.26 (t, 2H, pyrrole 3-H, 4-H), 6.06 (br. s, 1H, exchangeable with D_2O, NH), 4.42 (d, J = 5.9 Hz, 2H, CH_2), 2.23 (s, 3H, CH_3).

1-[2-(3-p-Chloro-benzamidomethyl-4-methyl)thien-2-yl]pyrrole (**8d**)

Method **A**, 82% yield from **5** and *p*-chloro-benzoyl chloride, white solid, m.p. 186°C (toluene).- $C_{17}H_{15}N_2OSCl$ (330.8) Calcd. C 61.7 H 4.53 N 8.5 S 9.7 Found C 61.5 H 4.72 N 8.3 S 9.7.- IR (KBr): 3300; 1620 cm^{-1}.- ^{1}H-NMR ([D_6]DMSO): δ = 8.59 (br. s, 1H, exchangeable with D_2O, NH), 7.79 (d, J = 8.6 Hz, 2H, benzene), 7.46 (d, 1H, benzene), 7.03-6.98 (m, 3H, pyrrole 2-H, 5-H and thiophene 5-H), 6.16 (t, J = 2.2 Hz, 2H, pyrrole 3-H, 4-H), 4.19 (d, J = 5.9 Hz, 2H, CH_2), 2.09 (s, 3H, CH_3).

1-[2-(3-p-Nitro-benzamidomethyl-4-methyl)thien-2-yl]pyrrole (**8e**)

Method **B**, 80% yield from **5** and *p*-nitro-benzoyl chloride, yellow crystals, m.p. 175-177°C (ethanol).- $C_{17}H_{15}N_3O_3S$ (341.4) Calcd. C 59.8 H 4.39 N 12.3 S 9.4 Found C 60.2 H 4.44 N 12.5 S 9.2.- IR (KBr): 3285; 1640 cm^{-1}.- ^{1}H-NMR ([D_6]DMSO): δ = 8.89 (br. s, 1H, exchangeable with D_2O, NH), 8.26 (d, J = 8.9 Hz, 2H, benzene), 8.03 (d, 2H, benzene), 7.03-6.98 (m, 3H, pyrrole 2-H, 5-H and thiophene 5-H), 6.19 (t, J = 2.1 Hz, 2H, pyrrole 3-H, 4-H), 4.26 (d, J = 5.9 Hz, 2H, CH_2), 2.13 (s, 3H, CH_3).

4-Phenyl-7-methyl-6H-pyrrolo[1,2-a]thieno[3,2-f][1,4]diazepine (**9a**)

A mixture of **8a** (1.5 g, 5 mmole) and $POCl_3$ (7.7 g, 50 mmole) was refluxed for 2 h. Excess of $POCl_3$ was removed at reduced pressure and the residue was cautiously treated with ice-water and basified with 20% NaOH. **9a** was extracted with chloroform and the extracts were washed with water, dried ($MgSO_4$) and evaporated. The residue was chromatographed on alumina, ethyl acetate-hexane 1:2, to give **9a** (22%) as white crystals, m.p. 138-140°C (ethanol).- $C_{17}H_{14}N_2S$ (278.4) Calcd. C 73.4 H 5.03 N 10.1 S 11.5 Found C 73.0 H 5.22 N 10.07 S 11.72.- IR (KBr): 1680, 1590 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 7.65-7.60 (m, 2H benzene), 7.41-7.33 (m, 3H, benzene), 7.22 (dd, J = 2.7 Hz, J = 1.7 Hz, 1H, 1-H), 6.61 (q, J = 1.1 Hz, 1H, 8-H), 6.42 (dd, 1H, 3-H), 6.36 (dd, 1H, 2-H), 4.55 (s, 2H, CH_2), 2.31 (d, J = 1.1 Hz, 3H, CH_3).- MS: m/z = 278 ($M^{+\cdot}$, 100%).

4-p-Nitrophenyl-7-methyl-6H-pyrrolo[1,2-a]thieno[3,2-f][1,4]diazepine (**9e**)

9e was prepared as described above from **8e** and $POCl_3$. It was purified by chromatography on alumina, ethyl acetate-hexane 1:6. Yellow crystals (27%). M.p. 176-178°C (ethanol).- $C_{17}H_{13}N_3O_2S$ (323.4) Calcd. C 63.2 H 4.02 N 13.0 S 9.9 Found C 63.2 H 4.21 N 12.9 S 9.7.- IR (KBr): 1650; 1590; 1570 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 8.21 (m, 2H benzene), 7.82 (d, J = 9.0 Hz, 2H, benzene), 7.27 (dd, J = 2.6 Hz, J = 1.7 Hz, 1H, 1-H), 6.66 (q, J = 1.1 Hz, 1H, 8-H), 6.40 (dd, 1H, 2-H), 6.37 (dd, 1H, 3-H), 4.60 (s, 2H, CH_2), 2.32 (d, J = 1.1 Hz, 3H, CH_3).- MS: m/z = 323 ($M^{+\cdot}$, 100%).

