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Further Studies on Arylpiperazinyl Alkyl Pyridazinones: Discovery of an Exceptionally Potent, Orally Active, Antinociceptive Agent in Thermally Induced Pain†

Claudio Biancalani,[†] Maria Paola Giovannoni,*[,†] Stefano Pieretti,[‡] Nicoletta Cesari,[†] Alessia Graziano,[†] Claudia Vergelli,[†] Agostino Cilibrizzi,[†] Amalia Di Gianuario,[‡] Mariantonella Colucci,[‡] Giorgina Mangano,[§] Beatrice Garrone,[§] Lorenzo Polenzani,[§] and Vittorio Dal Piaz[†]

[†]*Dipartimento di Scienze Farmaceutiche, via Ugo Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy,* [‡]*Department of Therapeutic Research and Medicine Evaluation, Viale Regina Elena 299, 00161 Roma, Italy, and* [§]*Angelini Research Center, Piazzale della Stazione, 00040 S. Palomba-Pomezia, Roma, Italy*

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A number of pyridazinone derivatives bearing an arylpiperazinylalkyl chain were synthesized and tested icv in a model of acute nociception induced by thermal stimuli in mice (tail flick). The most interesting and potent compound in this series was **6a**, which showed an $ED_{50} = 3.5\,\mu g$, a value about 3-fold higher with respect to morphine by the same route of administration. When administered per os, **6a** was 4-fold more potent than morphine in the same test, suggesting a significant bioavailability. The same compound also showed high potency in the hot plate test. The antinociceptive effect of **6a** was completely reversed by pretreatment with yohimbine both in the hot plate test and in the tail flick test. This demonstrated the involvement of the adrenergic system, which was confirmed by in vitro radioligand binding studies.

Introduction

Although rapid advances in medicinal chemistry research have greatly increased the options for analgesic therapy, traditional pain treatment, based on the use of NSAIDs and opioids, continues to dominate clinical analgesia. As is well-known, NSAIDs show adverse reactions at the gastrointestinal level together with inhibition of platelet aggregation and renal toxicity. Meanwhile, opioids induce severe side effects such as sedation, constipation, respiratory depression, and dependence.[1,2] In the 1990s, researchers discovered the two isoforms of cyclooxygenases, COX-1 and COX-2. It was initially observed that potent and selective COX-2 inhibitors had comparable analgesic potency with respect to the classic NSAIDs but a lower incidence of secondary effects.[3] This led to the development and commercialization of a large number of these selective inhibitors, collectively called "coxibs". Unfortunately, more recent studies have correlated an elevated risk of acute myocardial infarction with the use of COX-2 inhibitors[4,5] which remain a rational therapeutic approach for patients at high risk of serious gastrointestinal complications.[6] At present, a third class of analgesics, the "analgesic adjuvants", which are drugs with a different primary use, finds application for particular forms of pain. Neurophatic pain, for example, is particularly difficult to treat and is often refractory to conventional analgesics.[7] There is an active field of research into the numerous neuromodulators that are involved in complex pain-processing pathways (i.e., acetylcholine, adenosine, and adrenaline, NMDA, ion channels, and so on).[8−10] Thus, at the present, NMDA, mGLU1, Ca^{2+} channels, Na^{+} channels, and TRPV-1 are the most promising molecular targets for the treatment of neuropathic pain.[11]

Our work in the field of the analgesic agents has produced a large number of molecules, with pyridazine scaffold, showing an interesting level of antinociceptive potency.[12−21] We have synthesized two distinct series of compounds: one series is of 4-amino-5-vinyl-3(2*H*)-pyridazinones, for which we performed in-depth structure−activity relationship studies.[12−15,19−21] In this series, modifications at positions 2 and 6 of the pyridazine ring are well-tolerated, while an amino group or a low alkylamino group at position 4 is an essential requirement. Substitution of the vinyl group at position 5 led to inactive compounds, with the exception of the homologation (propenyl) and the introduction of a bromine at position β of the vinyl. But the most interesting modification performed at this position was the replacement of the vinyl group with five-membered heterocycles, which led to potent compounds, active per os and devoid of the potential toxicity related to the transformation of the vinyl group in epoxidic function in vivo.[19] Pretreatment of the above compounds with the α_2-antagonist yohimbine induced a complete reversal of the antinociceptive effect, suggesting involvement of the adrenergic system in the mechanism of analgesic action.

The second series includes a large number of pyridazinones and (hetero)condensed pyridazinones substituted with an arylpiperazinylalkyl moiety.[16−18] One of the most interesting terms showed an efficacy of 104% compared to morphine in the hot plate test and was able to completely abolish abdominal constrictions in the writhing test at the dose of 20 mg/kg sc[a].[17] The involvement of the adrenergic system and especially

†We are honored to contribute the present article to the special issue for the 100th anniversary of the Division of the Medicinal Chemistry that always worked for the diffusion of the more recent advances in Medicinal Chemistry.

*To whom correspondence should be addressed. Phone: +39-055-4573682. Fax: +39-055-4573671. E-mail: mariapaola.giovannoni@unifi.it.

[a] Abbreviations: ip, intra peritoneal; icv, intracerebral ventricular; sc, sub cutis; po, per os.

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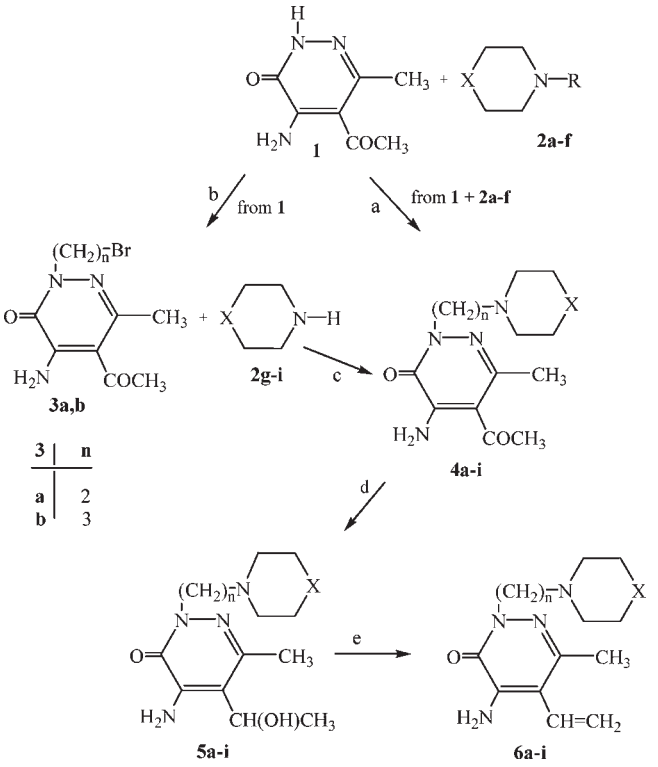
Scheme 1[a]



[a] (a) K_2CO_3, anhyd DMF, rt, 5−24 h; (b) Br-$(CH_2)_n$-Br, K_2CO_3 anhyd DMF rt, 2 h; (c) K_2CO_3, anhyd DMF, rt, 4−16 h; (d) $NaBH_4$, MeOH, rt, 0.5−3 h; (e) PPA 60−70 °C, 2−8 h.

indirect activation of the noradrenergic system through inhibition of noradrenaline reuptake was also demonstrated for this series.[18]

In this paper, we describe a new series of pyridazinones bearing an arylpiperazinylalkyl chain, designed and synthesized with the aim of completing the structure−activity relationship studies of this series. Analgesic activity was assessed by thermal nociceptive tests in mice. In addition, using a prototypical compound of the series, in vitro radioligand binding studies were performed on a panel of adrenergic receptors in order to define the pharmacological profile. These studies led us to identify compound **6a** as an exceptionally potent antinociceptive agent by oral route in the tail flick and hot plate tests.

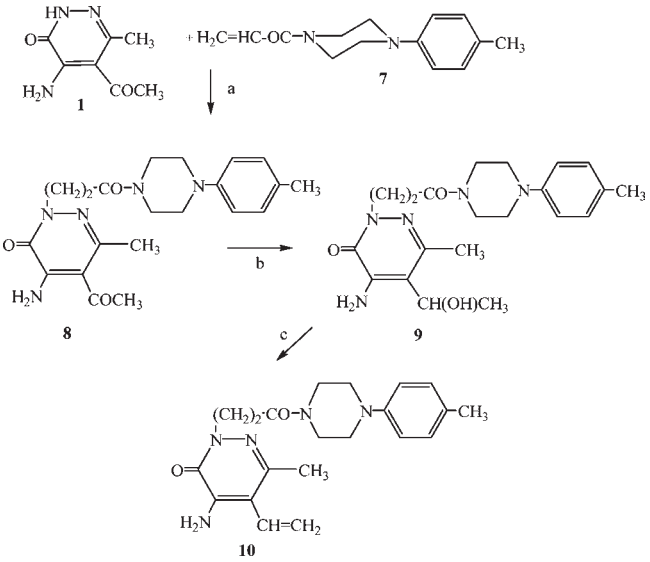
Chemistry

The synthetic pathways affording the final compounds **6a**−**i**, **10**, **16**, **20a**,**b**, **23**, **25a**−**c**, **30**, and **35** are reported in Schemes 1−8.

Scheme 1 shows the synthetic procedure, widely used in our laboratories, that produces the final 5-vinylpyridazinones **6a**−**i**. Compounds **4a**−**i**, which represent the key intermediates, were obtained starting from the 4-amino-5-acetylpyridazinone **1**,[22] following two different procedures:

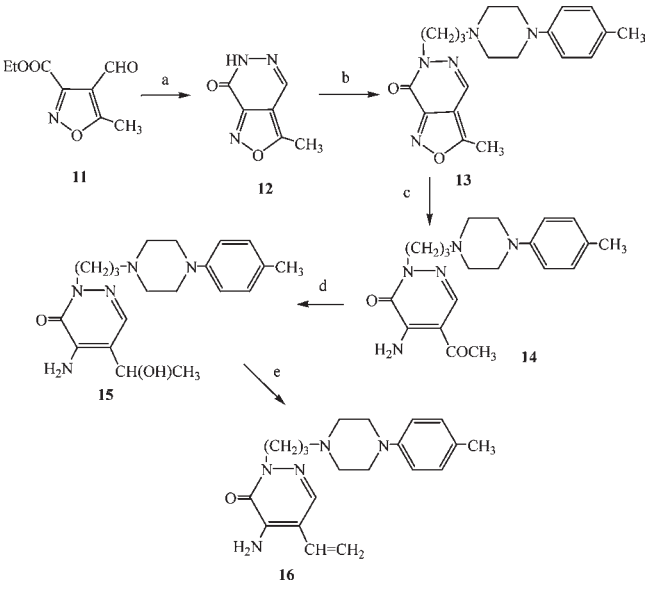
1. A direct alkylation of precursor **1** with the opportune arylpiperazinylpropyl bromide **2a**−**f** (route a, compounds **4a**−**f**, Table 1). Compounds **2a** and **2d** were previously described (**2a**,[16] **2d**[17]) while **2b**,**c**,**e**,**f** were prepared by condensing 1,3-dibromopropane with appropriate substituted-arylpiperazine in anhydrous acetone and K_2CO_3 at room temperature;
2. Two steps (route b,c compounds **4g**−**i**, Table 2), which consist of the synthesis of *N*-alkylbromide **3a**,**b** through a standard alkylation, followed by condensation with the appropriate aryl(alkyl)piperazine or piperidine in anhydrous DMF and K_2CO_3. Treatment of **4a**−**i** with sodium borohydride in anhydrous methanol afforded the secondary alcohols **5a**−**i**, which, in turn, were transformed into the 5-vinyl derivatives **6a**−**i** by dehydratation with polyphosphoric acid (PPA).

