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Our continuing interest in the chemistry and pharmacology of pyridazinone derivatives was recently oriented towards a more convenient synthesis of 5,6-dihydrobenzo[h]cinnolin-3(2H)-ones I and of the bioisosters 5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-one (II-A) and 5,6-dihydrothieno[3,2-h]cinnolin-3(2H)-one (II-B).
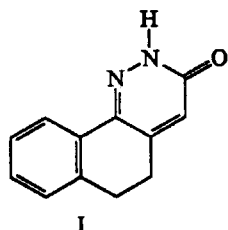
Thus, the previously reported synthesis of I involving a four step procedure [1] from 1-tetralone (1) was significantly improved by allowing 1 to condense with glyoxylic acid in alkaline medium at room temperature to give α-hydroxy-1-tetralone-2-acetic acid (2), which by refluxing in excess hydrazine hydrate for 0.5 hour was converted into I in 80% overall yield. The formation of the 4-hydroxy-4,4a,5,6-tetrahydrobenzocinnolinone 3 as intermediate was demonstrated by carrying out the condensation of 1 and equimolar hydrazine hydrate under milder reaction conditions (refluxing ethanol for 1 hour) to give 50% of 3, which was dehydrated to I in refluxing hydrazine hydrate.

IIA

IIB

Figure 1.

a) CHO-COOH, NaOH, H$_2$O, rt
b) NH$_2$NH$_2$•H$_2$O excess, Δ, 0.5 hour
c) NH$_2$NH$_2$•H$_2$O, EtOH, Δ, 1 hour
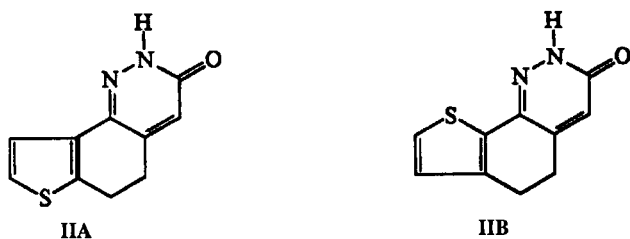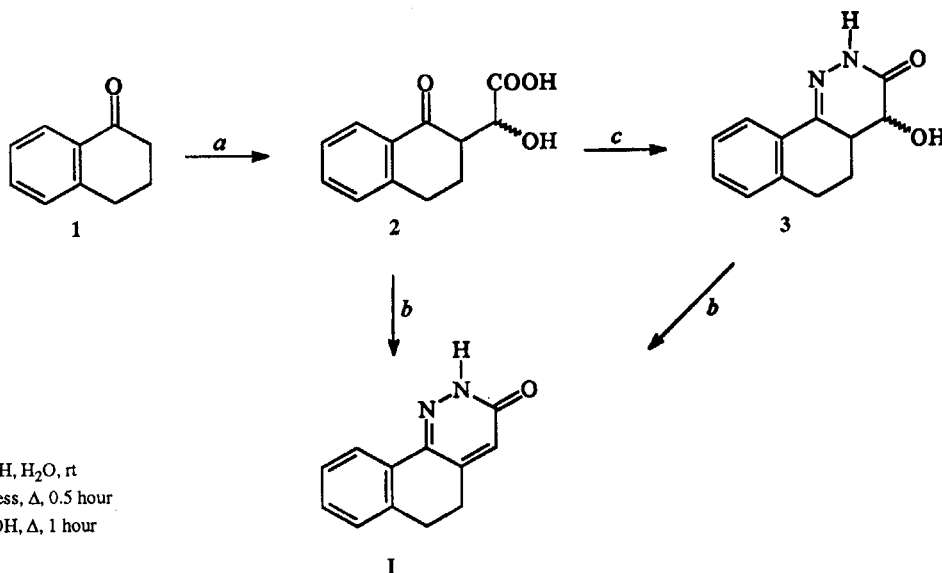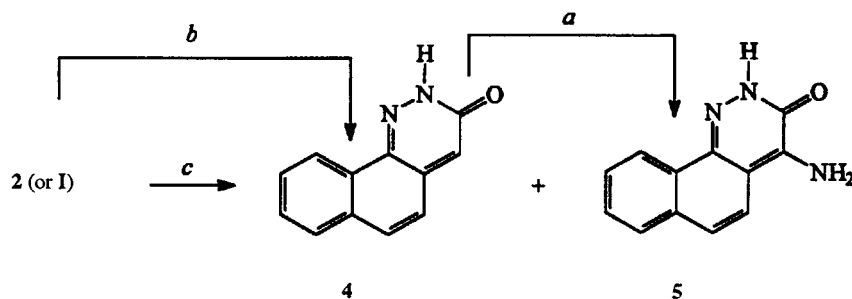
Figure 2.

