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4-Amino-5-vinyl-3(2*H*)-pyridazinones and analogues as potent antinociceptive agents: Synthesis, SARs, and preliminary studies on the mechanism of action

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Abstract—A series of 4-amino-5-vinyl-3(2*H*)-pyridazinones and analogues were synthesized and their antinociceptive effect was evaluated in the mouse abdominal constriction model. Several of the novel compounds showed ED_{50} values in the range 6–20 mg/kg/sc and demonstrated to be able to completely protect all the treated animals from the effect of the noxious stimulus at 30 mg/kg/sc. SAR studies confirmed the essential role played by an amino or substituted amino function at position 4 and by a vinyl group at position 5 of the diazine system.

1. Introduction

Current research in pain therapy concerns development of new potent antinociceptive agents endowed with the efficacy of morphine, but devoid of its adverse and use-limiting side-effects, such as respiratory depression, constipation, tolerance, and physical dependence liability. However, the second class of clinically dominant antinociceptive drugs, that of non-steroidal anti-inflammatory drugs (NSAIDS), whose effects are mediated by the peripheral inhibition of prostaglandin synthesis, suffers from strong limitations due to the induction of gastro-intestinal lesions and nephrotoxicity.[1] Development of selective COX-2 inhibitors only partially solved the problem because of their severe cardiovascular secondary effects.[2,3]

Current available drugs are able to control nociceptive and inflammatory pain, whereas neurogenic and psychogenic pain disorders lack an appropriate pharmacological treatment since only antidepressant, anticonvulsivant, and local anesthetic drugs have been reported to ameliorate these pain conditions.[4]

Thus, development of non-anti-inflammatory, non-opioid agents which are able to control pain from a broad range of causes represents a primary objective in analgesic drug research.

Pyridazine derivatives displaying antinociceptive activity, like compounds **1a**–**b**,[5] have been reported in the literature since the early 1950s (Fig. 1). Among this type of compounds, Emorfazone **2**,[6] which is characterized by the absence of either effects on the prostaglandin system or affinity for opioid receptors,[7,8] emerged and was launched in Japan.

More recently, a series of 4-carbamoyl-5-aryl-3(2*H*)-pyridazinones **3** were also reported as compounds displaying relevant analgesic activity both in writhing and hot plate test.[9] Compound **4** was patented for the therapy of mild to moderate pain[5] by ASTA Medica as a peripherally acting analgesic displaying low acute toxicity.[10]

In addition, very recently Couquelet et al.[11–13] described some series of pyridazines and their 1,2-oxazine

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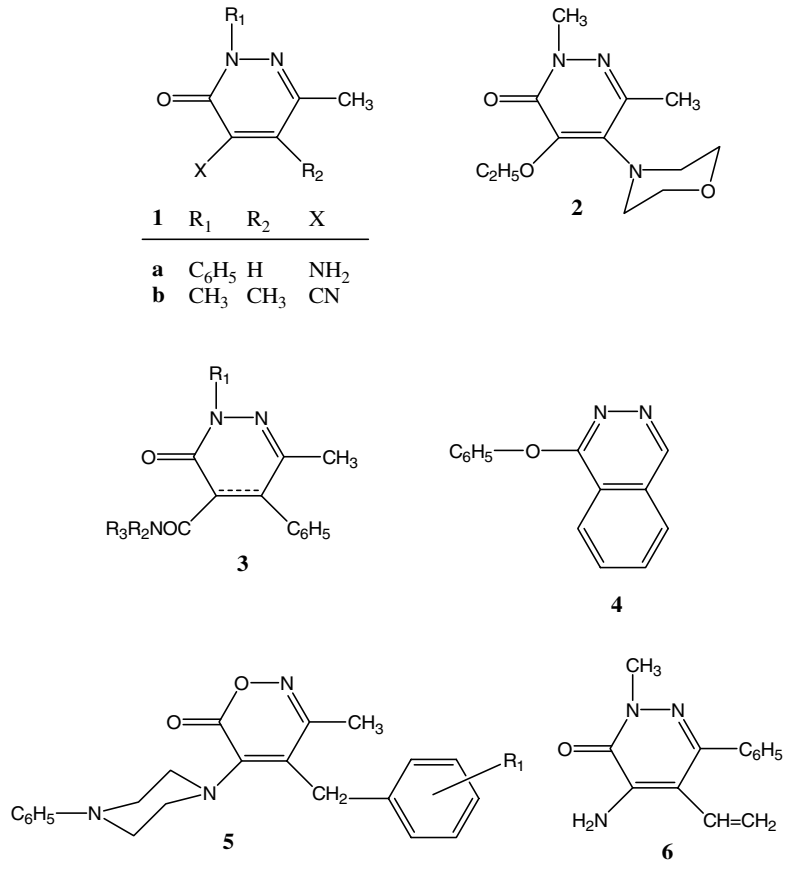


Figure 1. Pyridazine derivatives and analogues displaying alalgesic activity.

bioisosteres **5**, bearing arylpiperazine moieties, which display significant antinociceptive effects in animal models.

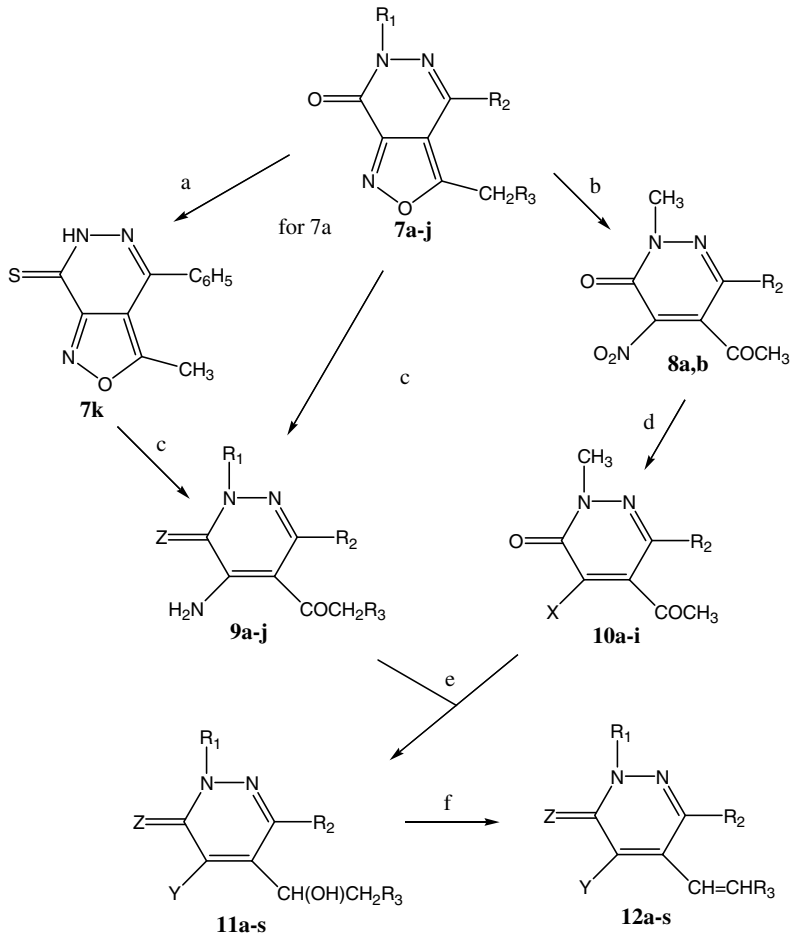
As a part of our efforts to discover new therapeutically useful antinociceptive agents bearing a pyridazine moiety, we previously described a series of 4,5-functionalized-6-phenyl-3(2*H*)-pyridazinones[14,15] structurally related to some of compounds **1**–**4** and we extensively studied the mechanism of action of the representative compound **6** (ED_{50} = 14.9 mg/kg/sc, quantal protection = 100% at 100 mg/kg) which seven times more potent than Emorfazone in the mouse abdominal constriction model.[16]

Further studies in this field led us to identify a group of pyridazine derivatives, bearing an arylpyperazinyl moiety, which showed very potent antinociceptive activity.[17–19]

In the present paper, we report our continued work on lead **6**. Extensive structure–activity relationship (SAR) studies were performed, taking into account that compound **6** is characterized by a very low degree of tolerance as regards the substitution at positions 4 and 5: replacement of $CH{=}CH_2$ with a variety of different functionalities ($COCH_3$, CN, $COOCH_3$, $CH(OC_2H_5)CH_3$), as well as the saturation of the double bond, caused a dramatic impairment of activity.[15] On this basis, we primarily focused our attention on position 4 by inserting a variety of amino groups. Moreover, we performed some modifications at positions 2 and 6, as well as the aromatization of the pyridazinonic ring in order to investigate the importance of the lactamic function.