Scheme 2[a]



[a] (a) K_2CO_3, anhyd DMF, 60 °C, 3.5 h; (b) $NaBH_4$, MeOH, rt, 15 min; (c) PPA, 70 °C, 3 h.

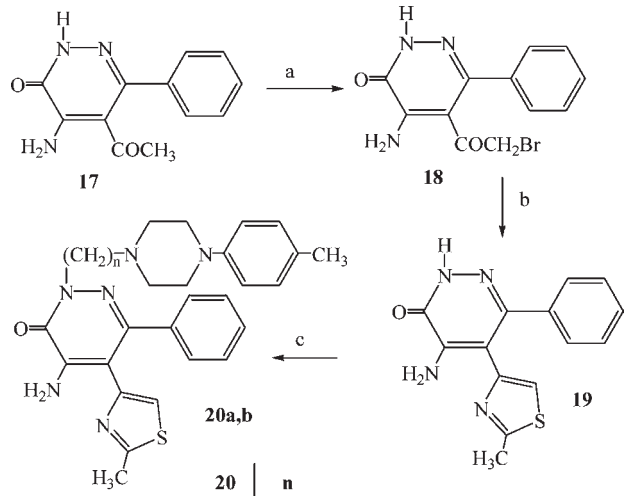
Scheme 3[a]



[a] (a) Hydrazine hydrate, EtOH, PPA, 70 °C, 30 min; (b) 1-(3-bromopropyl)-4-(*p*-tolyl)piperazine, K_2CO_3, anhyd DMF, 60°C, 4 h; (c) hydrazine hydrate, EtOH, 10% Pd/C, reflux, 1 h; (d) $NaBH_4$, MeOH, rt, 2 h; (e) PPA, 60 °C, 3 h.

Scheme 2 depicts a similar synthetic route for the final compound **10**. In this case, a basic side-chain was introduced through a Michael addition reaction using the 1-(4-*p*-tolylpiperazin-1-yl)propenone **7**, obtained from 1-*p*-tolylpiperazine and 3-chloropropanoyl chloride in THF and triethylamine. Then the 4-amino-5-acetylderivative **8** was converted into

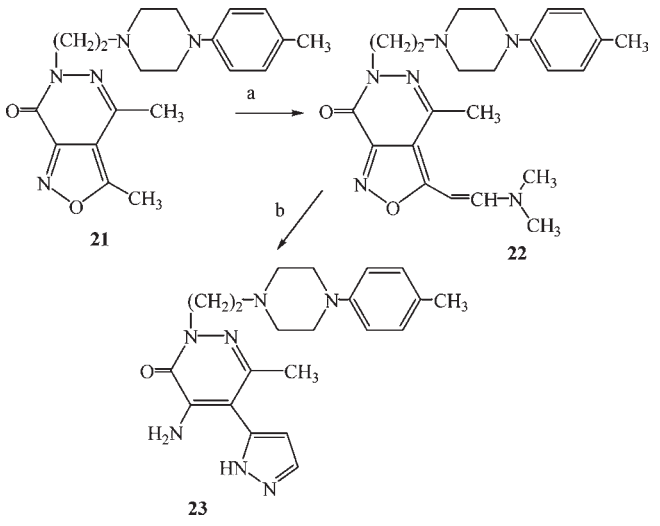
Scheme 4[a]



20	n
a	3
b	2

[a] (a) Br_2, AcOH, HBr, 50 °C, 5 h; (b) thioacetamide, anhyd EtOH, 80 °C, 5 h; (c) 1-(3-bromoalkyl)-4-(4-methylphenyl)-piperazine, K_2CO_3, anhyd DMF, rt, 24 h.

Scheme 5[a]



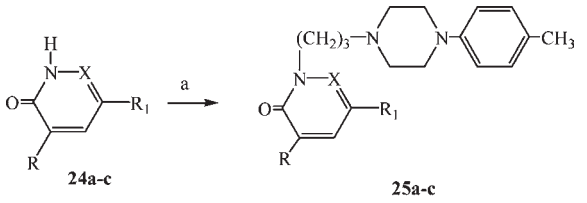
[a] (a) *N*,*N*-Dimethylformammide dimethylacetal, 100−110 °C, 3−4 h; (b) hydrazine hydrate, EtOH, 80 °C, 3 h.

final compound **10** using the same conditions described in Scheme 1.

For the synthesis of 4-amino-5-vinylpyridazinone **16** (Scheme 3), it was necessary to synthesize the 3-methylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one **12** starting from the isoxazole **11**[23] and hydrazine hydrate in PPA and ethanol. The intermediate **12** was alkylated with 3-bromopropyl-4-(*p*-tolyl)piperazine in standard conditions using K_2CO_3 in anhydrous DMF. Reductive cleavage with hydrazine hydrate and Pd/C in ethanol of the intermediate **13** gave rise to the corresponding 5-acetyl-4-aminopyridazinone **14**, which was transformed into the final **16** following the above-described two-step procedure.

Schemes 4 and 5 show the synthesis of 5-heterocyclic substituted pyridazinones. The 5-thiazolyl derivatives **20a**,**b** (Scheme 4) were prepared starting from the previously described compound **17**.[22] Treatment of **17** with bromine in acetic acid afforded product **18**, which, by treatment with thioacetamide in ethanol, gave rise to compound **19**. The final compounds **20a**,**b** were obtained by alkylation in standard conditions with the appropriate 1-(3-bromoalkyl)-4-(*p*-tolyl)-piperazine. The introduction of the pyrazole system at position 5 (Scheme 5) was performed by treatment of precursor **21**[16] with *N*,*N*-dimethylformamide dimethylacetal to afford the 3-dimethylaminovinyl derivative **22**, which, with hydrazine hydrate, gave rise to the final compound **23**.

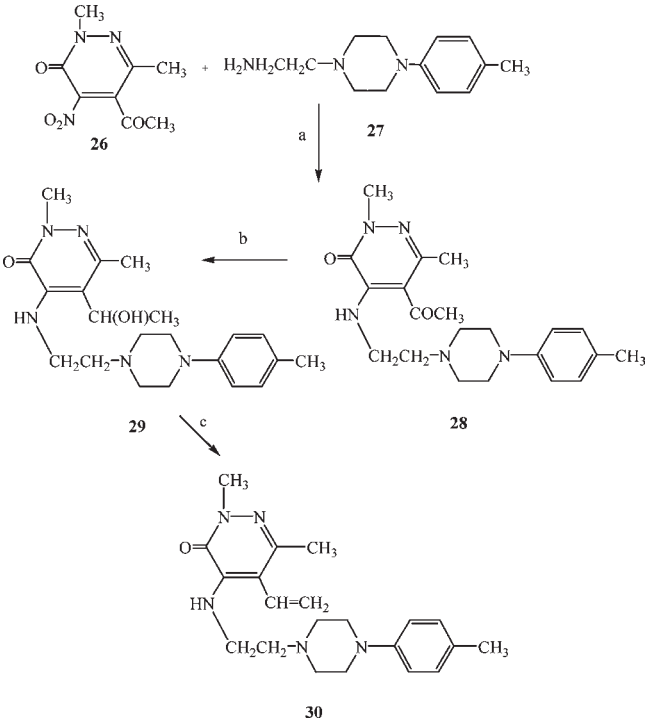
Scheme 6[a]



24, 25	R	X	R_1
a	H	N	CH_3
b	NH_2	N	CH_3
c	NH_2	CH	4-Py

[a] (a) 1-(3-Bromopropyl)-4-(4-methylphenyl)-piperazine, K_2CO_3, anhyd DMF, rt−60 °C, 2−24 h.

Scheme 7[a]

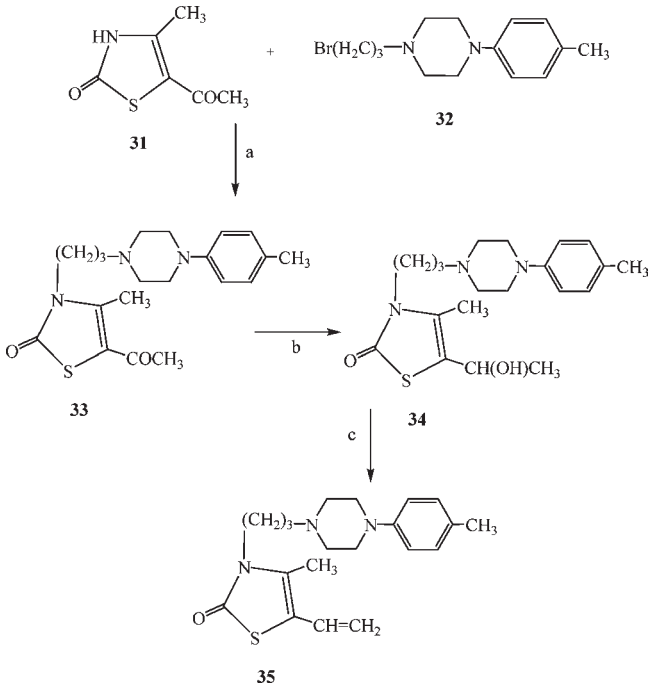


[a] (a) Anhyd EtOH, rt, 12 h; (b) $NaBH_4$, MeOH, rt, 20 min; (c) H_2SO_4 on silica gel, toluene, reflux, 4 h.

The synthetic step affording 5 and 4,5-unsubstituted final compounds (**25a**−**c**) is reported in Scheme 6. The reaction was carried out in standard conditions starting from the previously described[24−26] compounds **24a**−**c**.