We have however observed that if the mixture of **2** and hydrazine hydrate was kept at reflux for many hours the initially formed **I** underwent somewhat unexpected transformations we wish to report. In particular, by refluxing **2** and excess of hydrazine hydrate for 48 hours, 76% of benzo[*h*]cinnolinone **4** was obtained besides 10% of **I**. However, by prolonging the reaction time to 72 hours, a mixture of 26% of **4** and 68% of 4-aminobenzo[*h*]cinnolinone **5** was isolated.

Reagents: NH₂NH₂•H₂O excess, Δ, 24 hours (*a*), 48 hours (*b*), 72 hours (*c*)

Figure 3.

The structures of the previously unreported **4** and **5** were assigned by elemental and spectroscopic analyses. In addition, the structure of **5** was supported by single crystal X-ray analysis. As expected, **I** in refluxing hydrazine hydrate was converted into a mixture of **4** and **5** in a ratio proportional to the reaction time, thus suggesting a mechanism involving a hydrazine induced dehydrogenation of **I** to **4**, followed by amination of **4** to **5**. The conversion **4 → 5** was experimentally confirmed. Having ruled out the intervention of oxygen in the dehydrogenation of **I** to **4**, we hypothesized for this conversion the mechanism reported in Figure 4.
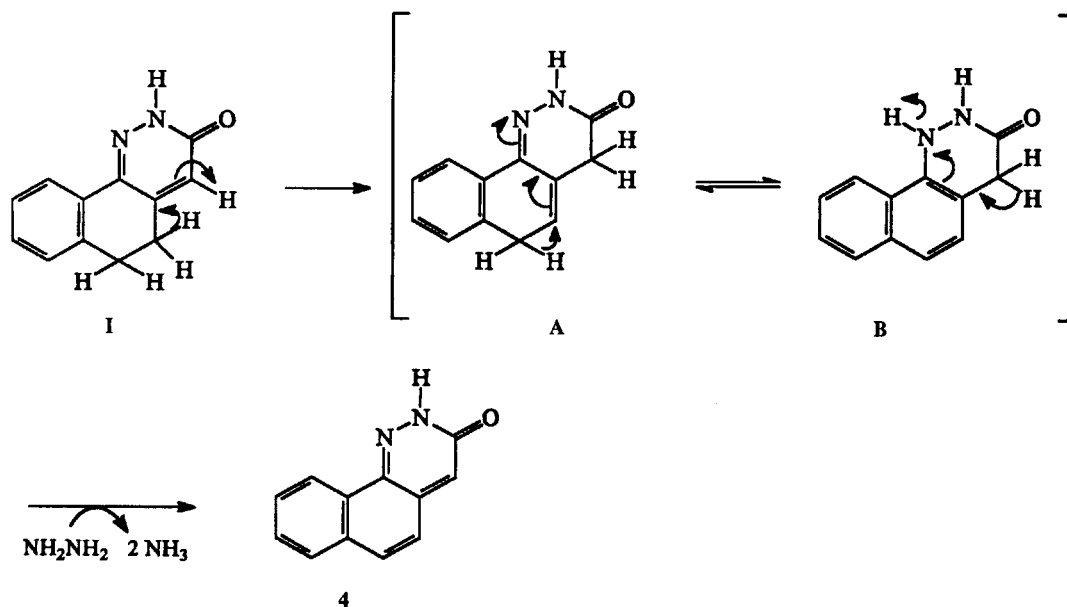

Figure 4.