2. Chemistry

The synthetic pathways affording the final 5-vinylpyridazinones **12a**–**s** are depicted in Scheme 1. With the exception of **7i** and **7k**, the isoxazolo[3,4-*d*]pyridazinones (Table 1), which represent the starting material, were previously described.[20–25] Compound **7i** was prepared by alkylation of the 3-methyl-4-phenylisoxazolo[3,4-*d*]pyridazin-3(2*H*)-one (**7a**) with 3-trifluorobenzyl chloride in acetone in the presence of K_2CO_3. Likewise, the 4-nitropyridazinones **8a**, **b** (**8a**, $R_2 = CH_3$, **8b**, R_2 = Ph), which originate from **7b** and **7c** by oxidative cleavage with CAN, are known compounds.[22] In the series of 4-amino (**9a**–**j**) and substituted amino analogues (**10a**–**i**), compounds **9a**,[20] **9g**[26], and **10d**[14] were previously described (Table 2). The other compounds of type **9** were obtained in good yields from the appropriate precursor by reductive cleavage with ammonium formate and Pd/C. In these reaction conditions the 4-chlorophenyl derivative **7e** underwent a concomitant dehalogenation; thus the desired **9d** was prepared by treatment of **7e** with $Mo(CO)_6$ in CH_3CN. The pyridazinthione **7k**, obtained by treatment of **7a** with Lawesson's reagent, afforded **9j** following the above procedure. The



Scheme 1. Synthetic procedure for compounds **12a**–**s**. Reagents and conditions: (a) Lawesson's reagent, toluene, 80 °C, 90 min; (b) CAN, 50% AcOH, 65% HNO_3, 60 °C, 30–60 min; (c) $HCOONH_4$, Pd/C, EtOH, reflux, 1–2 h; $Mo(CO)_6$, CH_3CN for **9d**; (d) appropriate nucleophile, EtOH, rt, 10–30 min; (e) $NaBH_4$, CH_3OH, rt, 10–90 min; (f) method A: PPA, rt −40 °C, 1–48 h; method B: H_2SO_4 on silica gel, toluene, 45–60 °C, 11–30 h.

Table 1.

Compound	R_1	R_2	R_3	Z
7a[a]	H	Ph	H	O
7b[b]	Me	Me	H	O
7c[b]	Me	Ph	H	O
7d[c]	Me	Ph	Me	O
7e[d]	Me	B[h]	H	O
7f[e]	*n*-But	Ph	H	O
7g[f]	Ph	Me	H	O
7h[b]	Ph	Ph	H	O
7i	A[g]	Ph	H	O
7j[e]	COOEt	Ph	H	O
7k	H	Ph	H	S

[a] Ref. 20.
[b] Ref. 21.
[c] Ref. 22.
[d] Ref. 23.
[e] Ref. 24.
[f] Ref. 25.
[g] A = 3-trifluoromethylbenzyl.
[h] B = 4-chlorophenyl.

nitroderivatives **8a**, **b** were treated with the appropriate nucleophile (amines or phenoxide) to give **10a**–**i**. Reduction of these intermediates with sodium borohydride in methanol at room temperature afforded the corresponding secondary alcohols **11a**–**s** in moderate yield (Table 3) which, in turn, were transformed into the final 5-vinyl derivatives **12a**–**s** in moderate yield by treatment with PPA (Method A) or with sulfuric acid on silica gel[27] (Method B) (Table 3).

Scheme 2 depicts the synthesis of compounds **12t**–**v** from their precursor **6** and **12e**. Compound **12t** was obtained by heating **6** with AcOH in pyridine; products **12u**, **v** were prepared by treatment of the appropriate precursor with bromine, followed by treatment of the intermediates **13a**, **b** with DBU in anhydrous THF.

Finally, the synthesis of 5-vinylpyridazines **18a**, **b** is shown in Scheme 3. Treatment of **7k** with iodomethane in acetone in the presence of K_2CO_3 gave regioselectively[28] the methylthio derivative **14** which, in turn, was transformed into the corresponding 3-methoxy analogue **15** by nucleophilic replacement with sodium methoxide. Treatment of the same precursor **14** with hydrogen in the presence of 10% Pd/C afforded a mixture of the corresponding open analogues **16a** and **16b** in which a concomitant desulfuration took place. Compounds **18a**, **b** were obtained from **16a** and **16c** following the synthetic procedure described in Scheme 1.

Table 2.

Compound	R_1	R_2	R_3	Z	X
9a[a]	H	Ph	H	O	
9b	Me	Me	H	O	
9c	Me	Ph	Me	O	
9d	Me	B[e]	H	O	
9e	*n*-But	Ph	H	O	
9f	Ph	Me	H	O	
9g[b]	Ph	Ph	H	O	
9h	A[d]	Ph	H	O	
9i	COOEt	Ph	H	O	
9j	H	Ph	H	S	
10a		Ph			NHMe
10b		Ph			NH*n*-But
10c		Ph			$N(Me)_2$
10d[c]		Ph			NHPh
10e		Me			NHPh
10f		Ph			NHBz
10g		Ph			$NH(CH_2)_2Ph$
10h		Ph			NHC_6H_{11}
10i		Ph			OPh

[a] Ref. 20.
[b] Ref. 26.
[c] Ref. 14.
[d] A = 3-trifluoromethylbenzyl.
[e] B = 4-chlorophenyl.

Table 3.

Compound	R_1	R_2	R_3	Z	Y
11, **12a**[a]	H	Ph	H	O	NH_2
11, **12b**	Me	Me	H	O	NH_2
11, **12c**	Me	Ph	Me	O	NH_2
11, **12d**	Me	B[c]	H	O	NH_2
11, **12e**	*n*-But	Ph	H	O	NH_2
11, **12f**	Ph	Me	H	O	NH_2
11, **12g**	Ph	Ph	H	O	NH_2
11, **12h**	A[b]	Ph	H	O	NH_2
11, **12i**	COOEt	Ph	H	O	NH_2
11, **12j**	H	Ph	H	S	NH_2
11, **12k**	Me	Ph	H	O	NHMe
11, **12l**	Me	Ph	H	O	NHBut(n)
11, **12m**	Me	Ph	H	O	$N(Me)_2$
11, **12n**	Me	Ph	H	O	NHC_6H_{11}(c)
11, **12o**	Me	Ph	H	O	OPh
11, **12p**	Me	Ph	H	O	NHPh
11, **12q**	Me	Me	H	O	NHPh
11, **12r**	Me	Ph	H	O	NHBn
11, **12s**	Me	Ph	H	O	$NH(CH_2)_2Ph$
12t	Me	Ph	H	O	NHCOMe
12u	Me	Ph	Br	O	NH_2
12v	*n*-But	Ph	Br	O	NH_2

[a] For compound **11a**, see Ref. 20.
[b] A = 3-trifluoromethylbenzyl.
[c] B = 4-chlorophenyl.

3. Results and discussion

The novel compounds were evaluated for their antinociceptive effect in the mouse abdominal constriction test and the results are reported in Table 4.

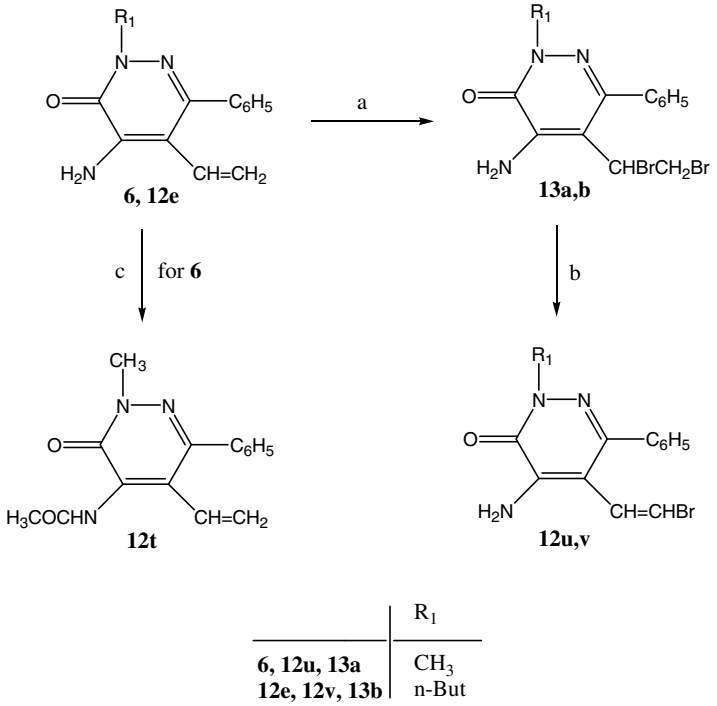
In order to identify the structural requirements capable of improving the antinociceptive effect of **6**, the SAR studies were carried out as a function of: (i) the nature of the substituents at positions 2, 4, and 6; (ii) introduction of substituents on the vinyl group; (iii) modification of the carbonyl dipole at position 3.