4-p-Clorophenyl-7-methyl-6H-pyrrolo[1,2-a]thieno[3,2-f][1,4]diazepine (**9d**)

9d was prepared in similar way from **8d** (1.65 g, 5 mmole), PCl_5 (1.01 g, 6 mmole) and $AlCl_3$ (0.66 g, 5 mmole). Chromatography on alumina with ethyl acetate-hexane 1:4 gave **9d** (41%), white solid, m.p. 176-177°C (ethanol).- $C_{17}H_{13}N_2SCl$ (312.7) Calcd. C 65.3 H 4.16 N 9.0 S 10.2 Found C 65.2 H 4.13 N 9.1 S 10.1.- IR (KBr): 1670; 1590; 1570 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ = 7.58 (d, J = 9.0 Hz, 2H benzene), 7.32 (d, J = 9.0 Hz, 2H, benzene), 7.23 (dd, J = 2.7 Hz, J = 1.6 Hz, 1H, 1-H), 6.63 (q, J = 1.1 Hz, 1H, 8-H), 6.40 (dd, 1H, 3-H), 6.36 (dd, J = 3.8 Hz, 1H, 2-H), 4.53 (s; 2H, CH_2), 2.31 (d, J = 1.1 Hz, 3H, CH_3).- MS: m/z = 312 ($M^{+\cdot}$, 100%).

4-Aryl-5,6-dihydro-7-methyl-4H-pyrrolo[1,2-a]thieno[3,2-f][1,4]diazepines **2**

Method **A**

The crude benzylidene derivative **6** was dissolved in absol. ethanol satd. with dry HCl (50 ml) and the solution stirred at room temp. for 45 min. The precipitated hydrochlorides **7** were filtered off, washed with dry ether and recrystallized from the appropriate solvent. The free bases **2** were obtained from **7** with dil. NaOH and extraction with ether or chloroform. Table 1 lists the yields, physical and analytical data and characteristic IR bands of compounds **2**. Their main ^{1}H-NMR spectroscopic features are summarized in Table 2.

Method **B**

To stirred suspension of $LiAlH_4$ (0.025 mole) in absol. ether (30 ml) was slowly added the pyrrolo-diazepine **9a**, **9d**, or **9e** (0.020 mole) dissolved in absol. ether (25 ml). The mixture was heated under reflux for 12 h, then cooled, treated with water and filtered. The org. layer was separated, washed with water and dried. Distillation of the solvent gave the diazepines **2a**, **2d**, and **2e** in practically quantitative yields.

Pharmacological material and methods

Male and female albino Swiss mice (10-25 g) were purchased from Interfauna (Barcelona, Spain) and housed in groups of 10/cage for at least three days prior to behavioural studies with free access to standard food and tap water and maintained on a 12/12 h light-dark circle (light from 8.00 a.m. to 8.00 p.m.). All animals were fasted overnight, tap water being available *ad libitum*, before p.o. dosing. Each dose group consisted of 5 animals unless otherwise stated. The temp. was 22 ± 1°C, except in the potentiation of barbiture sleeping time which was studied in a room of 27°C. Compounds were suspended in 1% Tween 80 and administered p.o. Control animals received 1% Tween 80 suspension. Chlordiazepoxide and diazepam were included in the tests for comparison.

Statistical analysis was performed using the Student's unpaired t-test[12]. A probability level of 0.05 or less was accepted as significant.

Effects on behaviour and LD_{50} in mice

According to *Irwin*[13], the behaviour of the mice was observed at 1 and 2 h after p.o. injection of test drugs. The LD_{50} was calculated from lethality within 3 days after p.o. administration by the method of *Litchfield* and *Wilcoxon*[14].

Effect on spontaneous motor activity in mice

Locomotor activity was recorded with a photocell activity meter for 15 min beginning 60 and 120 min after p.o. administration.

Effect on normal body temperature

The rectal temp. of the mice was measured with a thermistor thermometer (Panlab 0331) prior to the experiment and 1, 2 and 4 h after p.o. administration.