Two prototropic rearrangements converted **I** into the tautomer **I-B** which underwent hydrazine induced dehydrogenation to **4** with liberation of 2 moles of ammonia. This could appear as an improbable mechanism being that we were unable to isolate the 1,4-dihydrobenzocinnolinone **I-B** despite its naphthalene moiety. Nevertheless this mechanism was indirectly supported by the behavior towards hydrazine hydrate of the thienocinnolinones **II-A** and **II-B** isosters of **I**. Their synthesis recalls that employed for **I** starting from the appropriate 4(7)-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene **6a,b**, which were converted with glyoxylic acid into the α-hydroxyacetic acid derivatives **7a,b**. Condensation of **7** with equimolar hydrazine hydrate in refluxing ethanol led in 65-70% yield to the 4-hydroxythienocinnolinones **8a,b**, which were dehydrated to **II-A,B** in 80% yield by refluxing in ethanol in the presence of *para*-toluensulfonic acid.

a X = -CH-   Y = S
b X = S   Y = -CH-

i,   CHO-COOH, aqueous NaOH, rt
ii, N₂H₄•H₂O, EtOH, Δ, 1.5 hours
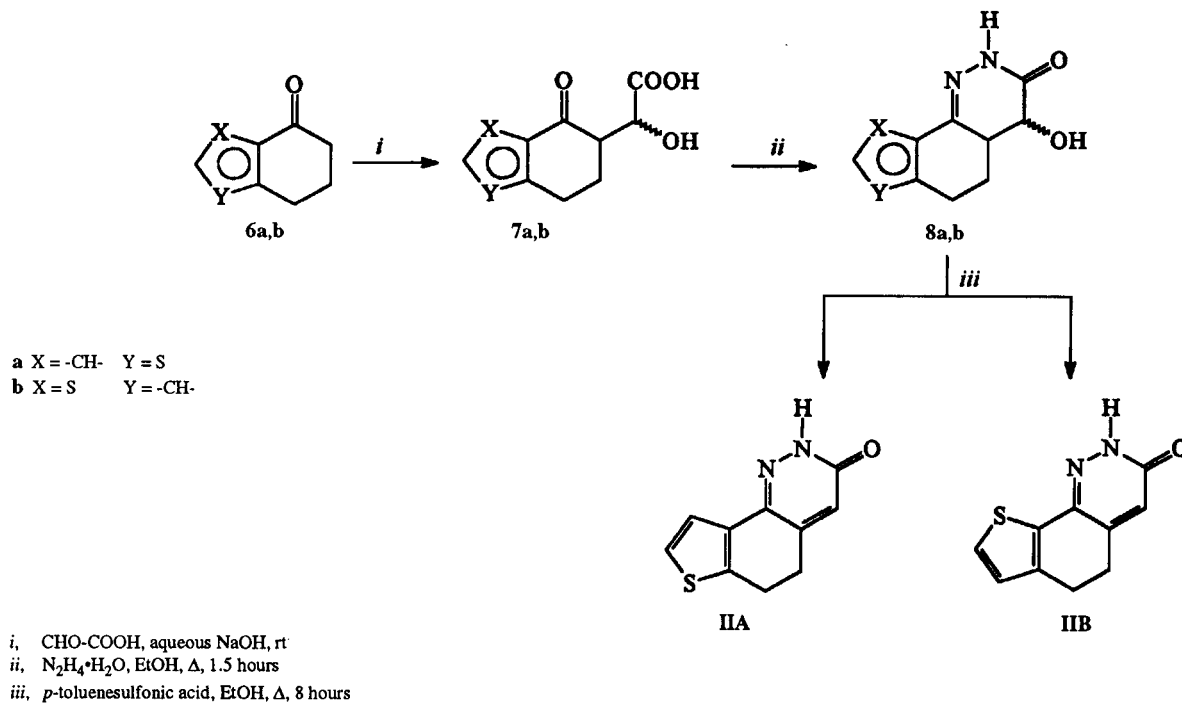*iii*, *p*-toluenesulfonic acid, EtOH, Δ, 8 hours

Figure 5.