The obtained data show that the methyl group at position 2 of the reference compound **6** (ED_{50} = 14.9 mg/kg/sc) can be replaced by other alkyl (**12e**), aryl (**12g**), or arylalkyl (**12h**) substituents, as well as by functional groups (**12i**), without significant reduction of activity. Among these analogues, compounds **12g** and **12i** exhibited a level of antinociceptive activity comparable with that of **6**. On the contrary, the 2-unsubstituted derivatives **12a** and **12j** proved to be significantly less potent. Derivative **12e** showed a slightly improved activity (ED_{50} = 12.3 mg/kg) but, interestingly, it was able to completely protect all the treated animals from the noxious stimulus (quantal protection = 100%) at a dose (30 mg/kg) 3-fold lower than that of **6**.[15]

Looking at the structural modifications of the primary amino group in compound **6**, the best results were obtained with the methylamino derivative (**12k**) (ED_{50} = 6.5 mg/kg). The butylamino analogue (**12l**) showed the same activity as **6**. The same level of activity of **12l** was displayed by the acetylamino analogue (**12t**), suggesting that the basic properties of the nitrogen function do not play an important role in eliciting the pharmacological effect. On the other hand, the 4-dimethylamino derivative (**12m**) proved 3-fold less potent than **6**.

The presence of an anilino residue at position 4 (**12p**) was associated with a considerable reduction of activity (ED_{50} = 55.5 mg/kg). Introduction in this compound of a methylenic spacer between the phenyl group and the 4-amino residue (**12r**) left the activity almost unchanged, confirming that the basic properties of the nitrogen function are not related to the antinociceptive activity. The insertion of a further CH_2 (**12s**) was associated with a relevant impairment of activity (ED_{50} = 89.4 mg/kg) and a similar weak level of activity was demonstrated by the 4-cyclohexylamino derivative (**12n**). These data clearly suggest that there are steric limitations for the substituent linked to position 4 of the pyridazine system. Moreover, the very weak activity of the dimethylamino derivative **12m** strongly suggests that the substituent at position 4 works as a hydrogen bond donor. This hypothesis is confirmed by the relevant loss of activity observed when the aromatic amino group in **12p** is replaced by the isosteric phenoxy group (**12o**).

It should be noted that the contemporary presence of an anilino group at position 4 and a methyl at position 6 (**12q**) provokes a complete loss of activity. This effect cannot be attributed to the introduction of the methyl instead of the phenyl in position 6, because the 2,6-dimethyl analogue **12b** is one of the most potent in the series (ED_{50} = 8.2 mg/kg/sc). Furthermore, compound **12f**, in which the substituents in positions 2 and 6 are interchanged, is also endowed with a good level of activity. Probably a different pharmacokinetic or metabolism could be invoked to explain the very different behavior of **12q**. Finally, the insertion of a chlorine in the para position of the phenyl ring (**12d**) resulted detrimental.



Scheme 2. Synthetic procedure for compounds **12t–v**. Reagents and conditions: (a) Br_2, CCl_4, rt, 5 min; (b) DBU, THF, rt −40 °C, 10 min −4 h; (c) Ac_2O, pyridine, 140 °C, 3 h.

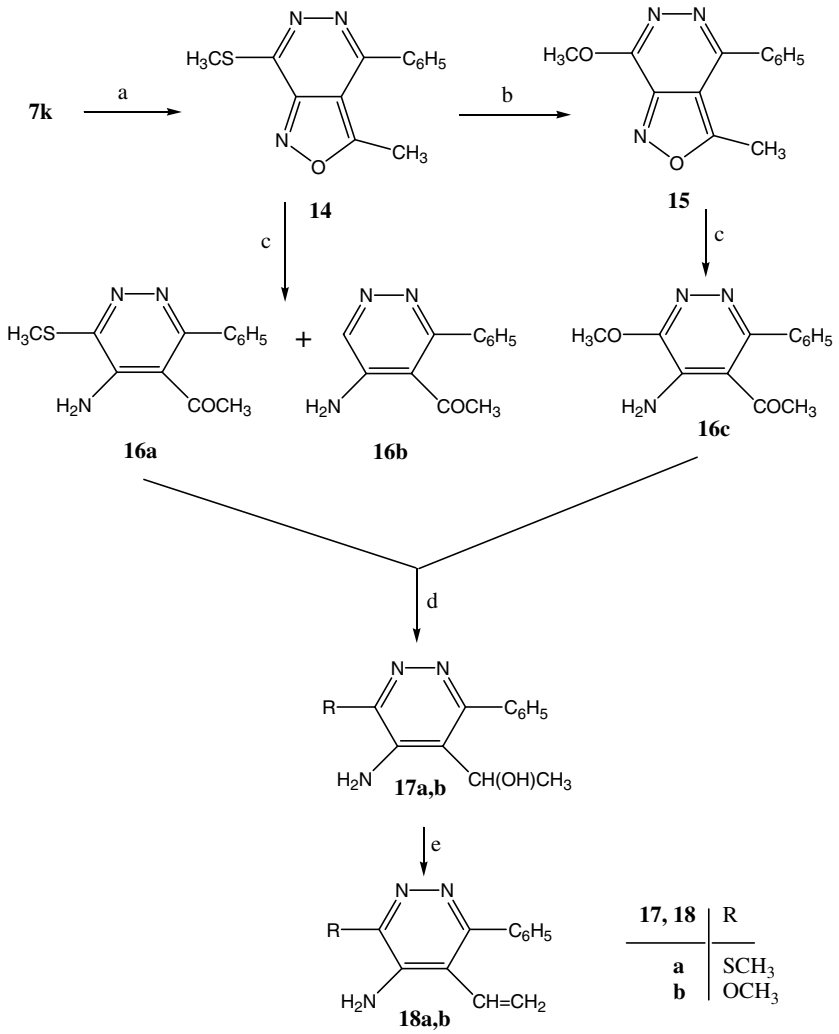
The effects of some structural modifications of the vinyl group were also studied: substitution of a hydrogen at the β-carbon with a methyl (**12c**) or the introduction of a bromine (**12u**) in the same position left the activity almost unchanged (ED_{50} = 20.6 and 10.3 mg/kg, respectively). Since, as mentioned, the presence of a *n*-butylamino group at position 2 is associated with a good level of activity, we synthesized a compound bearing this structural feature at position 2 and a bromine at the β-carbon of the vinyl group (**12v**). This compound proved to be significantly less potent both with respect to **12u** and **12e**.

Finally, the isosteric substitution of the oxygen at the amidic group of lead **6** with sulfur (**12j**) appears to be detrimental (ED_{50} = 40.9 mg/kg), suggesting a possible involvement of the oxygen in a hydrogen bond. The removal of the carbonyl dipole afforded conflicting data, since only methylthiopyridazine **18a** showed a level of antinociceptive potency (ED_{50} = 14.2 mg/kg) comparable to lead **6**.

Among the most interesting compounds, the 4-amino-2-*n*-butyl-6-phenyl-5-vinyl-3(2*H*)pyridazinone **12e** was selected as representative molecule and submitted to a series of studies in order to investigate its ability to modify the nociceptive threshold also in other tests and its effects on animal locomotor behavior; the mechanism of action of **12e** was also investigated. The results obtained in the formalin test (Fig. 2) demonstrated that **12e** was able to reduce the licking activity induced by formalin both in the first and second phase. It should be noted that **12e** was able to reduce the response to chemical nociceptive stimuli at doses lower than ibuprofen. In the hot plate test **12e** did not change the response to thermal stimuli (Fig. 3), whereas when the thermal stimuli were applied to the mouse tail, **12e** was able to raise the nociceptive threshold (Fig. 4). These effects were observed at doses that per se did not change the locomotor activity (Fig. 5) or nor motor coordination (Fig. 6) of the mice. Regarding the mechanism of action, binding studies showed the absence of affinity ($K_i > 10^{-5}$ M) for the opioid μ, *k* and *δ* receptors. Mechanisms based on the adenosine system were also ruled out on the basis of the absence of affinity for A_1 and A_2 receptors. A possible interaction with the central cholinergic system was also excluded ($K_i > 10^{-5}$ M), as well as interactions with GABA-B, α_2 adrenergic, 5-HT_2, and dopaminergic systems (data not shown).

In conclusion, several of the novel molecules exhibited greater antinociceptive activity than lead **6**. The present SAR studies revealed that modifications at positions 2 and 6, as well as at the β-carbon of the vinyl group, are tolerated, while for position 4 maximum activity is associated with amino or low alkylamino groups, being more hindered aryl-, arylalkyl-, and cycloalkylamino groups not tolerated.