Finally, with the synthesis of compound **30**, we obtained the shift of the alkylarylpiperazinyl chain from position 2 to position 4 (Scheme 7), whereas in compound **35**, we substituted the pyridazine scaffold with a thiazole ring bearing those fragments and functionalities important for activity (Scheme 8). Scheme 7

Scheme 8[a]



[a] (a) K_2CO_3, anhyd DMF, 40 °C, 2 h; (b) $NaBH_4$, MeOH, rt, 5 min; (c) H_2SO_4 on silica gel, toluene, reflux, 4 h.

Table 1. Legend for Compounds **2a**−**i**

compd **2**	R	X
a	$(CH_2)_3Br$	N−C_6H_4−CH_3 (p)
b	$(CH_2)_3Br$	N−C_6H_4−OCH_3 (p)
c	$(CH_2)_3Br$	N−C_6H_4−Cl (p)
d	$(CH_2)_3Br$	N−C_6H_4−Cl (m)
e	$(CH_2)_3Br$	N−C_6H_4−OCH_3 (m)
f	$(CH_2)_3Br$	N−C_6H_4−CH_3 (m)
g		$CHCH_3$
h		NCH_3
i		N−C_6H_4−OCH_3 (p)

depicts the synthesis of compound **30** for which the starting product is represented by the 4-nitropyridazinone **26**.[27] Nucleophilic displacement of the nitro group with the amine **27**[28] afforded the 4-substituted aminopyridazinone **28**, which underwent the same synthetic procedures reported in the previous schemes. Similarly, the thiazolone **35** was synthesized following the three-step procedure (alkylation, reduction, and dehydratation) reported for all the other final compounds, starting from product **31**.[29] In this case, the transformation of hydroxyethyl derivative **34** into the corresponding 5-vinyl derivatives **35** was performed using sulfuric acid adsorbed on silica gel following a method described in the literature.[30]

Results and Discussion

In the present study, the antinociceptive activity of the investigated compounds was first evaluated after central administration in a model of acute nociception induced by thermal stimuli in mice. All compounds were administered in the cerebral ventricle, and their activity was compared with that of morphine (Tables 3 and 4). Compound **6a** strongly increased the nociceptive threshold to thermal stimuli showing a potency in the tail flick test 3-fold lower with respect to the reference compound morphine, the most important analgesic used in humans. However, **6h**, **25b**, **25c**, and **35** were

Table 2. Legend for Compounds **4**−**6a**−**i**

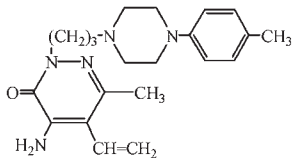
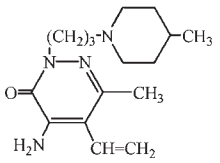
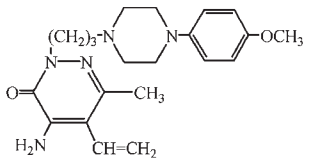
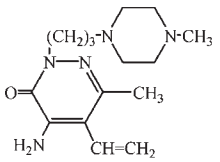
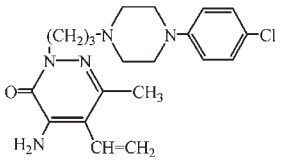
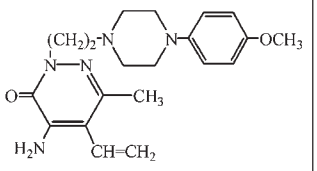
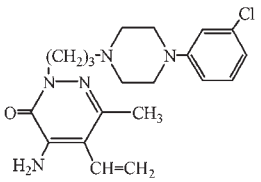
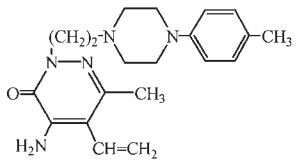
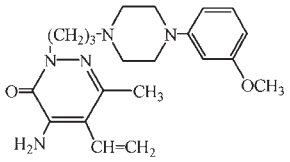
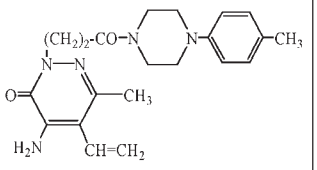
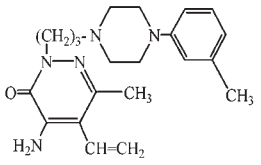
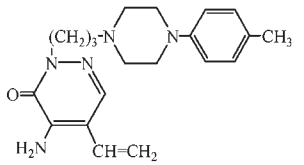
compd **4**−**6**	X	*n*
a	N−C_6H_4−CH_3 (p)	3
b	N−C_6H_4−OCH_3 (p)	3
c	N−C_6H_4−Cl (p)	3
d	N−C_6H_4−Cl (m)	3
e	N−C_6H_4−OCH_3 (m)	3
f	N−C_6H_4−CH_3 (m)	3
g	$CHCH_3$	3
h	NCH_3	3
i	N−C_6H_4−OCH_3 (p)	2

able to exhibit good antinociceptive activity because their ED_{50}s were less than 10 μg. The antinociceptive effect induced by these last compounds, although lower than those observed after morphine administration, was in any case interesting because they showed potency and efficacy comparable to that demonstrated by other analgesic drugs clinically employed such as diphenhydramine, baclofen, amitriptyline, etc.[31] Compounds **6b**, **6c**, **6i**, **23**, and **25a** showed ED_{50}s in the range of 10−20 μg, and compounds **6d**, **16**, **20b**, and **30** showed ED_{50}s in the range of 20−25 μg. ED_{50}s calculated for **6g**, **10**, and **20a** were higher than 25 μg and were significantly different from the ED_{50} for **6a**.

After the results obtained from the above experiments, the antinociceptive activity of **6a** was further investigated and demonstrated after oral administration to mice which underwent the hot plate and tail flick tests (Table 5). These effects were evident at low doses and the ED_{50} for **6a** was several fold lower than the morphine ED_{50} in both thermal nociceptive tests. These findings clearly indicate that **6a** is endowed with exceptionally potent antinociceptive activity and its bioavailability is significantly better than that of morphine. Previously we demonstrated that the analgesia induced by structurally related compounds[19] was completely prevented by pretreatment with the α_2-antagonist yohimbine. To clarify whether the α_2-adrenoceptor was involved in the mechanism of action of **6a**, we pretreated mice with yohimbine as antagonist. The dose of yohimbine employed to prevent the analgesia induced by the above-reported compound is the minimal dose able to antagonize antinociception induced by activation of α_2-adrenoceptor, as demonstrated by the block exerted on amitriptyline and imipramine antinociception.[32] A significant reversal of antinociceptive effect of compound **6a** by the α_2-antagonist yohimbine (2 mg kg^{-1} ip) was evidenced both in the hot plate and in the tail flick test (Table 5). In our experimental conditions, the α_2-antagonist yohimbine did not modify the pain threshold of mice in comparison with control animals. The lack of effect of this antagonist agrees with results of studies in which this compound did not modify the nociceptive threshold against both thermal (hot-plate) and chemical (writhing) noxious stimuli.[33] We can therefore exclude that prevention of the antinociception induced by the assayed compounds was due to a hyperalgesic effect of the α_2-adrenoceptor antagonist used.

Our further experiments performed to verify the involvement of α_1-receptors evidenced that the α_1-antagonist prazosin at both doses (0.1 and 1 mg/kg ip) administered 15 min before **6a** (1 mg/kg po) was able to potentiate **6a**-induced antinociception, increasing latencies to cutoff values from 30 to 120 min after **6a** administration. These last results might suggest that **6a** behaves also as an α_1-agonist. It was recently demonstrated, using a genetic and pharmacological approach, that α_1-adrenergic receptor agonist activity might

Table 3. Antinociceptive Effect of Final Compounds **6a**−**j**, **10**, **16** in the Tail Flick Test (icv)

Comp.	Structure	ED_{50} μg	Comp.	Structure	ED_{50} μg[a]
6a MW=367		3.5 (0.9-12.8)	**6g** MW= 290		26.4 (8.6-81.0)*^
6b MW= 383		12.6 (3.4-46.9)*	**6h** MW= 291		8.7 (3.7-20.6)*
6c MW= 388		15.4 (5.4-43.6)*	**6i** MW= 369		11.9 (4.9-29.6)*
6d MW= 388		23.7 (2.2-258.9)*	**6j**[b] MW= 353		26.1 (7.88-86.36)*
6e MW= 383		72.6 (12.9-406.2)*	**10** MW= 381		31.8 (8.4-119.5)*
6f MW= 325		51.55 (11.35-234.5)*	**16** MW= 353		25.1 (3.5-179.3)
Morphine		1.1 (0.4-2.9)			

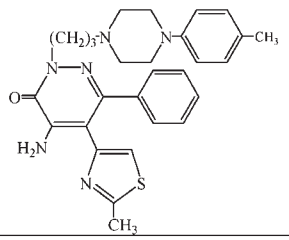
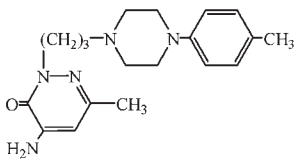
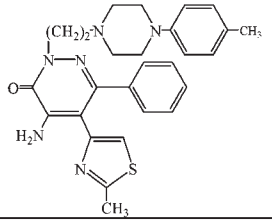
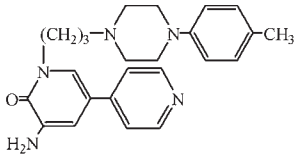
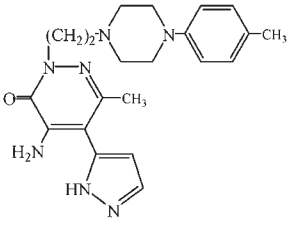
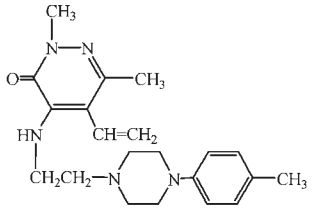
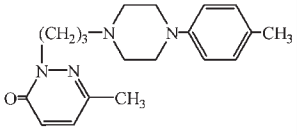
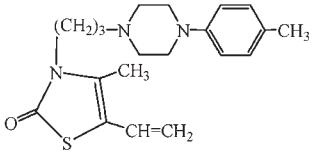
[a] At least four groups of six mice were used to generate dose−response curves and to estimate ED_{50} values. ED_{50s} and their significant differences were calculated with the aid of a computer program.[41] Statistical significance was assumed at $P < 0.05$. * is for a significant difference vs morphine; ^ is for a significant difference vs 6a. [b] Reference 16.

reduce α_2-mediated antinociception when the same compound is both an α_1 and an α_2 agonist.[34] Finally, to confirm the mechanism of **6a** action involving adrenergic receptors, we administered the opioid-antagonist naloxone, the $GABA_B$-antagonist CGP35348, and the nicotinic antagonist mecamylamine at doses reported to antagonize the antinociception induced by opioid, $GABA_B$, or nicotinic receptor activation before **6a**.[14] Neither naloxone nor CGP35348 or mecamylamine were able to reduce **6a** effects on thermal nociceptive threshold either in the hot plate or in the tail flick test.