Focusing on **II-A**, it was found that this compound when briefly refluxed (0.5 hour) with excess hydrazine hydrate was converted in about 90% yield into 1,4-dihydrothieno[2,3-*h*]cinnolin-3(2*H*)-one (**9-A**), whose structure was supported by elemental and spectroscopic analysis. In particular, the ¹H-nmr spectrum of **9-A** exhibited a singlet at δ 3.60, attributed to the 4-CH₂ and an AB quartet at δ 7.40 assigned to C₅-H and C₆-H. As expected, by prolonging the reaction time to 3 hours a hydrazine induced dehydrogenation of **9-A** followed by 4-amination took place, to give a mixture of 40% thieno[2,3-*h*]cinnolin-3(2*H*)-one (**10-A**) and of 10% of the 4-amino derivative **11-A**. Their structures were assigned on the basis of elemental and spectroscopic (¹H- and ¹³C-nmr) data. The conversion **10-A** → **11-A** was experimentally proved by isolating **11-A** in 67% yield from a reaction mixture of **10-A** and excess hydrazine hydrate refluxed for 5 hours.
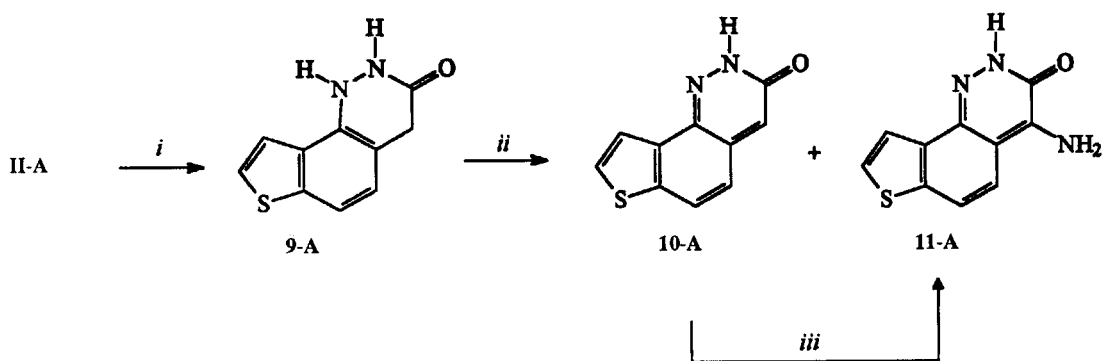
Reagents: NH$_2$NH$_2$·H$_2$O excess, Δ, 0.5 hour (*i*), 3 hours (*ii*), 5 hours (*iii*)

Figure 6.

A quite similar behavior towards refluxing hydrazine hydrate was shared by the isomer **II-B** which was progressively converted into **9-B**, **10-B** and **11-B**. In any case, despite the experimental support given by the isolation of **9-A,B** as the key intermediates in the hydrazine induced dehydrogenation of **II-A,B** into **10-A,B** we were unable to explain the non-isolation of **I-B** as the intermediate in the conversion of benzocinnolinone **I** into **4**. A possible reason could be in the higher heat of formation of **I-B** (ΔH = 35.98 kcal/mol) with respect to that of **I** (ΔH = 32.42 kcal/mol) thus suggesting that **I-B** is about 3.5

kcal/mol less stable than **I**. Conversely, in the thienocinnolinone series **10-A** (ΔH = 36.86 kcal/mol) and **10-B** (ΔH = 37.10 kcal/mol) are as stable as **II-A** (ΔH = 37.72 kcal/mol), respectively **II-B** (ΔH = 37.10 kcal/mol).

Finally, just a few words to comment on the amination at position 4 of the pyridazinone moiety of **4** and **10** to give **5** and **11**, respectively. We propose the following mechanism, involving hydrazine addition to the conjugated double bond to give **b**, which by intramolecular rearrangement with loss of ammonia led to **c**, eventually converted into **5** and **11**.
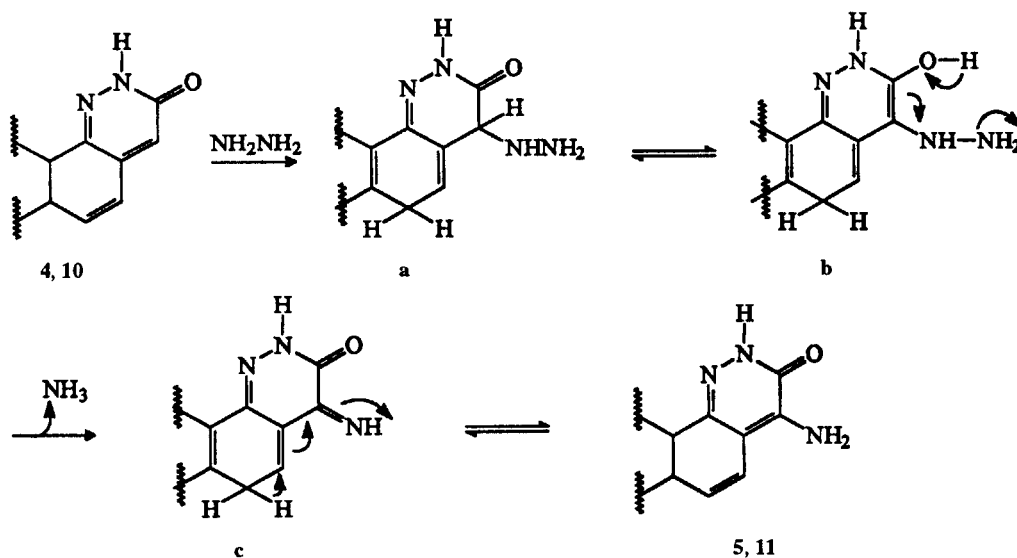

Figure 7.