For some of the tested compounds like **12b** and **12k**, IC_{50} values in the mouse abdominal constriction test were found to be 2-fold lower with respect to the previous lead **6**. Moreover for compound **12e**, which also showed activity in different animal models like tail flick and formalin test, a good balance between potency and behavioral effects was evidenced.



Scheme 3. Synthetic procedure for compounds **18a**, **b**. Reagents and conditions: (a) CH_3I, acetone, K_2CO_3, rt, 15 min; (b) CH_3ONa, CH_3OH, rt, 3 h; (c) H_2, Pd/C, EtOH, rt, 30–60 min; (d) $NaBH_4$, CH_3OH, rt, 15–30 min; (e) PPA, rt, 4–8 h.

Further studies on the mechanism of action of this novel lead are in progress.

4. Experimental

4.1. Chemistry

All melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Varian Gemini 200 instruments. Chemical shifts are reported in ppm, using the solvent as internal standard. Extracts were dried over Na_2SO_4 and the solvents were removed under reduced pressure. E. Merck F-254 commercial plates were used for analytical TLC to follow the course of reaction. Silica gel 60 (Merck 70–230 mesh) was used for column chromatography.

4.1.1. 6-(*m*-Trifluoro)-benzyl-3-methyl-4-phenylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one (7i). A mixture of 3-methylisoxazolo[3,4-*d*]pyridazin-3(2*H*)-one **7a** (0.6 mmol), (*m*-trifluoromethyl)benzyl chloride (1.2 mmol), and potassium carbonate (4 mmol) in acetone (3 mL) was heated under stirring at 40 °C for 6 h. After the mixture cooled, water (15 mL) was added and the final product **7i** was recovered by suction.

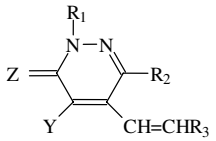
Yield = 65%; mp = 146–148 °C (EtOH); ^{1}H NMR ($CDCl_3$) 2.55 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 7.40–7.80 (m, 9H, 2Ar).

4.1.2. 3-Methyl-4-phenylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-thione (7k). A mixture of **7a** (0.6 mmol) and Lawesson's reagent (0.6 mmol) in toluene (8 mL) was heated at 90 °C for 90 min. After cooling the crude yellow precipitate was recovered by suction.

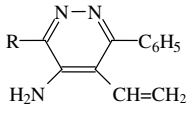
Yield = 93%; mp = 188 °C (EtOH); ^{1}H NMR ($CDCl_3$) 2.55 (s, 3H, CH_3), 7.55 (s, 5H, Ar), 11.0 (exch. br s, 1H, NH).

4.1.3. General procedure for compounds 9. The required isoxazolo[3,4-*d*]pyridazin-7(6*H*)-ones **7a**–**d** and **7f**–**k** (0.7 mmol) were suspended in EtOH (3.5 mL) and then $HCOONH_4$ (17 mmol) and 10% Pd/C (10 mg) were added. The mixture was refluxed for 1–2 h and after

Table 4.



12a-v



18a,b

Compound	R_1	R_2	R_3	Z/R	Y	ED_{50}(mg/kg/sc)[c]
12a	H	Ph	H	O	NH_2	28.8 (11.2–74.0)
12b	Me	Me	H	O	NH_2	8.2 (4.0–16.7)
12c	Me	Ph	Me	O	NH_2	20.6 (13.6–31.2)
12d	Me	B[b]	H	O	NH_2	32.7 (20.2–53.1)
12e	*n*-But	Ph	H	O	NH_2	12.3 (1.4–20.3)
12f	Ph	Me	H	O	NH_2	23.9 (14.1–40.7)
12g	Ph	Ph	H	O	NH_2	14.0 (9.2–21.1)
12h	A[a]	Ph	H	O	NH_2	22.2 (12.8–38.3)
12i	COOEt	Ph	H	O	NH_2	18.2 (12.2–27.2)
12j	H	Ph	H	S	NH_2	40.9 (19.1–87.0)
12k	Me	Ph	H	O	NHMe	6.5 (4.0–10.6)
12l	Me	Ph	H	O	NH*n*-But	15.6 (10.5–23.2)
12m	Me	Ph	H	O	$N(Me)_2$	45.7 (24.4–85.8)
12n	Me	Ph	H	O	NHC_6H_{11}	79.4 (46.0–137.1)
12o	Me	Ph	H	O	OPh	145.5 (58.1–364.6)
12p	Me	Ph	H	O	NHPh	55.5 (30.5–100.7)
12q	Me	Me	H	O	NHPh	400 (120–1331)
12r	Me	Ph	H	O	NHBn	47.2 (23.2–96.2)
12s	Me	Ph	H	O	$NH(CH_2)Ph$	89.4 (47.5–168.3)
12t	Me	Ph	H	O	NHCOMe	16.0 (11.3–22.7)
12u	Me	Ph	Br	O	NH_2	10.3 (6.6–16.1)
12v	*n*-But	Ph	Br	O	NH_2	61.0 (36.4–102.1)
18a				SCH_3		14.2 (9.2–20.8)
18b				OCH_3		54.7 (23.8–125.3)
6	Me	Ph	H	O	NH_2	14.9

[a] A = 3-trifluoromethylbenzyl.

[b] B = 4-chlorophenyl.

[c] Dose–response curves were constructed defining as significant an individual reaction lower than 3 SD of the control mean reaction time. Six to eight animals were used for each dose and at least 18 animals were used in each experiments. The parallel line assay of Finney[31] was used to estimate the ED_{50} values and their 95% confidence intervals with the aid of a computer.

cooling, CH_2Cl_2 (5 mL) was added. The solution was stirred for 5 min, then the catalyst was filtered off and the solvent was evaporated in vacuo.

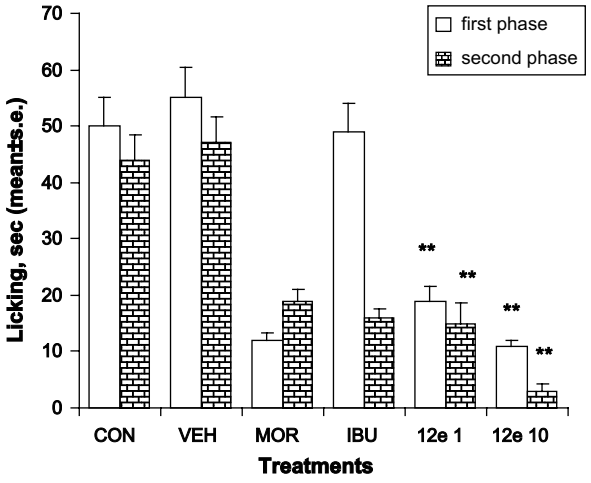


Figure 2. Effect of **12e** in the formalin test. CON, animals treated with formalin alone; VEH, animals treated with DMSO:saline 1:4 v/v; MOR, animals treated with morphine, 5 mg/kg; IBU, animals treated with ibuprofen 100 mg/kg; **12e**, animals treated with **12e** at 1 or 10 mg/kg. $^{**}p < 0.01$ versus VEH. $N = 10$.

4.1.3.1. 5-Acetyl-4-amino-2,6-dimethylpyridazin-3(2*H*)-one (9b). Yield = 92%; mp = 123–125 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, CH_3), 2.55 (s, 3H,

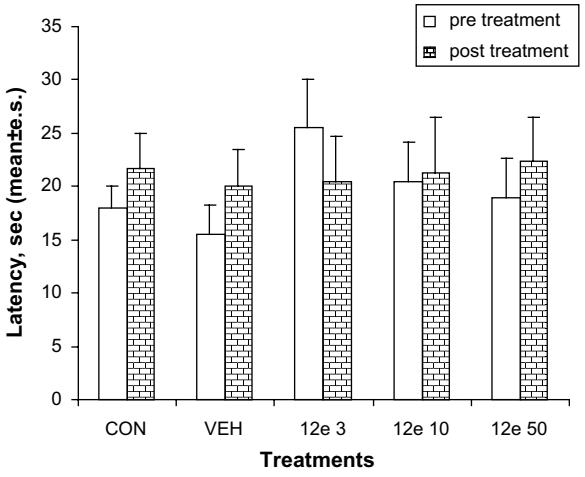


Figure 3. Effect of **12e** in the hot plate test. CON, non-treated animals; VEH, animals treated with DMSO:saline 1:4 v/v; **12e**, animals treated with **12e** at 3, 10 or 50 mg/kg. $N = 6$.