To further verify the pharmacological profile of compound **6a**, in vitro radioligand binding studies were performed on a panel of adrenergic receptors (Table 6). Compound **6a** showed selectivity for alpha adrenergic receptors α_{1A}, α_{1B}, and α_{2A}, with IC_{50} values in the range 56−320 nM, these data confirming an involvement of the adrenergic system in the analgesia produced by **6a**. On the contrary, evaluation of binding to subtype α_{2C}, also involved in the modulation of antinociception,[35] clearly demonstrated that compound **6a** showed low affinity for α_{2C} in comparison with the other examined subtypes, the inhibition being 71% at 10 μM.

SAR studies were performed by analyzing the results obtained after icv administration, where differences in pharmacokinetic properties among different compounds have a

Table 4. Antinociceptive Effect of Final Compounds **20a**,**b**, **23**, **25a**−**c**, **30**, **35** in the Tail Flick Test (icv)

Comp.	Structure	ED_{50} μg	Comp.	Structure	ED_{50} μg[a]
20a MW =500		48.3 (11.9-196.6)*^	**25b** MW =341		4.9 (1.7-14.5)*
20b MW =486		25.1 (3.5-179.3)*	**25c** MW =403		7.6 (1.8-31.6)*
23 MW =393		16.9 (2.6-110.5)*	**30** MW =367		24.1 (3.5-179.2)*
25a MW =326		13.8 (2.9-63.9)*	**35** MW =357		5.1 (0.3-88.1)*
	Morphine	1.1 (0.4-2.9)			

[a] At least four groups of six mice were used to generate dose−response curves and to estimate ED_{50} values. ED_{50s} and their significant differences were calculated with the aid of a computer program.[41] Statistical significance was assumed at $P < 0.05$. * is for a significant difference vs morphine; ∧ is for a significant difference vs 6a.

Table 5. Effect of Naloxone, CGP, Mecamilamine, and Yohimbine on Antinociception Induced by Compound **6a** in Hot Plate and Tail Flick Test

treatment[a]	hot plate, ED_{50} mg/kg[b]	tail flick, ED_{50} mg/kg[b]
morphine	6.9 (2.7−17.2)	3.5 (1.4−8.9)
6a	0.5 (0.1−3.3)	0.8 (0.1−6.7)
6a + NAL	1.1 (0.3−5.9)	1.2 (0.3−5.8)
6a + CGP	0.6 (0.1−5.9)	0.5 (0.02−13.0)
6a + MEC	0.7 (0.05−6.7)	2.3 (0.3−16.0)
6a + YOH	8.6 (0.8−91.7)*	6.1 (0.9−42.6)*

[a] In some experiments, **6a** was administered po alone or 30 min after ip treatment with naloxone (NAL, 1 mg/kg), CGP 35348 (CGP, 50 mg/kg), mecamylamine (MEC, 2 mg/kg), or yohimbine (YOH, 2 mg/kg) and the relative ED_{50} was then calculated. Morphine was administered po. [b] At least four groups of six mice were used to generate dose−response curves and to estimate ED_{50} values. ED_{50s} and their significant differences were calculated with the aid of a computer program.[41] Statistical significance was assumed at $P < 0.05$. * is for a significant difference vs **6a**; ∧ is for a significant difference vs morphine

reduced impact on the activity. The most potent compound was **6a** (ED_{50} = 3.5 μg). By comparing **6a** with compound **16**, where the methyl group in position 6 of the heterocycle was eliminated, we found that the nor derivative had strongly reduced activity (ED_{50} = 25.1 μg), suggesting that the presence of a small lipophilic group in this part of the molecule is an important structural requirement for antinociceptive activity. The nature and length of the arylpiperazinylpropyl side chain in position 2 also plays a relevant role for the activity. Thus, replacement of the CH_2 attached at piperazine N_1 with a CO group (compound **10**) was associated with about a 10-fold reduction in activity. This finding strongly suggests that nitrogen is an essential requirement of this piperazine, its ability being to be protonated at a physiological pH critical for activity. Likewise, replacement of the *p*-tolylpiperazine with methylpiperidine (compound **6g**) led to similar results, suggesting a fundamental role also for piperazine N_4. This hypothesis was clearly confirmed by examining the behavior

Table 6. Binding Experiments Performed on **6a**

assay ID[a]	species	IC_{50} (nM)	$E_{max10\ \mu M}$, %	reference compound	IC_{50} (nM)
adrenergic α_{1A}	rat	56	101	prazosin	0.254
adrenergic α_{1B}	rat	102	99	prazosin	0.190
adrenergic α_{2A}	human	320	100	yohimbine	3.67
adrenergic α_{2C}	human	nc[b]	71	yohimbine	27.1

[a] All the procedures were performed according to standard radioligand binding technique. The α_{1A}-adrenoceptor and α_{1B}-adrenoceptor binding assays were performed on Wistar rat submaxillary gland and Wistar rat liver membrane extracts, respectively, using 0.25 nM [3H]-prazosin as radioligand. α_{2A}-adrenoceptor binding assay was performed on human recombinant insect Sf9 cell extracts using 1nM of [3H]-MK-912 as radioligand. The compound was dissolved in dimethyl sulfoxide (DMSO) and diluted in incubation buffer, then tested in duplicate at 1 μM concentration. [b] α_{2C} IC_{50} was not calculated and maximal inhibition effect at the highest tested concentration ($E_{max10\ \mu M}$) is reported.

of **6h**, where the piperazine N_4 basicity was restored. This compound's activity (ED_{50} = 8.7 μg) was at a level comparable to **6a**. The nature of the substituent in the phenyl group was also critical. Thus, replacement of CH_3 with Cl was associated with reduced activity (compound **6c**, ED_{50} = 15.4 μg) as well as the substitution with a methoxy group (**6b**), which led to a compound 3-fold less potent than **6a**. When the above-mentioned groups (chlorine, methoxy, and methyl) were inserted at the meta position of the phenyl ring, we observed reduced activity as demonstrated by ED_{50} values which are 23.7 μg for **6d**, 72.6 μg for **6e**, and 51.5 μg for **6f**.

Finally, to evaluate the importance of the length of the alkyl chain, we tested compound **6j**, the lower homologue of **6a**, which we have previously described.[16] This compound, which showed potent activity in the writhing test by sc administration to mice (ED_{50} = 2.5 mg/kg)[16] when tested in the tail flick test (icv), is more than 7-fold less potent than **6a**. This trend was not observed for the methoxy derivative because the lower homologue of **6b** (compound **6i**) showed the same activity. These data indicate that antinociceptive activity is significantly affected by electronic and/or steric properties of the substituent and the related position on the aromatic ring.

Compounds **20a**−**b** and **23** were synthesized as metabolically more stable analogues of **6a** in which the vinyl group in position 5 was replaced with five-membered heterocycles as possible bioisosters. We applied this type of structural modification successfully to a series of structurally related pyridazinones.[19] In this case, we found that the thiazole derivatives **20a** and **20b** were almost devoid of antinociceptive activity (ED_{50} = 48.3 and 25.1 μg, respectively), whereas the pyrazole **23** showed weak activity (ED_{50} = 16.9 μg). When we analyzed the role of the amino and the vinyl groups at positions 4 and 5, interesting results emerged: compound **25b**, where the vinyl group was eliminated, showed antinociceptive activity at a similar level as **6a** (ED_{50} = 4.9 μg), while when both the substituents were eliminated (compound **25a**), a reduction in activity was observed (ED_{50} = 13.8 μg). The analogue with the pyridine scaffold **25c** exhibited comparable activity (ED_{50} = 7.6 μg), suggesting that the presence of the NH_2 in position 4 is more crucial to activity than the $CH{=}CH_2$ group in position 5. To evaluate the role played by the heterocyclic scaffold, we synthesized the thiazolone **35** in which the side chain, and the $CH{=}CH_2$ of **6a** were inserted in a different heterocycle. This compound showed almost the same potency as **6a** (ED_{50} = 5.1 μg). This result disagrees with that found in the pyridazinone series, where the role played by the vinyl group appeared less important compared with that of NH_2. On the other hand, as previously observed, the side-chain also contributes to the antinociceptive activity because **25a**, lacking both the functional groups, maintains a significant level of activity (ED_{50} = 13.8 μg). Better interaction of the thiazolone system with the biological target counterbalances the absence at the NH_2, which might explain the good antinociceptive effect observed for **25a**. Finally, compound **30**, where a side chain similar to that of **6a** at position 4 and a methyl group at position 2 of the pyridazinone were inserted, showed low activity (ED_{50} = 24.1 μg).

In conclusion, we have identified the very potent antinociceptive agent **6a**, whose structure is characterized by a 6-methylpyridazinone scaffold substituted with a *p*-tolylpiperazinylpropyl side chain at position 2, a primary amino group at position 4, and a vinyl group at position 5. This compound was several times more potent than morphine both in the tail flick and hot plate test when orally administered to mice. SAR studies performed on this prototype showed that in this series the above side chain and NH_2 are essential requirements for the activity of **6a**.

Experimental Section

Chemistry. All melting points were determined on a Buchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Avance 400 instruments (Bruker Biospin version 002 with SGU). 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. 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. Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for C, H, N and the results were within ±0.4% of the theoretical values, unless otherwise stated. Reagents and starting materials **2e**−**g** were commercially available.

General procedure for 2b,c,e,f (2a,[16] 2d[17]). To a suspension of 1,3-dibromopropane (3.0 mmol) and anhydrous K_2CO_3 (3.9 mmol) in anhydrous acetone, a solution of appropriate commercially available (substituted)phenylpiperazine (1.9 mmol), in anhydrous acetone was slowly added. The suspension was stirred for 3−4 h at room temperature. After evaporation of the solvent and dilution with cold water (20−30 mL), the mixture was extracted with CH_2Cl_2 (3 × 20 mL) and the solvent was evaporated in vacuo to afford compounds **2b**,**c**,**e**,**f**, which were purified by flash chromatography using cyclohexane/ethyl acetate 1:1 as eluent for compound **2b**, cyclohexane/ethyl acetate 1:2 for compound **2c**,**e**, and cyclohexane/ethyl acetate 2:1 for compound **2f**.

1-(3-Bromopropyl)-4-(4-methoxyphenyl)piperazine 2b. Yield = 21%; mp = 55−58 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 2H, $BrCH_2\underline{CH_2}CH_2N$), 2.55 (t, 2H, $BrCH_2CH_2\underline{CH_2}N$), 2.55−2.65 (m, 4H, piperazine), 3.05−3.15 (m, 4H, piperazine), 3.50 (t, 2H, $Br\underline{CH_2}CH_2CH_2N$), 3.75 (s, 3H, OCH_3), 6.85 (d, 2H, Ar), 6.90 (d, 2H, Ar). Anal. ($C_{14}H_{21}BrN_2O$) C, H, N.