This mechanism which closely recalls that hypothesized by Shemyakin *et al.* [2] for the formation of osazones, differs from that proposed by Sing *et al.* to explain the hydrazine induced amination of 6-arylpyridazinones, which is based on 1-4 addition of hydrazine to the pyridazinone ring followed by loss of ammonia and final tautomerization [3].
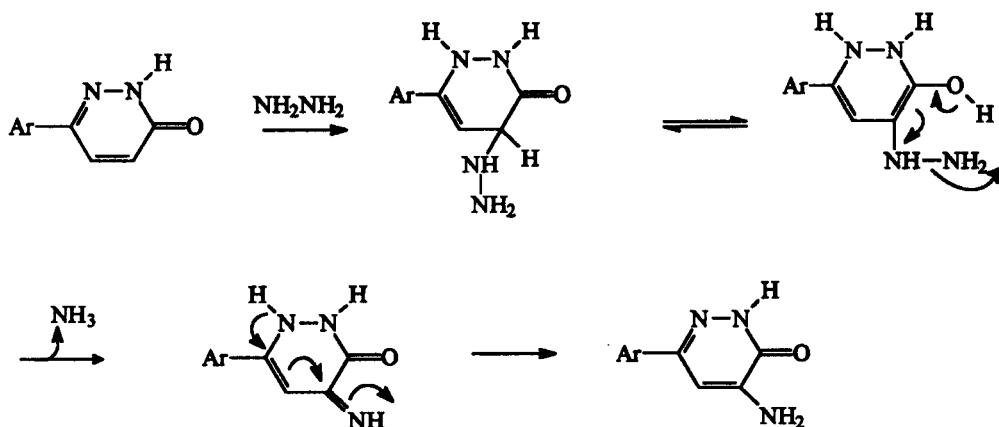

Figure 8.

However, if the Sing's mechanism could be operative even for tricyclic pyridazinones, we cannot explain the observed failure of the known indenopyridazinone **12** [4] to give 4-amination in refluxing hydrazine.
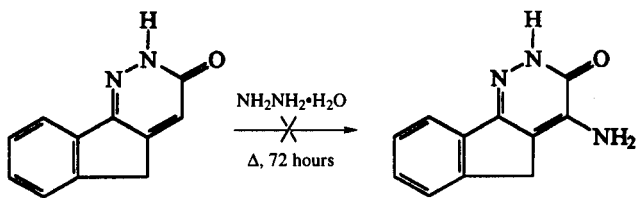

12

Figure 9.

The above presented substrates were investigated as potential starting materials for new biologically active compounds. Though several fields were investigated, today I am only dealing with two of them, namely: 1. α_1-Adrenoceptor antagonists. 2. Non opioid analgesics.

α_1-Adrenoceptor Antagonists.

α_1-Adrenergic receptors (α_1-AR) are members of the superfamily of G protein coupled receptors that transduce the signal across the cell membrane, thus initiating a variety of intracellular biochemical events. Like all G protein-coupled receptors, the adrenergic receptor family shares the presence of seven hydrophobic regions that are believed to form a bundle of α-helical transmembrane domains, connected by alternating intracellular and extracellular hydrophilic loops. They are at present classified into three subtypes, designated as α_{1A} (α_{1a}), α_{1B} (α_{1b}), and α_{1D} (α_{1d}), with lower case subscripts being used to indicate the recombinant receptors, and upper case subscripts to denote the native receptors [5-7]. The presence of these different α_1- adrenoceptor subtypes in blood vessels and other smooth muscles points to the importance of developing selective drugs for receptor classification and characterization as well as for therapeutic effectiveness. Among the compounds showing high affinity towards α_1-adrenergic receptors attention has recently been devoted to a series of phenylpiperazines of general structure **III**, where aryl represents several aromatic residues non structurally related. Among others, the tricyclic pyridazine system **IV** was investigated by us [8] as a possible pendant for piperazinyl derivatives.
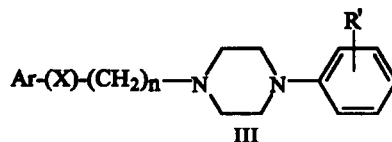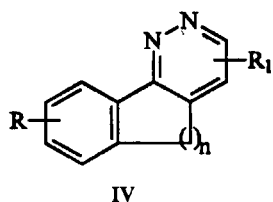

III

Figure 10.