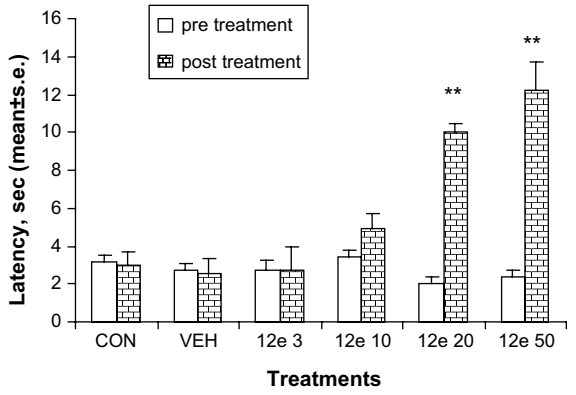


Figure 4. Effect of **12e** in the tail flick test. CON, non-treated animals; VEH, animals treated with DMSO:saline 1:4 v/v; **12e**, animals treated with **12e** at 3, 10, 20, and 50 mg/kg. $^{**}p < 0.01$ versus VEH. $N = 10$.

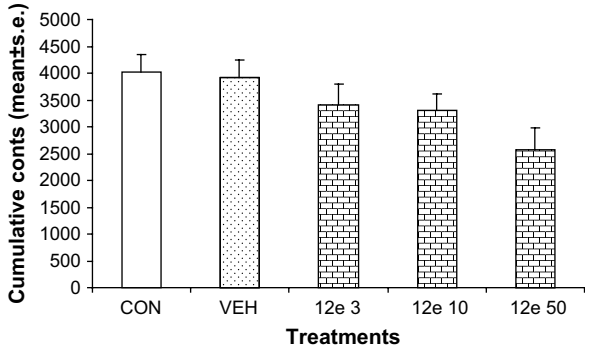


Figure 5. Effect of **12e** in the activity cage test. CON, non-treated animals; VEH, animals treated with DMSO:saline 1:4 v/v; **12e**, animals treated with **12e** at 3, 10, and 50 mg/kg. $N = 8$.

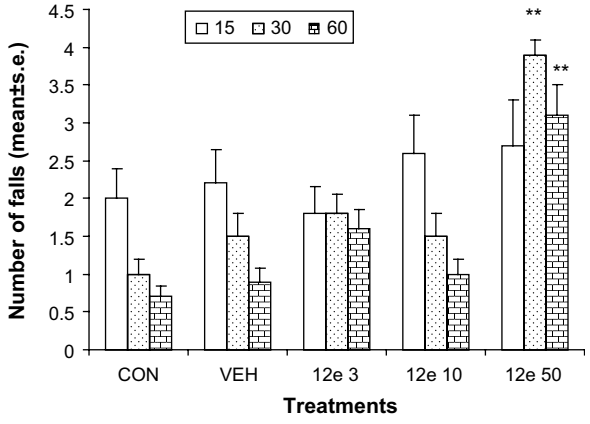


Figure 6. Effect of **12e** in the rotarod test. CON, non-treated animals; VEH, animals treated with DMSO:saline 1:4 v/v; **12e**, animals treated with **12e** at 3, 10, and 50 mg/kg. $^{**}p < 0.01$ versus VEH. $N = 6$.

CO—CH_3), 3.70 (s, 3H, N—CH_3), 7.65 (exch. br s, 2H, NH_2).

4.1.3.2. 5-Acetyl-4-amino-6-(*p*-chlorophenyl)-2-methylpyridazin-3(2*H*)-one (9d). A mixture of **7e** (0.2 mmol), $Mo(CO)_6$ (0.25 mmol), water (0.1 mL), and CH_3CN (4 mL) was heated at 100 °C for 2 h. After cooling the solution was diluted with CH_3CN (2 mL) and 10 mg of charcoal was added. After suction, the solvent was removed in vacuo and the residue, treated with water, afforded compound **9d**.

Yield = 66%; mp = 235–236 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 3H, $COCH_3$), 3.80 (s, 3H, NCH_3), 7.50–7.35 (m, 4H, Ar).

4.1.4. General procedure for compounds 10a–h. A mixture of the required 5-acetyl-2-methyl-4-nitropyridazinone **8a** or **b** (0.2 mmol), the appropriate amine (0.5 mmol) in EtOH (2 mL) was stirred at room temperature for 10–30 min. Compounds **10b** and **10d–h** were directly recovered by suction; dilution with ice-water of reaction mixture afforded compounds **10a** and **10c**.

4.1.4.1. 5-Acetyl-2-methyl-4-methylamino-6-phenylpyridazin-3(2*H*)-one (10a). Yield = 73%; mp = 163–164 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.60 (m, 1H, NH), 1.90 (s, 3H, $COCH_3$), 2.95 (d, 3H, NHCH_3), 3.80 (s, 3H, NCH_3), 7.40 (m, 5H, Ar).

4.1.4.2. 5-Acetyl-2-methyl-4-phenoxy-6-phenylpyridazin-3(2*H*)-one (10i). To a solution of **8b** (0.5 mmol) in ethyl ether (6 mL) sodium phenoxide (1 mmol) was added. After stirring for 90 min at room temperature ice-water was added (20 mL) and the mixture was extracted with CH_2Cl_2 (3× 15 mL). The organic layer was washed with 0.5 N NaOH (2× 5 mL), dried on Na_2SO_4, and evaporated in vacuo to furnish compound **10i**.

Yield = 79%; mp = 78–80 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, $COCH_3$), 3.85 (s, 3H, NCH_3), 7.00–7.40 (m, 5H, Ar), 7.45 (s, 5H, Ar).

4.1.5. General procedure for compounds 11a–s and 17a, b. Sodium borohydride (2.2 mmol) was added portionwise to a stirred solution of **9a–j** and **10a–i** (0.35 mmol) in CH_3OH (2 mL) for 10–90 min at root temperature. The reaction mixture was diluted with ice-water (8 mL). Compounds were collected by filtration, with the exception of **11d**, **11m**, and **11o** which were obtained by extraction with CH_2Cl_2 (3× 15 mL) and evaporation of the solvent.

4.1.5.1. 4-Amino-2,6-dimethyl-5-(1-hydroxyethyl)pyridazin-3(2*H*)-one (11b). Yield = 95%; mp = 93–94 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 3H, CH(OH)CH_3), 3.65 (s, 3H, NCH_3), 4.95 (q, 1H, CH(OH)CH_3), 5.70 (exch. br s, 1H, OH).

4.1.6. General procedure for compounds 12a–s and 18a, b. *Method A.* The appropriate 5-hydroxyalkylpyridazine **11a–o** and **17a**, **b** (0.4 mmol) were treated with PPA (40 mmol) at root temperature for 1–48 h. After treatment with ice-water (20 mL) the crude precipitate of the desired compound was collected by suction.

4.1.6.1. 4-Amino-6-phenyl-5-vinylpyridazin-3(2*H*)one (12a). Yield = 44%; mp = 277–278 °C (EtOH); ^{1}H

NMR ($CDCl_3$) 5.30 (m, 2H, CH=CH_2), 6.25 (m, 1H, CH=CH_2), 7.40 (s, 5H, Ar). Anal. Calcd for $C_{12}H_{11}N_3O$ (213,09): C, 67.59; H, 5.20; N, 19.71. Found: C, 67.83; H, 5.34; N, 19.47.

4.1.6.2. 4-Amino-2,6-dimethyl-5-vinylpyridazin-3(2*H*)-one (12b). Yield = 64%; mp = 112–113 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, CH_3), 3.70 (s, 3H, NCH_3), 5.20 (exch. br s, 2H, NH_2), 5.65 (dd, J = 9.6 Hz, J = 16.7 Hz, 2H, CH=CH_2), 6.45 (dd, J = 9.6 Hz, J = 16.7 Hz, 1H, CH=CH_2). Anal. Calcd for $C_8H_{11}N_3O$ (165,19): C, 58.17; H, 6.71; N, 25.44. Found: C, 58.01; H, 6.66; N, 25.25.

4.1.6.3. 4-Amino-2-methyl-6-phenyl-5-propenylpyridazin-3(2*H*)-one (12c). Yield = 81%; mp = 151–153 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 1.80 (d, 3H, CH=CH–CH_3), 3.80 (s, 3H, NCH_3), 5.25 (m, 1H, CH=CH–CH_3), 5.95 (m, 1H, CH=CH–CH_3), 7.40 (m, 5H, Ar). Anal. Calcd for $C_{14}H_{15}N_3O$ (241,29): C, 69.69; H, 6.27; N, 17.41. Found: C, 70.00; H, 5.99; N, 17.63.