1-(3-Bromopropyl)-4-(4-chlorophenyl)piperazine 2c. Yield = 70%; oil. ^{1}H NMR ($CDCl_3$) δ 2.10−2.20 (m, 2H, $BrCH_2\underline{CH_2}CH_2N$), 2.60−2.70 (m, 2H, $BrCH_2CH_2\underline{CH_2}N$), 2.65−2.80 (m, 4H, piperazine), 3.20−3.30 (m, 4H, piperazine), 3.50 (t, 2H, $Br\underline{CH_2}CH_2CH_2N$), 6.85 (d, 2H, Ar), 7.20 (d, 2H, Ar). Anal. ($C_{13}H_{18}BrClN_2$) C, H, N.

1-(3-Bromopropyl)-4-(3-methoxyphenyl)piperazine 2e. Yield = 35%; oil. ^{1}H NMR ($CDCl_3$) δ 2.11 (m, 2H, $BrCH_2\underline{CH_2}CH_2N$), 2.59 (m, 2H, $BrCH_2CH_2\underline{CH_2}N$), 2.65 (m, 4H, piperazine), 3.23 (m, 4H, piperazine), 3.51 (t, 2H, $Br\underline{CH_2}CH_2CH_2N$), 3.81 (s, 3H, OCH_3), 6.44 (d, 2H, Ar), 6.48 (s, 1H, Ar), 6.55 (s, 1H, Ar), 7.19 (t, 1H, Ar). Anal. ($C_{14}H_{21}BrN_2O$) C, H, N.

1-(3-Bromopropyl)-4-(3-methylphenyl)piperazine 2f. Yield = 35%; oil. ^{1}H NMR ($CDCl_3$) δ 2.10 (quint, 2H, $BrCH_2\underline{CH_2}CH_2N$), 2.35 (s, 3H, CH_3), 2.57 (t, 2H, $BrCH_2CH_2\underline{CH_2}N$), 2.64 (t, 4H, piperazine), 3.22 (t, 4H, piperazine), 3.52 (t, 2H, $Br\underline{CH_2}$-CH_2CH_2N), 6.68−6.75 (m, 2H, Ar), 6.78 (s, 1H, Ar), 7.19 (t, 1H, Ar). Anal. ($C_{14}H_{21}BrN_2$) C, H, N.

General Procedure for 3a,b. To a suspension of the appropriate dibromoalkane (2.6 mmol) and K_2CO_3 (3.5 mmol) in anhydrous DMF (2 mL) a solution of **1**[22] (1.7 mmol) in anhydrous DMF was slowly added. The mixture was stirred at room temperature for 2 h. After dilution with cold water, the suspension was extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the final compounds **3a**,**b**, which were purified by column chromatography using cyclohexane/ethyl acetate 1:1 as eluent for **3b** and the mixture petroleum ether/Et_2O/$CHCl_3$/abs EtOH/NH_4OH 10:4:4:1:0.5 for compound **3a**.

5-Acetyl-4-amino-2-(2-bromoethyl)-6-methylpyridazin-3(2H)-one 3a. Yield = 25%; mp = 102−104 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, 6-CH_3), 2.60 (s, 3H, $COCH_3$), 3.70 (t, 2H, $NCH_2\underline{CH_2}Br$), 4.50 (t, 2H, $N\underline{CH_2}CH_2Br$), 7.80 (exch br s, 2H, NH_2). Anal. ($C_9H_{12}BrN_3O_2$) C, H, N.

5-Acetyl-4-amino-2-(3-bromopropyl)-6-methylpyridazin-3(2H)-one 3b. Yield = 20%; mp = 220−223 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.35−2.45 (m, 2H, $NCH_2\underline{CH_2}CH_2Br$), 2.55 (s, 3H, 6-$CH_3$), 2.60 (s, 3H, $COCH_3$), 3.45 (t, 2H, $NCH_2CH_2\underline{CH_2}Br$), 4.25 (t, 2H, $N\underline{CH_2}CH_2CH_2Br$), 7.80 (exch br s, 2H, NH_2). Anal. ($C_{10}H_{14}BrN_3O_2$) C, H, N.

General Procedure for 4a−f. A mixture of 5-acetyl-4-aminopyridazin-3(2H)-one **1**[22] (0.84 mmol), anhydrous K_2CO_3 (1.7 mmol), and the appropriate 1-(3-bromoalkyl)(substituted)arylpiperazine **2a**−**f**, (**2a**[16] and **2d**[17]) (0.84 mmol) in anhydrous DMF (1−2 mL) was stirred for 5−24 h at room temperature. After dilution with cold water (20−30 mL), the crude precipitate was recovered by suction.

5-Acetyl-4-amino-6-methyl-2-[3-(4-*p*-tolylpiperazin-1-yl)-propyl]pyridazin-3(2*H*)-one 4a. Yield = 56%; mp = 138−140 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.05−2.20 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.50 (s, 3H, 6-$CH_3$), 2.60 (s, 3H, $COCH_3$), 2.65−2.85 (m, 6H: 2H, $CONCH_2CH_2\underline{CH_2}N$ and 4H, piperazine), 3.15−3.35 (m, 4H, piperazine), 4.20 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 7.75 (exch br s, 2H, NH_2). Anal. ($C_{21}H_{29}N_5O_2$) C, H, N.

5-Acetyl-4-amino-2-{3-[4-(4-methoxyphenyl)piperazin-1-yl]-propyl}-6-methylpyridazin-3(2*H*)-one 4b. Yield = 65%; mp = 142−144 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.05−2.15 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.50 (s, 3H, 6-$CH_3$), 2.55 (s, 3H, $COCH_3$), 2.60 (m, 2H, $CONCH_2CH_2\underline{CH_2}N$), 2.65−2.75 (m, 4H, piperazine), 3.10−3.20 (m, 4H, piperazine), 3.80 (s, 3H, OCH_3), 4.20 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 6.85 (d, 2H, Ar), 6.95 (d, 2H, Ar), 7.80 (exch br s, 2H, NH_2). Anal. ($C_{21}H_{29}N_5O_3$) C, H, N.

5-Acetyl-4-amino-2-{3-[4-(4-chlorophenyl)-piperazin-1-yl]propyl}-6-methylpyridazin-3(2*H*)-one 4c. Yield = 93%; mp = 159−161 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.05−2.15 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.55 (s, 3H, 6-$CH_3$), 2.60−2.75 (m, 9H: 2H, $CONCH_2CH_2\underline{CH_2}N$ and 4H, piperazine and 3H, $COCH_3$), 3.15−3.25 (m, 4H, piperazine), 4.20 (t, 2H, $CON\underline{CH_2}$-CH_2CH_2N), 6.85 (d, 2H, Ar), 7.20 (d, 2H, Ar), 7.75 (exch br s, 2H, NH_2). Anal. ($C_{20}H_{26}ClN_5O_2$) C, H, N.

5-Acetyl-4-amino-2-{3-[4-(3-chlorophenyl)-piperazin-1-yl]propyl}-6-methylpyridazin-3(2H)-one 4d. Yield = 76%; mp = 138−140 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.00−2.15 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.50 (s, 3H, 6-$CH_3$), 2.50−2.60 (m, 2H, $CONCH_2CH_2\underline{CH_2}N$), 2.65 (s, 3H, $COCH_3$), 2.65−2.75 (m, 4H, piperazine), 3.15−3.25 (m, 4H, piperazine), 4.20 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 6.75−6.85 (m, 2H, Ar), 6.90 (m, 1H, Ar), 7.20 (m, 1H, Ar), 7.75 (exch br s, 2H, NH_2). Anal. ($C_{20}H_{26}ClN_5O_2$) C, H, N.

5-Acetyl-4-amino-2-{3-[4-(3-methoxyphenyl)-piperazin-1-yl]propyl}-6-methylpyridazin-3(2H)-one 4e. Yield = 75%; mp = 111−113 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.08 (m, 2H, $CON\underline{CH_2}CH_2CH_2N$), 2.53 (s, 3H, 6-$CH_3$), 2.59 (s, 3H, $COCH_3$), 2.60−2.73 (m, 6H (2H, $CONCH_2CH_2\underline{CH_2}N$; 4H, piperazine)), 3.23 (m, 4H, piperazine), 3.81 (s, 3H, OCH_3), 4.18 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 6.44 (d, 1H, Ar), 6.48 (s, 1H, Ar), 6.55 (d, 1H, Ar), 7.19 (t, 1H, Ar), 7.76 (exch br s, 2H, NH_2). Anal. ($C_{21}H_{29}N_5O_3$) C, H, N.

5-Acetyl-4-amino-2-{3-[4-(3-methoxyphenyl)-piperazin-1-yl]-propyl}-6-methylpyridazin-3(2H)-one 4f. Yield = 85%; mp = 133−134 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.06−2.20 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.33 (s, 3H, CH_3−Ar), 2.50−2.60 (m, 2H, $CONCH_2CH_2\underline{CH_2}N$), 2.53 (s, 3H, 6-$CH_3$), 2.59 (s, 3H, $COCH_3$), 2.61−2.72 (m, 4H, piperazine), 3.15−3.33 (m, 4H, piperazine), 4.18 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 6.68−6.72 (m, 2H, Ar), 6.76 (s, 1H, Ar), 7.17 (t, 1H, Ar), 7.75 (exch br s, 2H, NH_2). Anal. ($C_{21}H_{28}N_5O_2$) C, H, N.

General Procedure for 4g−i. To a suspension of **3a**,**b** (0.43 mmol) and K_2CO_3 (0.86 mmol) in anhydrous DMF, the appropriate (substituted)cycloalkylamine **2g**−**i**, commercially available, (0.43−0.86 mmol) was added. The mixture was stirred for 4−16 h at room temperature. After dilution with cold water (20−30 mL), the suspension was extracted with CH_2Cl_2 (3 × 15 mL) and the solvent was evaporated in vacuo to afford compounds **4g**−**i**, which were purified by column chromatography (eluents: $CHCl_3$/CH_3OH 9:1 for compounds **4g**,**h** and the mixture petroleum ether/Et_2O/$CHCl_3$/abs EtOH/NH_4OH 10:4:4:1:0.5 for compound **4i**).