Figure 11.

To better identify the really essential features of this structure we progressively simplified it and we are now working on a monocyclic pyridazinone system having the phenylpiperazinylalkylamino chain at position 4. In particular we are evaluating the following:
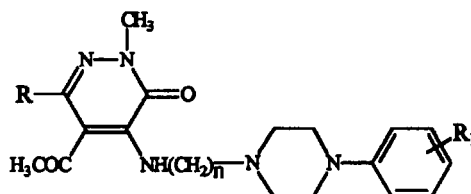
(a) The role of the substituents at position 5 and 6 of the pyridazinone ring.

(b) The role of the length of the spacer in the piperazinyl chain.

(c) The role of the substituents on the phenyl ring of the side chain.

(d) The shifting piperazinyl chain from position 4 to 5.

As far as their synthesis is concerned, compounds having a 5-acetyl group were prepared from the corresponding 4-nitropyridazinone [9] by condensing with the appropri-

ate piperazinylalkylamine in ethanol at room temperature, while the other derivatives were obtained from known halopyridazinines [10] by refluxing overnight with excess of the required amine. Among the 5-acetyl-6-phenyl derivatives, compounds having a substituted phenylpiperazinylethyl chain (13a-d) displayed remarkable affinity towards the α_1-receptor, in some case (13b,c) accompanied by a significant selectivity towards the 5-hydroxytryptamine serotoninergic receptor ($5HT_{1A}$). In contrast, no appreciable selectivity was recorded among the three α_1-subtypes. Substitution of the 6-phenyl by a methyl group (13g) was detrimental to both potency and selectivity. In all cases, elongation of the side chain by insertion of one (13e,f,h,i) or two (13j) methylenes led to less active derivatives compared with their corresponding ethyl analogs (See Table I).
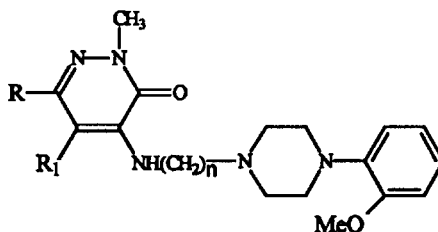
When the 5-acetyl group was replaced by a hydrogen (13k-m), the potency and the selectivity were lowered, independent from the substituent at 6-position and the length of the side chain. However, insertion at the 5-position of a bromine (13n) restored a significant affinity towards α_1-receptor and selectivity with respect to 5-hydroxytryptamine receptor, thus suggesting that the presence of an electron withdrawing group at this position should be an essential requirement (See Table II).

Table I

Compound	R	R_1	n	Ki (nM) Native Receptors Rat Brain		Ki (nM) Cloned Receptors Human Brain		
				α_1	$5HT_{1A}$	α_{1a}	α_{1b}	α_{1d}
13a	C_6H_5	2-OMe	2	1.2	9.3	0.6	3.3	0.13
13b	C_6H_5	2-OMe, 5-Cl	2	1.9	228	0.3	3.9	0.4
13c	C_6H_5	2-Cl	2	3.3	113.1	0.3	0.75	0.25
13d	C_6H_5	2-F	2	4.7	24	0.3	3.8	0.49
13e	C_6H_5	2-OMe	3	10.7	244.1	1.5	20.3	4.3
13f	C_6H_5	2-OMe, 5-Cl	3	10.5	1000	0.4	1.4	3.9
13g	CH_3	2-OMe	2	14.5	13	2.3	49.8	3.2
13h	CH_3	2-OMe	3	40.9	1.2	0.9	53.4	34.4
13i	CH_3	2-OMe, 5-Cl	3	11.3	12.2	0.7	8.04	5.3
13j	CH_3	2-OMe	4	38	1.4	3.0	49.1	5.96
prazosin	-	-	-	0.74	2360	0.58	0.28	0.29
BMY7378	-	-	-	282	0.37	378	70.9	1.28
8-OH-DPAT	-	-	-	18000	2.33	-	-	-