4.1.6.4. 4-Amino-6-(4-chlorophenyl)-2-methyl-5-vinylpyridazin-3(2*H*)-one (12d). Yield = 80%; mp = 109–111 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H, NCH_3), 5.35–5.55 (m, 4H, 2H, NH_2; 2H CH=CH_2), 6.25 (dd, J = 16.0 Hz, J = 10.0 Hz, 1H, CH=CH_2), 7.40 (s, 4H, Ar). Anal. Calcd for $C_{13}H_{12}ClN_3O$ (261,71): C, 59.66; H, 4.62; N, 16.06. Found: C, 59.28; H, 4.44; N, 15.91.

4.1.6.5. 4-Amino-2-butyl-6-phenyl-5-vinylpyridazin-3(2*H*)-one (12e). Yield = 64%; mp = 74–78 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, CH_2CH_3), 1.30–1.65 (m, 4H, $CH_2CH_2CH_2CH_3$), 3.25 (t, 2H, NHCH_2), 3.80 (s, 3H, NCH_3), 4.95 (d, J = 14.9 Hz, 1H, CH=CH_2), 5.40 (d, J = 9.0 Hz, 1H, CH=CH_2), 6.00 (exch. br s, 1H, NH), 6.45 (dd, J = 15.0 Hz, J = 9.0 Hz, 1H, CH=CH_2), 7.40 (s, 5H, Ar). Anal. Calcd for $C_{16}H_{19}N_3O$ (269,34): C, 71.35; H, 7.11; N, 15.60. Found: C, 71.60; H, 6.88; N, 15.84.

4.1.6.6. 4-Amino-6-methyl-2-phenyl-5-vinylpyridazin-3(2*H*)-one (12f). Yield = 62%; mp = 104–105 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 5.30 (exch. br s, 2H, NH_2), 5.70 (m, 2H, CH=CH_2), 6.55 (dd, J = 17.4 Hz, J = 11.0 Hz, 1H, CH=CH_2), 7.50 (m, 5H, Ar). Anal. Calcd for $C_{13}H_{13}N_3O$ (227,26): C, 68.70; H, 5.77; N, 18.49. Found: C, 69.04; H, 6.01; N, 18.31.

4.1.6.7. 4-Amino-2,6-diphenyl-5-vinylpyridazin-3(2*H*)-one (12g). Yield = 90%; mp = 142–143 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 5.65 (m, 2H, CH=CH_2), 6.35 (m, 1H, CH=CH_2), 7.30–7.70 (m, 10H, 2Ar). Anal. Calcd for $C_{18}H_{15}N_3O$ (289,33): C, 74.72; H, 5.23; N, 14.52. Found: C, 74.55; H, 5.48; N, 14.39.

4.1.6.8. 4-Amino-6-phenyl-2-(3-trifluoromethylbenzyl)-5-vinylpyridazin-3(2*H*)-one (12h). Yield = 84%; mp = 124–127 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 5.35–5.65 (m, 6H: 2H, CH_2Ph; 2H, NH_2; 2H, CH=CH_2), 6.25 (dd, J = 17.7 Hz, J = 11.4 Hz, 1H, CH=CH_2), 7.50 (m, 5H, Ar). Anal. Calcd for $C_{20}H_{16}F_3N_3O$ (371,36): C, 64.69; H, 4.34; N, 11.32. Found: C, 65.03, H, 4.59; N, 11.58.

4.1.6.9. 5-Amino-6-oxo-3-phenyl-4-vinyl-6*H*-pyridazine-1-carboxylic acid ethyl ester (12i). Yield = 62%; mp = 102–104 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, OCH_2CH_3), 4.25 (q, 2H, OCH_2CH_3), 5.40 (exch. br s, 2H, NH_2) 5.50–5.65 (dd, J = 16.7 Hz, J = 11.0 Hz, 2H, CH=CH_2), 6.25 (dd, J = 16.7 Hz, J = 11.0 Hz, 1H, CH=CH_2), 7.35–7.50 (m, 5H, Ar). Anal. Calcd for $C_{15}H_{15}N_3O_3$ (285,30): C, 63.15; H, 5.30; N, 14.73. Found: C, 62.93; H, 4.97; N, 15.08.

4.1.6.10. 4-Amino-6-phenyl-5-vinylpyridazine-3(2*H*)-thione (12j). Yield = 79%; mp = >300 °C dec. (cyclohexane); yield = 62%; mp = 102–104 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 5.30 (d, J = 17 Hz, 1H, CH=CH_2), 5.50 (d, J = 12 Hz, 1H, CH=CH_2), 6.30–6.50 (m, 1H, CH=CH_2), 6.90 (exch. br s, 2H, NH_2), 7.30–7.55 (m, 5H, Ar). Anal. Calcd for $C_{12}H_{11}N_3S$ (229,30): C, 62.86; H, 4.84; N, 18.33. Found: C, 63.04; H, 4.97; N, 15.08.

4.1.6.11. 2-Methyl-4-methylamino-6-phenyl-5-vinylpyridazin-3(2*H*)-one (12k). Yield = 71%; mp = 114–116 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.95 (s, 3H, NHCH_3), 3.80 (s, 3H, NCH_3), 4.95 (d, J = 14.9 Hz, 1H, CH=CH_2), 5.45 (d, J = 9.0 Hz, 1H, CH=CH_2), 6.45 (dd, J = 15.0 Hz, J = 9.0 Hz, 1H, CH=CH_2), 7.35 (s, 5H, Ar). Anal. Calcd for $C_{14}H_{15}N_3O$ (241,29): C, 69.69; H, 6.27; N, 17.41. Found: C, 69.78; H, 6.39; N, 17.77.

4.1.6.12. 4-Butylamino-2-methyl-6-phenyl-5-vinylpyridazin-3(2*H*)-one (12l). Yield = 71%; mp = 56–58 °C (EtOH/H_2O 1:1); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, CH_2CH_3), 1.25–1.70 (m, 4H, $CH_2CH_2CH_3$), 3.15–3.30 (m, 2H, NHCH_2), 3.80 (s, 3H, NCH_3), 4.95 (d, J = 15.0 Hz, 1H, CH=CH_2), 5.40 (d, J = 9.0 Hz, 1H, CH=CH_2), 6.00 (exch. br s, 2H, NH_2), 6.45 (dd, J = 15.0 Hz, J = 9.0 Hz, 1H, CH=CH_2), 7.40 (s, 5H, Ar). Anal. Calcd for $C_{17}H_{21}N_3O$ (283,37): C, 72.06; H, 7.47; N, 14.83. Found: C, 71.85; H, 7.71; N, 15.08.

4.1.6.13. 4-Dimethylamino-2-methyl-6-phenyl-5-vinylpyridazin-3(2*H*)-one (12m). Yield = 88%; mp = 57–60 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.95 (s, 6H, N(CH_3)$_2$), 3.80 (s, 3H, NCH_3), 4.85 (d, J = 17.7 Hz, 1H, CH=CH_2), 5.40 (d, J = 11.4 Hz, 1H, CH=CH_2), 6.35 (dd, J = 17.7 Hz, J = 11.4 Hz, 1H, CH=CH_2), 7.40 (s, 5H, Ar). Anal. Calcd for $C_{15}H_{17}N_3O$ (255,31): C, 70.56; H, 6.71; N, 16.46. Found: C, 70.90; H, 6.96; N, 16.73.

4.1.6.14. 4-Cyclohexylamino-2-methyl-6-phenyl-5-vinylpyridazin-3(2*H*)-one (12n). Yield = 49%; mp = 79–81 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.10–1.90 (m, 11H, NHC_6H_{11}), 3.80 (s, 3H, NCH_3), 5.00 (d, J = 16.0 Hz, 1H, CH=CH_2), 5.40 (d, J = 10.0 Hz, 1H, CH=CH_2), 6.45 (dd, J = 16.0 Hz, J = 10.0 Hz, 1H, CH=CH_2), 7.40 (s, 5H, Ar). Anal. Calcd for

$C_{19}H_{23}N_3O$ (309,41): C, 73.76; H, 7.49; N, 13.58. Found: C, 74.04; H, 7.80; N, 13.78.

4.1.6.15. 2-Methyl-4-phenoxy-6-phenyl-5-vinylpyridazin-3(2*H*)-one (12o). Yield = 78%; mp = 97–100 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H, NCH_3), 5.55 (d, J = 9.2 Hz, 1H, CH=CH_2), 5.90 (d, J = 15.3 Hz, 1H, CH=CH_2), 6.30 (dd, J = 15.3 Hz, J = 9.2 Hz, 1H, CH=CH_2), 6.95–7.40 (m, 5H, Ar), 7.45 (s, 5H, Ar). Anal. Calcd for $C_{19}H_{16}N_2O_2$ (304,34): C, 74.98; H, 5.30; N, 9.20. Found: C, 75.23; H, 5.49; N, 8.87.