Muscle relaxant activity

Effect on muscle relaxant activity was studied by

a) *Traction test* according to *Courvoisier et al.*[15]: Mice were forced to hang with their forelegs on a wire of 1 mm in diameter, which was stretched horizontally at a height of 35 cm, 60 and 120 min after p.o. drug administration. When they fell off the wire within 5 s or their failed to grasp the wire with their hind legs 3 times succesively, muscle relaxation was judged to be positive.

b) *Chimney test:* In a pyrex tube (30 cm long and 28 mm diameter) marked at 20 cm from base a mouse was introduced at the end nearing the mark. When the animal reached the other end of the tube, the tube was moved to the vertical position and immediately the mouse tried to climb the tube backwards. Only those mice which reached the mark within 30 seconds were selected for further testing. The operation was repeated 60 and 120 min after p.o. administration[16].

Interaction with barbiturate-induced sleep in mice

Sodium pentobarbital at a subhypnotic dose of 20 mg/kg or at a hypnotic dose of 40 mg/kg dissolved in 0.9% saline was injected i.p. to groups of 8 mice 60 min after p.o. injection of the drug. The number of mice which lose righting reflex at subhypnotic dose, latency and duration of sleep (loss and recovery of the righting reflex) were recorded

Anticonvulsant activity: Effect on chemically induced seizures

Convulsions were induced by an i.p. injection of pentetrazol (120 mg/kg) or strychnine sulphate (2.5 mg/kg) dissolved in 0.9% saline. Test drugs were administered orally 60 min before the convulsants' injection, and anticonvulsive activity was determined by loss of clonic and tonic seizures and mortality.

Exploratory behaviour pattern: Holeboard test and plus-maze test

The holeboard apparatus consisted of a wooden board (40 x 40 cm) with 16 equally spaced holes. The holeboard test involved placing the mouse 55 min after p.o. injection with the control vehicle or test material in the center of the floor and counting the number of head-dips during 5 min trials[17].

The plus-maze apparatus was made of plexiglas and consisted of two open (30 x 5 cm) and two enclosed (30 x 5 x 15 cm) arms, connected by a central platform (5 x 5 cm). The open arms, the central platform and the floor of the closed arms were made of black plexiglas, and the sides of the closed arms were made of clear plexiglas. The apparatus was mounted on a base raising it 38.5 cm above the floor.

The test consisted of injecting p.o. mice with the drug or its vehicle 55 min before being tested individually in the holeboard for 5 min. Immediately after the holeboard test each animal was placed in the center of the plus-maze facing an open arm and allowed to freely explore for 5 min. The number of entries made on the open and closed arms and the time spent on each type of arms were recorded. Three measures were obtained from the test: the total number of arm entries, the % of arms entries made on the open arms, and the time spent on the open arms expressed as % of time spent on both the open and closed arms. The last two measures are used as indices of anxiety: they increase following the administration of anxiolytics and decrease following the administration of anxiogenic drugs[18,19].

Binding of [^{3}H]flunitrazepam to GABA/benzodiazepine receptor complex

The binding of [^{3}H]flunitrazepam to GABA/benzodiazepine receptor complex of bovine cerebral cortex was determined as described by *Sigel et al.*[20], with minor modifications.

Preparation of membranes

Bovine brain was obtained fresh from a slaughterhouse, the cortex was rapidly removed, chopped, frozen inmediately and stored at -80°C until use. Cortex (199 g) was thawed, chopped and homogenized in 10 mM HEPES (pH 7.5), 1 mM EDTA, 300 mM sucrose, 0.5 mM dithiothreitol, 1 mM benzamidine/HCl, 0.3 mM phenylmethylsulfonyl fluoride (1 liter) at 0-4°C. The homogenate was centrifuged at 1,000 x g for 12 min. The supernatant was centrifuged at 27,000 x g for 35 min. The pellet was resuspended, using a glass-teflon homogenizer, in 500 ml of the same medium without sucrose and phenylmethylsulfonyl fluoride. After recentrifugation the pellet was resuspended in the latter medium (final volume 150 ml; 15 mg protein/ml).

Binding assay in solution

Membranes were diluted at 1 mg protein/ml in 20 mM K phosphate (pH 7.4), 0.1 mM EDTA, 0.1% (w/v) Triton X-100. Diluted membranes (140 ml) were incubated with [^{3}H]flunitrazepam (10 nM final concentration) without and with unlabelled ligands at different concentrations with incubation at 0°C for one. Duplicates of incubations were filtered on GF/C glass fiber filters (Whatman) under suction. The filters were washed 3 times with 4 ml of 20 mM K phosphate (pH 7.4), 0.1 mM EDTA, dried and counted in a liquid scintillation fluid. Non-specific binding was determined in the presence of cold 10 mM flunitrazepam and represented <q 5% of the total binding. The results were estimated by considering the maximal specific binding obtained with 10 nM flunitrazepam as the 100%.

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