5-Acetyl-4-amino-6-methyl-2-[3-(4-methylpiperidin-1-yl)propyl]pyridazin-3(2H)-one 4g. Yield = 38%; mp = 96−98 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 0.90−1.00 (m, 3H, $CH\underline{CH_3}$), 1.40−1.50 (m, 3H, piperidine), 1.60−1.70 (m, 2H, piperidine), 2.05−2.20 (m, 4H: 2H, $CONCH_2\underline{CH_2}CH_2N$ and 2H, piperidine), 2.50 (s, 3H, 6-CH_3), 2.50−2.60 (m, 2H, $CON\underline{CH_2}CH_2\underline{CH_2}N$), 2.60 (s, 3H, $COCH_3$), 3.00−3.10 (m, 2H, piperidine), 4.15 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 7.75 (exch br s, 2H, NH_2). Anal. ($C_{16}H_{26}N_4O_2$) C, H, N.

5-Acetyl-4-amino-6-methyl-2-[3-(4-methylpiperazin-1-yl)propyl]pyridazin-3(2H)-one 4h. Yield = 13%; mp = 98−100 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.95−2.10 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.40 (s, 3H, NCH_3), 2.50 (s, 3H, 6-CH_3), 2.60 (s, 3H, $COCH_3$), 2.60−2.75 (m, 10H: 8H, piperazine and 2H, $CONCH_2CH_2\underline{CH_2}N$), 4.15 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 7.75 (exch br s, 2H, NH_2). Anal. ($C_{15}H_{25}N_5O_2$) C, H, N.

5-Acetyl-4-amino-2-{2-[4-(4-methoxyphenyl)piperazin-1-yl]-ethyl}-6-methylpyridazin-3(2H)-one 4i. Yield = 42%; mp = 135−137 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, 6-CH_3), 2.60 (s, 3H, $COCH_3$), 2.70−2.80 (m, 4H, piperazine), 2.80−2.90(m, 2H, $CONCH_2\underline{CH_2}N$), 3.05−3.15 (m, 4H, piperazine), 3.80 (s, 3H, OCH_3), 4.30 (t, 2H, $CON\underline{CH_2}CH_2N$), 6.85 (d, 2H, Ar), 6.90 (d, 2H, Ar), 7.75 (exch br s, 2H, NH_2). Anal. ($C_{20}H_{27}N_5O_3$) C, H, N.

General Procedure for 5a−i. Sodium borohydride (2−5.5 mmol) was added portionwise to a stirred solution of appropriate 4-amino-5-acetyl derivatives **4a**−**i** (0.37−0.47 mmol) in CH_3OH (5−7 mL). The reaction mixture was stirred for 0.5−3 h at room temperature. After concentration in vacuo, the residue was diluted with ice water (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded final compounds **5a**−**i**.

4-Amino-5-(1-hydroxyethyl)-6-methyl-2-[3-(4-*p*-tolylpiperazin-1-yl)propyl]pyridazin-3(2*H*)-one 5a. Yield = 88%; mp = 176−178 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3H, CH-(OH)$\underline{CH_3}$), 2.05−2.15 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.20 (s,

3H, 6-CH_3), 2.30 (s, 3H, Ph-CH_3), 2.50−2.60 (m, 2H, CON-$CH_2CH_2CH_2$N), 2.65−2.75 (m, 4H, piperazine), 2.85 (exch br s, 1H, OH), 3.10−3.25 (m, 4H, piperazine), 4.15 (t, 2H, CON-$CH_2CH_2CH_2$N), 5.00 (q, 1H, CH(OH)CH_3), 5.80 (exch br s, 2H, NH_2), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{21}H_{31}N_5O_2$) C, H, N.

4-Amino-5-(1-hydroxyethyl)-2-{3-[4-(4-methoxyphenyl)-piperazin-1-yl]propyl}-6-methylpyridazin-3(2*H*)-one 5b. Yield = 99%; mp = 147−150 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 3H, CH(OH)CH_3), 2.10−2.20 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.20 (s, 3H, 6-CH_3), 2.60−2.95 (m, 6H: 2H, CONCH$_2$CH$_2$-CH_2N and 4H, piperazine), 3.00 (exch br s, 1H, OH), 3.20−3.30 (m, 4H, piperazine), 3.75 (s, 3H, OCH_3), 4.10−4.20 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.00 (q, 1H, CH(OH)CH_3), 5.80 (exch br s, 2H, NH_2), 6.80 (d, 2H, Ar), 7.00 (d, 2H, Ar). Anal. ($C_{21}H_{31}N_5O_3$) C, H, N.

4-Amino-2-{3-[4-(4-chlorophenyl)piperazin-1-yl]propyl}-5-(1-hydroxyethyl)-6-methylpyridazin-3(2*H*)-one 5c. Yield = 99%; mp = 193−195 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3H, CH(OH)CH_3), 2.05−2.15 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.20 (s, 3H, 6-CH_3), 2.55−2.80 (m, 6H: 2H, CONCH$_2$CH$_2$CH$_2$N and 4H, piperazine), 2.85 (exch br s, 1H, OH), 3.15−3.30 (m, 4H, piperazine), 4.20 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.05 (q, 1H, CH-(OH)CH_3), 5.80 (exch br s, 2H, NH_2), 6.85 (d, 2H, Ar), 7.20 (d, 2H, Ar). Anal. ($C_{20}H_{28}ClN_5O_2$) C, H, N.

4-Amino-2-{3-[4-(3-chlorophenyl)piperazin-1-yl]propyl}-5-(1-hydroxyethyl)-6-methylpyridazin-3(2*H*)-one 5d. Yield = 86%; mp = 124−126 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3H, CH(OH)CH_3), 2.00−2.10 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.20 (s, 3H, 6-CH_3), 2.50−2.60 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.60−2.70 (m, 4H, piperazine), 2.80 (exch br s, 1H, OH), 3.20−3.30 (m, 4H, piperazine), 4.15 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.00−5.10 (m, 1H, CH(OH)CH_3), 5.80 (exch br s, 2H, NH_2), 6.75−6.85 (m, 2H, Ar), 6.90 (m, 1H, Ar), 7.20 (t, 1H, Ar). Anal. ($C_{20}H_{28}ClN_5O_2$) C, H, N.

4-Amino-5-(1-hydroxyethyl)-2-{3-[4-(3-methoxyphenyl)-piperazin-1-yl]propyl}-6-methylpyridazin-3(2*H*)-one 5e. Yield = 92%; oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (d, 3H, CH(OH)CH_3), 1.97−2.09 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.18 (s, 3H, 6-CH_3), 2.50 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.62 (m, 4H, piperazine), 3.20 (m, 4H, piperazine), 3.80 (s, 3H, OCH_3), 4.06−4.19 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 4.99 (q, 1H, CH(OH)CH_3), 5.79 (exch br s, 2H, NH_2), 6.43 (d, 1H, Ar), 6.47 (s, 1H, Ar), 6.54 (s, 1H, Ar), 7.18 (t, 2H, Ar). Anal. ($C_{21}H_{31}N_5O_3$) C, H, N.

4-Amino-5-(1-hydroxyethyl)-2-{3-[4-(3-methylphenyl)-piperazin-1-yl]propyl}-6-methylpyridazin-3(2*H*)-one 5f. Yield = 95%; mp = 55−57 °C. ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3H, CH-(OH)CH_3), 2.08 (q, 2H, CONCH$_2CH_2$CH$_2$N), 2.20 (s, 3H, 6-CH_3), 2.33 (s, 3H, CH_3−Ar), 2.56 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.60−2.70 (m, 4H, piperazine), 3.17−3.28 (m, 4H, piperazine), 4.16 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.02 (q, 1H, CH(OH)CH_3), 5.79 (exch br s, 2H, NH_2), 6.67−6.71 (m, 2H, Ar), 6.76 (s, 1H, Ar), 7.17 (t, 1H, Ar). Anal. ($C_{21}H_{31}N_5O_2$) C, H, N.

4-Amino-5-(1-hydroxyethyl)-6-methyl-2-[3-(4-methylpiperidin-1-yl)propyl]pyridazin-3(2*H*)-one 5g. Yield = 83%; mp = 77−80 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 3H, CHCH_3), 1.15−1.40 (m, 3H, piperidine), 1.45 (d, 3H, CH(OH)CH_3), 1.55−1.65 (m, 2H, piperidine), 1.90−2.10 (m, 4H: 2H, piperidine and 2H, CONCH$_2CH_2$CH$_2$N), 2.20 (s, 3H, 6-CH_3), 2.35−2.45 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.80−2.90 (m, 2H, piperidine), 3.10 (exch br s, 1H, OH), 4.00−4.15 (t, 2H, CON-$CH_2CH_2CH_2$N), 4.90−5.00 (m, 1H, CH(OH)CH_3), 5.80 (exch br s, 2H, NH_2). Anal. ($C_{16}H_{28}N_4O_2$) C, H, N.

4-Amino-5-(1-hydroxyethyl)-6-methyl-2-[3-(4-methylpiperazin-1-yl)propyl]pyridazin-3(2*H*)-one 5h. Yield = 93%; mp = 103−105 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3H, CH-(OH)CH_3), 1.95−2.05 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.20 (s, 3H, 6-CH_3), 2.40 (s, 3H, NCH_3), 2.40−2.80 (m, 10H: 8H, piperazine and 2H, CONCH$_2$CH$_2$CH$_2$N), 3.05 (exch br s, 1H, OH), 4.05−4.20 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.05 (q, 1H, CH(OH)CH_3), 5.80 (exch br s, 2H, NH_2). Anal. ($C_{15}H_{27}N_5O_2$) C, H, N.

4-Amino-5-(1-hydroxyethyl)-2-{2-[4-(4-methoxyphenyl)-piperazin-1-yl]ethyl}-6-methylpyridazin-3(2*H*)-one 5i. Yield = 85%; mp = 158−160 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3H, CH(OH)CH_3), 2.20 (s, 3H, 6-CH_3), 2.55 (exch br s, 1H, OH), 2.75 (m, 4H, piperazine), 2.90 (m, 2H, CONCH$_2$CH$_2$N), 3.05−3.15 (m, 4H, piperazine), 3.75 (s, 3H, OCH_3), 4.20−4.30 (m, 2H, CONCH$_2$CH$_2$N), 5.00 (q, 1H, CH(OH)CH_3), 5.80 (exch br s, 2H, NH_2), 6.85 (d, 2H, Ar), 6.90 (d, 2H, Ar). Anal. ($C_{20}H_{29}N_5O_3$) C, H, N.

General Procedure for 6a−i. The appropriate pyridazinones **5a−i** (0.4 mmol) were reacted with PPA (40 mmol) for 2−8 h at 60−70 °C. After treatment with ice water, the mixture was neutralized with 6N NaOH, extracted with CH_2Cl_2 (3 × 15 mL), and the solvent was evaporated in vacuo to afford final compound **6a−i**.