4.1.6.16. 3-Methylsulfanyl-6-phenyl-5-vinyl-pyridazin-4-ylamine (18a). Yield = 70%; mp = 170–172 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.80 (s, 3H, SCH_3), 4.75 (exch. br s, 2H, NH_2), 5.65 (d, J = 17.0 Hz, 1H, CH=CH_2), 5.65 (d, J = 12.0 Hz, 1H, CH=CH_2), 6.40 (dd, J = 17.0 Hz, J = 12.0 Hz, 1H, CH=CH_2), 7.35–7.65 (m, 5H, Ar). Anal. Calcd for $C_{13}H_{13}N_3S$ (243,33): C, 64.17; H, 5.39; N, 17.27. Found: C, 64.00; H, 5.12; N, 17.04.

4.1.6.17. 3-Methoxy-6-phenyl-5-vinyl-pyridazin-4-ylamine (18b). Yield = 68%; mp = 110–112 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 4.20 (s, 3H, OCH_3), 4.85 (exch. br s, 2H, NH_2), 5.60 (d, J = 17.9 Hz, 1H, CH=CH_2), 5.65 (d, J = 12.3 Hz, 1H, CH=CH_2), 6.35 (dd, J = 17.9 Hz, J = 12.3 Hz, 1H, CH=CH_2), 7.35–7.55 (m, 5H, Ar). Anal. Calcd for $C_{13}H_{13}N_3O$ (227,26): C, 68.70; H, 5.77; N, 18.49. Found: C, 69.04; H, 6.03; N, 18.24.

Method B: The appropriate 5-hydroxyalkylpyridazinones **11p–s** (0.4 mmol) dissolved in toluene (4.5 mL) and SiO_2/H_2SO_4 (1 g) prepared following the procedure described in Ref. 23 was added portionwise over 3 h. The mixture was heated at 45–60 °C for 11–30 h and after cooling acetone (20 mL) was added. Silica gel was removed by suction and washed with acetone (3× 15 mL). The organic layers were collected and evaporated in vacuo to afford the vinyl derivatives **12p–s**.

4.1.6.18. 2-Methyl-6-phenyl-4-phenylamino-5-vinylpyridazin-3(2*H*)-one (12p). Yield = 99%; mp = 127–130 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H, NCH_3), 4.75 (d, J = 17.7 Hz, 1H, CH=CH_2), 5.05 (d, J = 11.4 Hz, 1H, CH=CH_2), 6.15 (dd, J = 17.7 Hz, J = 11.4 Hz, 1H, CH=CH_2), 6.85–7.10 (m, 3H, Ar), 7.20–7.45 (m, 7H, Ar), 7.80 (exch. br s, 1H, NH). Anal. Calcd for $C_{19}H_{17}N_3O$ (303,36): C, 75.23; H, 5.65; N, 13.85. Found: C, 74.87; H, 5.49; N, 14.07.

4.1.6.19. 2,6-Dimethyl-4-phenylamino-5-vinylpyridazin-3(2*H*)-one (12q). Yield = 38%; mp = 100–112 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, CH_3), 3.80 (s, 3H, NCH_3), 5.00 (d, J = 17.2 Hz, 1H, CH=CH_2), 5.20 (d, J = 10.3 Hz, 1H, CH=CH_2), 6.20 (dd, J = 17.2 Hz, J = 10.3 Hz, 1H, CH=CH_2), 6.80–6.90 (m, 2H, Ar), 6.95–7.05 (m, 1H, Ar), 7.20–7.30 (m, 2H, Ar). Anal. Calcd for $C_{14}H_{15}N_3O$ (241,29): C, 69.69; H, 6.27; N, 17.41. Found: C, 70.08; H, 5.95; N, 17.20.

4.1.6.20. 4-Benzylamino-2-methyl-6-phenyl-5-vinylpyridazin-3(2*H*)-one (12r). Yield = 34%; mp = 123 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H, NCH_3), 4.50 (s, 2H, CH_2), 5.05 (d, J = 17.2 Hz, 1H, CH=CH_2), 5.40 (d, J = 9.1 Hz, 1H, CH=CH_2), 6.35 (dd, J = 17.2 Hz, J = 9.1 Hz, 1H, CH=CH_2), 7.25–7.40 (m, 2H, Ar). Anal. Calcd for $C_{20}H_{19}N_3O$ (317,38): C, 75.69; H, 6.03; N, 13.24. Found: C, 75.99; H, 5.73; N, 12.91.

4.1.6.21. 2-Methyl-4-phenethylamino-6-phenyl-5-vinylpyridazin-3(2*H*)-one (12s). Yield = 53%; mp = 116–118 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.85 (t, 2H, CH_2Ph), 3.55 (q, 2H, CH_2NH), 3.85 (s, 3H, NCH_3), 5.00 (d, J = 15.0 Hz, 1H, CH=CH_2), 5.40 (d, J = 9.1 Hz, 1H, CH=CH_2), 6.1 (exch. br s, 1H, NH), 6.45 (dd, J = 15.0 Hz, J = 9.1 Hz, 1H, CH=CH_2), 7.15–7.40 (m, 10H, 2Ar). Anal. Calcd for $C_{21}H_{21}N_3O$ (331,41): C, 76.11; H, 6.39; N, 12.68. Found: C, 75.89; H, 6.71; N, 12.88.

4.1.7. 4-Acetamide-2-methyl-6-phenyl-3-oxo-5-vinylpyridazine (12t). A mixture of compound **6** (0.2 mmol), pyridine (5 mL), and acetic anhydride (20 mmol) was stirred at 140 °C for 3 h and 30 min. After cooling, dilution with water, and acidification with 2 N HCl, the solution was extracted with CH_2Cl_2 (3× 15 mL). After the solvent was dried and evaporated in vacuo, treatment of the residue with EtOH/H_2O 1:1 afforded compound **12t**.

Yield = 56%; mp = 84–86 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H, $COCH_3$), 3.85 (s, 3H, NCH_3), 5.50 (m, J = 17.6 Hz, J = 11.5 Hz, 2H, CH=CH_2), 6.20 (dd, J = 17.6 Hz, J = 11.5 Hz, 1H, CH=CH_2), 7.55–7.35 (m, 5H, Ar). Anal. Calcd for $C_{15}H_{15}N_3O_2$ (269,30): C, 66.90; H, 5.61; N, 15.60. Found: C, 67.13; H, 5.90; N, 15.83.

4.1.8. General procedure for 12u, v. The appropriate 2-alkyl-4-amino-6-phenyl-5-vinylpyridazinones **6** and **12e** (0.3 mmol) were suspended in CCl_4 (3.5 mL) and, after cooling at −5 °C, bromine (1 mmol) was added and the mixture was stirred for 10 min. The crude precipitate 2-alkyl-4-amino-5-(1,2-dibromoethyl)-6-phenylpyridazinone (**13a**, **b**) was recovered by suction and washed with ethyl acetate. The dried product was dissolved in anhydrous THF (7.5 mL), DBU (10 mmol) was added, and the reaction was carried out under nitrogen for 10 min at 20 °C (**12u**) and for 4 h at 40 °C (**12v**). After evaporation of the solvent, the residue was treated with ice-water and acidified with 2 N HCl. The crude precipitate **12u** and **12v** was recovered by suction.

4.1.8.1. 4-Amino-5-(2-bromovinyl)-2-methyl-6-phenylpyridazin-3(2*H*)-one (12u). Yield = 50%; mp = 148–150 °C (EtOH/H_2O 1:1); ^{1}H NMR ($CDCl_3$) δ 3.70 (s, 3H, NCH_3), 6.35 (d, 1H, CH=CHBr), 6.80 (d, 1H, CH=CHBr), 7.40 (m, 5H, Ar). Anal. Calcd for $C_{13}H_{12}BrN_3O$ (306,16): C, 51.00; H, 3.95; N, 13.72. Found: C, 50.75; H, 4.18; N, 14.03.

4.1.8.2. 4-Amino-5-(2-bromovinyl)-2-butyl-6-phenylpyridazin-3(2*H*)-one (12v). Yield = 74%; mp = 103–105 °C

(EtOH); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, CH_2CH_3), 1.30–1.50 (m, 2H, $CH_2CH_2CH_3$), 1.65–1.90 (m, 2H, $CH_2CH_2CH_3$), 4.20 (t, 2H, NCH_2), 5.35 (exch. br s, 2H, NH_2), 6.55 (d, J = 16.7 Hz, 1H, CH=CHBr), 6.70 (d, J = 16.7 Hz, 1H, CH=CHBr), 7.45 (s, 5H, Ar). Anal. Calcd for $C_{16}H_{18}BrN_3O$ (348,24): C, 55.18; H, 5.21; N, 12.07. Found: C, 54.81; H, 4.99; N, 11.83.