Table II

| Compound | R | R_1 | n | Ki (nM) Native Receptors Rat Brain | | Ki (nM) Cloned Receptors Human Brain | | |
				α_1	$5HT_{1A}$	α_{1a}	α_{1b}	α_{1d}
13k	C_6H_5	H	2	16.5	20.4	-	-	11.8
13l	C_6H_5	H	3	22.6	229	-	-	11.3
13m	CH_3	H	2	183	32	18.5	33.1	17.7
13n	H	Br	2	5.9	37.2	0.6	5.7	0.4
prazosin	-	-	-	0.74	2360	0.58	0.28	0.29
BMY7378	-	-	-	282	0.37	378	70.9	1.28
8-OH-DPAT	-	-	-	18000	2.33	-	-	-

Shifting the piperazinyl side-chain from position 4 to 5 led to compounds with very weak activity (**13o-r**) (See Table III).

Table III
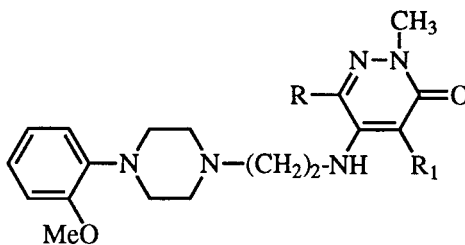
| Compound | R | R_1 | Ki (nM) Native Receptors Rat Brain | | Ki (nM) Cloned Receptors Human Brain | | |
			α_1	$5HT_{1A}$	α_{1a}	α_{1b}	α_{1d}
13o	H	H	427	42	-	-	23
13p	H	Br	30	-	11.5	23	32
13r	Cl	H	247	87	-	-	34
prazosin	-	-	0.74	2360	0.58	0.28	0.29
BMY7378	-	-	282	0.37	378	70.9	1.28
8-OH-DPAT	-	-	18000	2.33	-	-	-

Finally a short comment should be devoted to our attempts to prepare the 4,6-dichloropyridazinone **14**, whose synthesis was planned starting from the known [11] 2-methyl-6-hydroxypyridazin-3(2*H*)-one (**15**). According to literature data on the 2-phenyl analog [12], treatment of **15** by phosphoryl chloride and phosphorus pentachloride should give **14**. However, no trace of it was evident in the reaction mixture, the only recovered compound being **16**, whose structure was assigned through spectroscopic investigation. Treatment of **16** by *o*-methoxyphenylpiperazinylethylamine selectively led to **17**, as clearly indicated by the nmr spectra (See Scheme 1).

Scheme 1
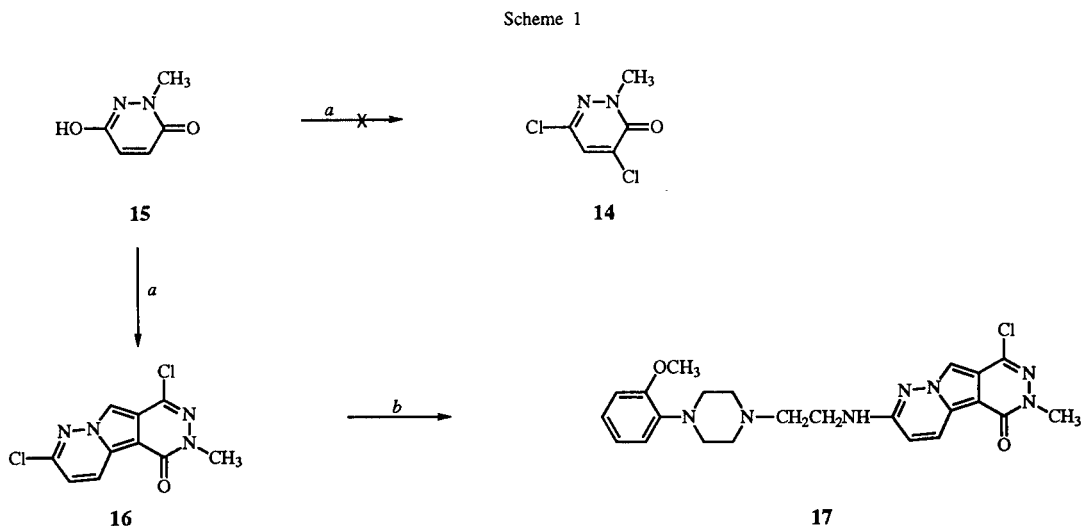
(*a*) 1. POCl$_3$, Δ; 2. PCl$_5$, Δ
(*b*) 2-[4-(2-methoxyphenyl)piperazin-1-yl]ethylamine, EtOH, Δ

When tested in binding studies, **17** displayed significant potency for both α_1-adrenergic and 5-hydroxytryptamine serotoninergic receptor (See Table IV).