4-Amino-6-methyl-2-[3-(4-*p*-tolylpiperazin-1-yl)propyl]-5-vinylpyridazin-3(2*H*)-one 6a. Yield = 98%; mp = 99−101 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.05−2.15 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.20 (s, 3H, 6-CH_3), 2.30 (s, 3H, Ph−CH_3), 2.50−2.65 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.65−2.80 (m, 4H, piperazine), 3.15−3.30 (m, 4H, piperazine), 4.20 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.20 (exch br s, 2H, NH_2), 5.60 (d, 1H, CH=CH_2), 5.70 (d, 1H, CH=CH_2), 6.50 (dd, 1H, CH=CH_2), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{21}H_{29}N_5O$) C, H, N.

4-Amino-2-{3-[4-(4-methoxyphenyl)-piperazin-1-yl]propyl}-6-methyl-5-vinylpyridazin-3(2*H*)-one 6b. Yield = 73%; mp = 97−99 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.10−2.20 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.25 (s, 3H, 6-CH_3), 2.60−2.80 (m, 6H: 4H, piperazine and 2H, CONCH$_2$CH$_2$CH$_2$N), 3.10−3.25 (m, 4H, piperazine), 3.80 (s, 3H, OCH_3), 4.20 (m, 2H, CON-$CH_2CH_2CH_2$N), 5.20 (exch br s, 2H, NH_2), 5.60 (d, 1H, CH=CH_2), 5.70 (d, 1H, CH=CH_2), 6.50 (dd, 1H, CH=CH_2), 6.85 (d, 2H, Ar), 6.90 (d, 2H, Ar). Anal. ($C_{21}H_{29}N_5O_2$) C, H, N.

4-Amino-2-{3-[4-(4-chlorophenyl)-piperazin-1-yl]propyl}-6-methyl-5-vinylpyridazin-3(2*H*)-one 6c. Yield = 70%; mp = 127−129 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.00−2.10 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.25 (s, 3H, 6-CH_3), 2.50−2.60 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.60−2.70 (m, 4H, piperazine), 3.10−3.20 (m, 4H, piperazine), 4.20 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 4.75 (exch br s, 2H, NH_2), 5.60 (d, 1H, CH=CH_2), 5.70 (d, 1H, CH=CH_2), 6.50 (dd, 1H, CH=CH_2), 6.85 (d, 2H, Ar), 7.20 (d, 2H, Ar). Anal. ($C_{20}H_{26}ClN_5O$) C, H, N.

4-Amino-2-{3-[4-(3-chlorophenyl)-piperazin-1-yl]propyl}-6-methyl-5-vinylpyridazin-3(2*H*)-one 6d. Yield = 56%; mp = 86−88 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.10−2.20 (m, 2H, CONCH$_2CH_2$CH$_2$N), 2.25 (s, 3H, 6-CH_3), 2.50−2.66 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.65−2.75 (m, 4H, piperazine), 3.20−3.30 (m, 4H, piperazine), 4.20 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.20 (exch br s, 2H, NH_2), 5.60 (d, 1H, CH=CH_2), 5.70 (d, 1H, CH=CH_2), 6.50 (dd, 1H, CH=CH_2), 6.75−6.85 (m, 2H, Ar), 6.90 (s, 1H, Ar), 7.20 (m, 1H, Ar). Anal. ($C_{20}H_{26}ClN_5O$) C, H, N.

4-Amino-2-{3-[4-(3-methoxyphenyl)-piperazin-1-yl]propyl}-6-methyl-5-vinylpyridazin-3(2*H*)-one 6e. Yield = 40%; oil. ^{1}H NMR ($CDCl_3$) δ 2.06 (quint, 2H, CONCH$_2CH_2$CH$_2$N), 2.23 (s, 3H, 6-CH_3), 2.53 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.61−2.66 (m, 4H, piperazine), 3.21 (t, 4H, piperazine), 3.80 (s, 3H, OCH_3), 4.19 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.21 (exch br s, 2H, NH_2), 5.59 (dd, 1H, CH=CH_2), 5.71 (dd, 1H, CH=CH_2), 6.40−6.58 (m, 4H: 1H, CH=CH_2, and 3H Ar), 7.17 (t, 1H, Ar). Anal. ($C_{21}H_{29}N_5O_2$) C, H, N.

4-Amino-2-{3-[4-(3-methylphenyl)-piperazin-1-yl]propyl}-6-methyl-5-vinylpyridazin-3(2*H*)-one 6f. Yield = 45%; oil. ^{1}H NMR ($CDCl_3$) δ 2.06 (quint, 2H, CONCH$_2CH_2$CH$_2$N), 2.23 (s, 3H, 6-CH_3), 2.33 (s, 3H, CH_3−Ar), 2.53 (t, 2H, CONCH$_2$-CH_2CH_2N), 2.63 (t, 4H, piperazine), 3.20 (t, 4H, piperazine), 4.19 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.21 (exch br s, 2H, NH_2), 5.59 (dd, 1H, CH=CH_2), 5.71 (dd, 1H, CH=CH_2), 6.47 (dd, 1H,

CH=CH$_2$), 6.67−6.73 (m, 2H, Ar), 6.76 (s, 1H, Ar), 7.16 (t, 1H, Ar). Anal. ($C_{21}H_{29}N_5O$) C, H, N.

4-Amino-6-methyl-2-[3-(4-methylpiperidin-1-yl)propyl]-5-vinylpyridazin-3(2*H*)-one 6g. Yield = 64%; mp = 80−83 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 3H, CHCH$_3$), 1.20−1.40 (m, 3H, piperidine), 1.60−1.70 (m, 2H, piperidine), 1.90−2.00 (m, 2H, piperidine), 2.00−2.10 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.20 (s, 3H, 6-CH$_3$), 2.40−2.50 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.90−3.00 (m, 2H, piperidine), 4.10 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.20 (exch br s, 2H, NH$_2$), 5.60 (d, 1H, CH=CH$_2$), 5.70 (d, 1H, CH=CH$_2$), 6.45 (dd, 1H, CH=CH$_2$). Anal. ($C_{16}H_{26}N_4O$) C, H, N.

4-Amino-6-methyl-2-[3-(4-methylpiperazin-1-yl)propyl]-5-vinylpyridazin-3(2*H*)-one 6h. Yield = 53%; oil. ^{1}H NMR ($CDCl_3$) δ 1.95−2.05 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.20 (s, 3H, 6-CH$_3$), 2.40 (s, 3H, NCH$_3$), 2.40−2.80 (m, 10H: 8H, piperazine and 2H, CONCH$_2$CH$_2$CH$_2$N), 4.15 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.20 (exch br s, 2H, NH$_2$), 5.60 (d, 1H, CH=CH$_2$), 5.80 (d, 1H, CH=CH$_2$), 6.50 (dd, 1H, CH=CH$_2$). Anal. ($C_{15}H_{25}N_5O$) C, H, N.

4-Amino-2-{2-[4-(4-methoxyphenyl)piperazin-1-yl]ethyl}-6-methyl-5-vinylpyridazin-3(2*H*)-one 6i. Yield = 90%; mp = 133−135 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, 6-CH$_3$), 2.75−3.10 (m, 6H: 4H, piperazine and 2H, CONCH$_2$CH$_2$N), 3.10−3.25 (m, 4H, piperazine), 3.80 (s, 3H, OCH$_3$), 4.30−4.40 (m, 2H, CONCH$_2$CH$_2$N), 5.20 (exch br s, 2H, NH$_2$), 5.60 (d, 1H, CH=CH$_2$), 5.70 (d, 1H, CH=CH$_2$), 6.50 (dd, 1H, CH=CH$_2$), 6.85 (d, 2H, Ar), 6.90 (d, 2H, Ar). Anal. ($C_{20}H_{27}N_5O_2$) C, H, N.

1-(4-*p*-Tolylpiperazin-1-yl)-propenone 7. To a mixture of 200 mg (1.14 mmol) of 1-*p*-tolylpiperazine and 110 mg (1.14 mmol) of triethylammine in anhydrous THF (3 mL), 110 mg (1.14 mmol) of 3-chloropropionylchloride were slowly added. The reaction mixture was stirred for 30 min at room temperature. After concentration in vacuo, it was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). After evaporation of the solvent, the residue was dissolved in anhydrous DMF (1 mL), 165 mg (1.19 mmol) of K_2CO_3 was added, and the mixture was stirred at 50 °C for 3 h. After cooling, 20 mL of water was added and the solution was extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded compound **7**.

Yield = 97%; mp = 150−152 °C (cyclohexane). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH$_3$), 3.15 (t, 4H, piperazine), 3.70−3.80 (m, 2H, piperazine), 3.85−3.95 (m, 2H, piperazine), 5.75 (d, 1H, CH=CH$_2$), 6.35 (d, 1H, CH=CH$_2$), 6.65 (dd, 1H, CH=CH$_2$), 6.95 (m, 2H, Ar), 7.15 (m, 2H, Ar). Anal. ($C_{14}H_{18}N_2O$) C, H, N.

5-Acetyl-4-amino-6-methyl-2-[3-oxo-3-(4-*p*-tolylpiperazin-1-yl)-propyl]pyridazin-3(2*H*)-one 8. Compound **8** was obtained from compound **1**[22] following the procedure described for **4a**−**d**. For this compound, the reaction was carried out at 60 °C for 4 h.

Yield = 98%; mp = 198−200 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, Ph-CH$_3$), 2.55 (s, 3H, 6-CH$_3$), 2.60 (s, 3H, COCH$_3$), 2.90 (t, 2H, CONCH$_2$CH$_2$CO), 3.05−3.20 (m, 4H, piperazine), 3.60−3.75 (m, 2H, piperazine), 3.75−3.90 (m, 2H, piperazine), 4.60 (t, 2H, CONCH$_2$CH$_2$CO), 6.80−6.95 (m, 2H, Ar), 7.15 (d, 2H, Ar), 7.80 (exch br s, 2H, NH$_2$). Anal. ($C_{21}H_{27}N_5O_3$) C, H, N.

4-Amino-5-(1-hydroxyethyl)-6-methyl-2-[3-oxo-3-(4-*p*-tolylpiperazin-1-yl)propyl]pyridazin-3(2*H*)-one 9. Compound **9** was obtained from compound **8** following the procedure described for **5a**−**i**.

Yield = 90%; mp = 201−204 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3H, CH(OH)CH$_3$), 2.20 (s, 3H, 6-CH$_3$), 2.30 (s, 3H, Ph-CH$_3$), 2.80−2.95 (t, 2H, CONCH$_2$CH$_2$CO), 3.05−3.20 (m, 4H, piperazine), 3.60−3.75 (m, 2H, piperazine), 3.75−3.90 (m, 2H, piperazine), 4.40−4.50 (m, 2H, CONCH$_2$CH$_2$CO), 5.05 (q, 1H, CH(OH)CH$_3$), 5.80 (exch br s, 2H, NH$_2$), 6.85−6.95 (m, 2H, Ar), 7.15 (d, 2H, Ar). Anal. ($C_{21}H_{29}N_5O_3$) C, H, N.