4.1.9. 3-Methyl-7-methylthio-4-phenylisoxazolo[3,4-*d*]-pyridazine (14). A mixture of compound **7k** (0.2 mmol), acetone (3 mL), anhydrous K_2CO_3 (20 mmol), and iodomethane (4 mmol) was stirred at room temperature for 15 min. After dilution with ice-water the crude precipitate was recovered by suction.

Yield = 74%; mp = 188–190 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 3H, C—CH_3), 2.80 (s, 3H, SCH_3), 7.55 (s, 5H, Ar).

4.1.10. 3-Methyl-7-methoxy-4-phenylisoxazolo[3,4-*d*]pyridazine (15). CH_3ONa (0.4 mmol) in abs CH_3OH (10 mL) was added portionwise to a solution of **14** (0.2 mmol) in CH_3OH (10 mL) and the reaction mixture was stirred at room temperature for 3 h. After neutralization with 2 N HCl, the mixture was concentrated and after cooling, the precipitate was recovered by suction.

Yield = 58%; mp = 187–189 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 2.65 (s, 3H, C—CH_3), 4.40 (s, 3H, OCH_3), 7.45–7.75 (m, 5H, Ar).

4.1.11. General procedure for 16a–c. A mixture of the appropriate 3-methyl-4-phenylisoxazolo[3,4-*d*]pyridazine **14** and **15** (0.5 mmol), 10% Pd/C (30 mg), and EtOH (25 mL) was shaken under hydrogen at room temperature and 2 bar for 30–120 min. The catalyst was filtered off and the solvent was evaporated in vacuo.

Reduction of **14** in this experimental condition afforded a mixture of **16a** and **16b**, which were separated by column chromatography.

4.1.11.1. 5-Acetyl-4-amino-3-methylthio-6-phenylpyridazine (16a). Yield = 83%; mp = 113–115 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3H, $COCH_3$), 2.80 (s, 3H, SCH_3), 6.20 (exch. br s, 2H, NH_2), 7.40–7.65 (m, 5H, Ar).

4.2. Biological assays

4.2.1. Animals. Male CD-1 mice (Charles River, Italy) weighing 25–30 g were used for all experiments. The animals were housed in colony cages (5 mice each) under standard light (alight from 7.00 a.m. to 7.00 p.m.), temperature (22 ± 1°C), and relative humidity (60 ± 10%) conditions for at least 1 week before the experimental sessions. Food and water were available ad libitum. All experiments were carried out according to the guidelines of the European Community Council for Experimental Animal Care (86/609/EEC).

4.2.2. Hot plate test. The hot plate test was performed as previously reported[29] with some modifications. The device (Socrel Mod. DS-37, Ugo Basile, Italy) consisted of a metal plate (25 × 25 cm) heated to a constant temperature (50 ± 0.1°C) on which a plastic cylinder (20 cm diameter, 16 cm high) was placed. The time of latency in second was recorded between the moment the animal was placed on the hot plate surface and when it licked its paw or jerked it strongly and then lifted it off. The measurement was terminated if the latency exceeded the cut-off time (40 s) or if the mouse jumped off the hot plate. Baseline latency was of 15–17 s. Hot plate data were recorded as latency to respond in sec both 30 and 15 min before the treatment for baseline determination and six more times—15, 30, 45, 60, 75, and 90 min, after treatment.

4.2.3. Tail flick test. Tail flick latency[29] was obtained using a tail flick unit (Socrel Mod DS-20, Ugo Basile, Italy) consisting of an infrared source, radiant light (100 W bulb) with adjustable intensity which was focused by an aluminized parabolic mirror on a photocell. Radiant heat was focused 1–2 cm from the tip of the tail, and the latency time in second until the mouse flicked its tail was recorded. The measurement was terminated if the latency exceeded the cut-off time (15 s). Beam intensity was adjusted to produce a tail flick latency of 3–4 s in the baseline determination. The animals were restrained during trials by means of plastic cylinder 4 cm in diameter and 8 cm long. Tail flick data were recorded as latency to respond in sec both 30 and 15 min before the treatment for baseline determination and six more times—15, 30, 45, 60, 75, and 90 min, after treatment.

4.2.4. Writhing test. Writhing test was performed as previously reported.[30] Mice were given an intraperitoneal (ip) injection of 0.6% v/v acetic acid in a volume of 10 mL/kg. Acetic acid induces a series of writhes, consisting in abdominal contraction and hind limb extension and the number of writhes was recorded over a 10-min period beginning 5 min after acetic acid injection. Compounds were subucutaneously (sc) administered 30 min before acetic acid injection.

4.2.5. Formalin test. In the formalin test[30] 20 μL of a 0.5% solution of formalin in saline was injected subcutaneously (sc) into the dorsal surface of the right hind paw of the mouse using a microsyringe with a 27-gauge needle. The formalin injection produced a distinct biphasic response consisting of licking or biting the paw. The first phase occurred from 0 to 5 min after formalin injection and the second phase from 15–20 to 30–40 min. The mouse was then put into a plexiglas cage (30 × 14 × 12 cm) which served as an observation chamber, and the total amount of time in second the animal spent licking or biting the paw after formalin injection was recorded from 0 to 5 (first phase) min and from 20 to 40 (second phase) min. Compounds were intraperitoneally (ip) administered 30 min before formalin injections.

4.2.6. Motor coordination. Motor coordination was evaluated as previously reported,[16] by using a rotarod

apparatus (Ugo Basile, Italy) consisting of a bar with a diameter of 3.0 cm, subdivided into five compartments by a disk of 24 cm in diameter. The bar rotated at a constant speed of 16 rev/min. A preliminary selection of mice was made on the day of experiment excluding those that did not remain on the rotarod bar for two consecutive periods of 45 s each. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 45 s. The performance time was measured before and 15, 30, and 60 min after ip treatments.

4.2.7. Locomotor activity. In the locomotor activity evaluation[16] mice were randomly assigned to one experimental group and tested in an activity cage (Ugo Basile, Italy) in groups of four. The cage consisted of three parts: the animal cage, the electric unit, and the counter and recorder which were incorporated into the electric unit. The dimensions of the cage were $28 \times 32 \times 40$ cm. The sides were opaque and the ceiling transparent to allow observation. The floor consisted of stainless steel bars 3 mm in diameter and 11 mm apart. The odd bars were grounded and the even bars were active so that movements of the animals were recorded continuously on a counter as well as on paper. Temperature, sound, and light conditions were maintained constant during the course of the experiments. Measurements of locomotor activity following treatments were carried out at 10-min intervals and cumulative counts were recorded after 2 h. The animals were placed in the activity cage for at least 30 min before receiving the treatment so as to reduce the possible contribution of exploratory activity to the total count to a minimum.

4.2.8. Drug and treatment procedure. On each day of testing compounds were freshly dissolved in 0.9% NaCl solution for ip or sc administration and injected in a volume of 5 mL/kg. As necessary, solutions were prepared by dissolving the compound in dimethylsulfoxide; aliquots of this solution were used for subsequent dilution in saline (dimethylsulfoxide:saline 1:4 v/v) and were administered immediately after sonication.

4.2.9. Data analysis and statistics. Data obtained in the hot plate and in tail flick test were analyzed for each mouse as the mean of two latencies measured in second before drug administration for baseline latency (values recorded at 30 and 15 min before the first treatment) and as the mean of the six latencies measured in second after compounds, administration (values recorded at 15, 30, 45, 60, 75, and 90 min) and are reported as means ± SE. Writhing data were recorded as the mean number of writhes ± SE recorded during the 10-min observation period after acetic acid administration. Following, the animal responses were made quantal by defining as significant an individual reaction lower than 3 SD of the control mean reaction time of all the animals in the group for each dose. Six to 8 animals were used for each dose and at least 18 animals were used in each experiment. Dose–response curves were constructed from the percent of animals showing significant antinociception at various doses. The parallel line assay of Finney[31] was used to estimate the ED_{50} values and their 95% confidence intervals with the aid of a computer. Data obtained in the formalin test were analyzed as means of the total time in second the animal spent licking or biting the injected paw both in the first and in second phase after formalin injection and are reported as means ± SE. Data obtained in the rotarod experiments were analyzed as means of the number of falls from the rotating rod in 45 s measured before and 15, 30, and 60 min after **18a** treatment and are reported as means ± SE. Locomotor activity data were analysed as means of the cumulative counts recorded 2 h after **18a** administration and are reported as means ± SE. Data obtained in the experiments were statistically analyzed by using analysis of variance followed by Newman–Keul's procedure for verifying significance between two means. Statistical significance was always assumed at least at 5% level.

Supplementary data

^{1}H NMR spectral data for derivatives **9c–f**, **9h–j**, **10b**, **c** and **10e–h**, **11b–f**, **11h–s**, **12b–o**, **12q–s**, **16b**, **c**, **17a**, **b**, **18a**, **b**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.05.035.

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