Table IV

| Compound | Ki (nM) Native Receptors Rat Brain | | Ki (nM) Cloned Receptors Human Brain | | |
	α_1	5-HT$_{1A}$	α_{1a}	α_{1b}	α_{1d}
17	13.3	16.3	1.10	15.2	3.04
prazosin	0.74	2360	0.58	0.28	0.29
BMY7378	282	0.37	378	70.9	1.28
8-OH-DPAT	18000	2.33	-	-	-

Non Opioid Analgesics.

Despite an ever growing knowledge of endogenous nociceptive and antinociceptive systems, several pain syndromes, including chronic pain, are still unsatisfactorily treated. Though the availablity of the three main opioid receptors (μ, δ, κ) recently cloned provides favorable prospects for the synthesis of new compounds similar to morphine in activity but possibly devoided of its side effects, this target is still unattained. On the other hand, non opioid analgesics, though free of drawbacks such as tolerance and dependence, are often accompanied by severe complications of a different nature.

Recently, several 4,6-diarylpyridazine derivatives having an arylpiperazinyl moiety at position 3 were reported to possess remarkable analgesic properties [13]. On the basis of these facts, we planned a series of tricyclic derivatives **18** as new potential antinociceptive agents. Their synthesis (see Scheme 2) started from the appropriate chloropyridazines, which were condensed with the required aminoalkylpiperazinyl reagent. Quite surprisingly, besides the

Scheme 2
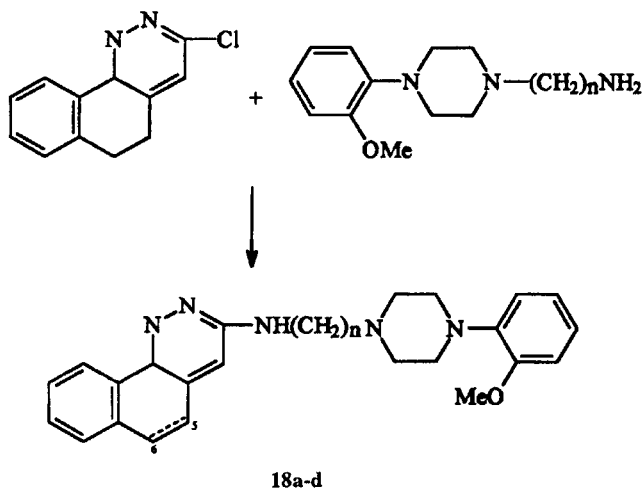
expected **18a** (n = 2) and **18b** (n = 3) having a single bond in position 5-6, also the corresponding 5,6-dehydro compounds **18c** and **18d** were obtained. All compounds were tested for their analgesic properties on the hot plate method. The most interesting derivative was **18a**, which at a dose of 50 mg/kg remarkably increased the licking latency time with respect to the control (See Table V).

Table V

Hot Plate: Licking Time (s) after Treatment [a]

Compound	Dose mg/kg po	Before Treatment	15 minutes	30 minutes	45 minutes
Saline		14.6±0.7	15.6±1.1	15.2±1.2	13.9±1.5
18a	10	13.7±0.8	17.6±1.1	14.8±1.4	16.7±1.5
	30	15.2±1.1	19.7±1.5*	24.4±1.3**	26.5±1.5**
	50	15.6±0.8	23.3±1.4**	33.7±2.1**	32.5±1.4**
18b	25	13.6±0.7	23.7±1.3**	20.8±1.5*	18.2±1.1
	50	13.4±0.8	26.2±1.4*	22.7±.2**	19.5±1.6
18c	20	13.9±1.1	19.2±1.3*	21.5±1.8**	16.4±1.3
	40	14.0±0.8	23.6±1.5**	26.7±1.6**	18.7±1.0
18d	50	14.0±0.9	19.6±1.2*	19.3±1.0*	17.6±1.6

[a] Groups of at least 6 animals were used. Trazodone is reported [13] to enhance by 28% the licking time in a similar test.

Analgesic properties were displayed also in the mouse abdominal constriction test. As far as their mechanism of action is concerned, they do not interact with opioid receptors, as is clearly proven by the inability of naloxone to prevent their antinociceptive action.

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