4-Amino-6-methyl-2-[3-oxo-3-(4-*p*-tolylpiperazin-1-yl)propyl]-5-vinylpyridazin-3(2*H*)-one 10. Compound **10** was obtained from compound **9** following the procedure described for **6a**−**i**.

Yield = 50%; mp = 129−131 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, 6-CH$_3$), 2.30 (s, 3H, Ph-CH$_3$), 2.90 (t, 2H, CONCH$_2$CH$_2$CO), 3.05−3.15 (m, 4H, piperazine), 3.60−3.70 (m, 2H, piperazine), 3.75−3.85 (m, 2H, piperazine), 4.65 (m, 2H, CONCH$_2$CH$_2$CO), 5.20 (exch br s, 2H, NH$_2$), 5.60 (d, 1H, CH=CH$_2$), 5.70 (d, 1H, CH=CH$_2$), 6.45 (dd, 1H, CH=CH$_2$), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{21}H_{27}N_5O_2$) C, H, N.

3-Methylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one 12. To a cooled and stirred mixture of 650 mg (3.58 mmol) of **11**[23] and 3 g of PPA (30 mmol) in EtOH (5 mL), 390 mg (7.97 mmol) of hydrazine hydrate were added. The reaction was carried out at 70 °C for 30 min. After cooling, cold water was added and the precipitate was recovered by suction.

Yield = 60%; mp = 261−263 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.85 (s, 3H, 3-CH$_3$), 8.05 (s, 1H, 4-H), 9.50 (exch br s, 1H, NH). Anal. ($C_6H_5N_3O_2$) C, H, N.

3-Methyl-6-[3-(4-*p*-tolylpiperazin-1-yl)propyl]isoxazolo[3,4-*d*]-pyridazin-7(6*H*)-one 13. Compound **13** was obtained starting from compound **12** following the procedure described for **4a**−**d**. For this compound, the reaction was carried out at 60 °C for 4 h.

Yield = 72%; mp = 130−132 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.15−2.25 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.30 (s, 3H, Ph-CH$_3$), 2.60−2.85 (m, 9H: 3H, 3-CH$_3$ and 4H, piperazine and 2H, CONCH$_2$CH$_2$CH$_2$N), 3.15−3.35 (m, 4H, piperazine), 4.30 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 8.05 (s, 1H, 4-H). Anal. ($C_{20}H_{25}N_5O_2$) C, H, N.

5-Acetyl-4-amino-2-[3-(4-*p*-tolylpiperazin-1-yl)propyl]pyridazin-3(2*H*)-one 14. To a suspension of 150 mg (0.49 mmol) of compound **13** in EtOH (10 mL) 60 mg (1.20 mmol) of hydrazine hydrate and 30 mg of 10% Pd/C were added and the mixture was refluxed for 1 h. Then, after cooling, the catalyst was filtered off and the solvent was evaporated in vacuo to afford **14**.

Yield = 80%; mp = 144−146 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.10−2.20 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.30 (s, 3H, Ph-CH$_3$), 2.55 (s, 3H, COCH$_3$), 2.60−2.90 (m, 6H: 4H, piperazine and 2H, CONCH$_2$CH$_2$CH$_2$N), 3.15−3.35 (m, 4H, piperazine), 4.25 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 6.80 (exch br s, 1H, NH$_2$), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 8.00 (s, 1H, 6-H), 9.00 (exch br s, 1H, NH$_2$). Anal. ($C_{20}H_{27}N_5O_2$) C, H, N.

4-Amino-5-(1-hydroxyethyl)-2-[3-(4-*p*-tolylpiperazin-1-yl)-propyl]pyridazin-3(2*H*)-one 15. Compound **15** was obtained from compound **14** following the procedure described for **5a**−**i**.

Yield = 96%; mp = 148−151 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.55 (d, 3H, CH(OH)CH$_3$), 2.05−2.15 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.30 (s, 3H, Ph-CH$_3$), 2.50−2.60 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.60−2.75 (m, 4H, piperazine), 2.90 (exch br s, 1H, OH), 3.15−3.25 (m, 4H, piperazine), 4.20 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 4.85 (q, 1H, CH(OH)CH$_3$), 5.60 (exch br s, 2H, NH$_2$), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 7.45 (s, 1H, 6-H); Anal. ($C_{20}H_{29}N_5O_2$) C, H, N.

4-Amino-2-[3-(4-*p*-tolylpiperazin-1-yl)propyl]-5-vinylpyridazin-3(2*H*)-one 16. Compound **16** was obtained from compound **15** following the procedure described for **6a**−**i**.

Yield = 23%; mp = 126−128 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.15−2.25 (m, 2H, CONCH$_2$CH$_2$CH$_2$N), 2.30 (s, 3H, Ph-CH$_3$), 2.55−2.85 (m, 6H: 2H, CONCH$_2$CH$_2$CH$_2$N and 4H, piperazine), 3.15−3.35 (m, 4H, piperazine), 4.25 (t, 2H, CONCH$_2$CH$_2$CH$_2$N), 5.15 (exch br s, 2H, NH$_2$), 5.50 (d, 1H, CH=CH$_2$), 5.70 (d, 1H, CH=CH$_2$), 6.50 (dd, 1H, CH=CH$_2$), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 7.70 (s, 1H, 6-H). Anal. ($C_{20}H_{27}N_5O$) C, H, N.

4-Amino-5-(2-bromoacetyl)-6-phenylpyridazin-3(2*H*)-one 18. To a stirred suspension of 300 mg (0.13 mmol) of **17**[22] and a catalytic amount of HBr in AcOH (1.9 mL), a solution of Br_2 (210 mg, 0.13 mmol) in acetic acid (1 mL) was slowly added. The mixture was heated for 5 h at 50 °C, and after dilution with 30 mL of cold water, the precipitate was recovered by suction.

Yield = 64%; mp = 228−230 °C (EtOH). ^{1}H NMR ($CDCl_3$) 3.50 (s, 2H, CH$_2$Br), 6.80 (exch br s, 2H, NH$_2$), 7.40 (exch br s, 1H, NH); 7.45−7.60 (m, 5H, Ar). Anal. ($C_{12}H_{10}BrN_3O_2$) C, H, N.

4-Amino-5-(2-methylthiazol-4-yl)-6-phenylpyridazin-3(2*H*)-one 19. A suspension of **18** (160 mg, 0.52 mmol) and 40 mg (0.52 mmol) of thioacetamide in anhydrous EtOH (2 mL) was heated at 80 °C for 5 h. After cooling, 15 mL of water was added and the final product was recovered by suction.

Yield = 68%; mp = 189−191 °C (EtOH). ^{1}H NMR ($CDCl_3$) 2.80 (s, 3H, thiazole-CH_3), 6.25 (s, 1H, Ar), 6.70 (exch br s, 2H, NH_2), 7.30−7.40 (m, 5H, Ar), 7.45 (exch br s, 1H, NH). Anal. ($C_{14}H_{12}N_4OS$) C, H, N.

General Procedure for 20a,b. Compounds **20a**,**b** were obtained from compound **19** following the general procedure described for **4a**−**d**. In this case, the final compounds **20a**,**b** were purified by column chromatography using the mixture petroleum ether/Et_2O/$CHCl_3$/abs EtOH/NH_4OH 10:4:4:1:0.5 as eluent.

4-Amino-5-(2-methylthiazol-4-yl)-6-phenyl-2-[3-(4-*p*-tolylpiperazin-1-yl)-propyl]pyridazin-3(2*H*)-one 20a. Yield = 20%; oil. ^{1}H NMR ($CDCl_3$) δ 2.15−2.25 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.70 (m, 2H, $CONCH_2CH_2\underline{CH_2}N$), 2.75 (s, 3H, thiazole-$\underline{CH_3}$), 2.80−2.90 (m, 4H, piperazine), 3.15−3.30 (m, 4H, piperazine), 4.35 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 6.20 (s, 1H, Ar), 6.60 (exch br s, 2H, NH_2), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 7.30−7.40 (m, 5H, Ar). Anal. ($C_{28}H_{32}N_6OS$) C, H, N.

4-Amino-5-(2-methylthiazol-4-yl)-6-phenyl-2-[2-(4-*p*-tolylpiperazin-1-yl)ethyl]pyridazin-3(2*H*)-one 20b. Yield = 15%; mp = 181−183 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3Ph), 2.75 (s, 3H, thiazole-$\underline{CH_3}$), 2.80−2.90 (m, 4H, piperazine), 2.95−3.05 (m, 2H, $CONCH_2\underline{CH_2}N$), 3.15−3.30 (m, 4H, piperazine), 4.40−4.50 (m, 2H, $CON\underline{CH_2}CH_2N$), 6.20 (s, 1H, Ar), 6.60 (exch br s, 2H, NH_2), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 7.30−7.40 (m, 5H, Ar). Anal. ($C_{27}H_{30}N_6OS$) C, H, N.

3-(2-Dimethylaminovinyl)-4-methyl-6-[2-(4-*p*-tolyl-piperazin-1-yl)ethyl]isoxazolo[3,4-*d*]pyridazin-7(6*H*)-one 22. A suspension of 100 mg of compound **21**[16] (0.27 mmol) in 1.03 mL (7.55 mmol) of *N*,*N*-dimethylformammide dimethyl acetal was stirred at 90 °C for 1 h. After cooling, 15 mL of water were added and the precipitate was recovered by suction.

Yield = 61%; mp = 150−152 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.45 (s, 3H, 4-CH_3), 2.90−3.60 (m, 16H: 8H, piperazine and 2H, $CONCH_2\underline{CH_2}N$ and 6H, $N(\underline{CH_3})_2$), 4.40−4.60 (m, 2H, $CON\underline{CH_2}CH_2N$), 5.20 (d, 1H, $\underline{CH}$=CH-N), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 7.60 (d, 1H, CH=$\underline{CH}$-N). Anal. ($C_{23}H_{30}N_6O_2$) C, H, N.

4-Amino-6-methyl-5-(1*H*-pyrazol-3-yl)-2-[2-(4-*p*-tolylpiperazin-1-yl)ethyl]pyridazin-3(2*H*)-one 23. A suspension of compound **22** (70 mg, 0.17 mmol) and 150 mg of hydrazine hydrate (3 mmol) in ethanol (2−3 mL) was stirred at 70 °C for 3 h. The mixture was concentrated in vacuo, diluted with cold water, and extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the desired **23**, which was purified by column chromatography using cycloexane/ethyl acetate 1:4 as eluent.

Yield = 46%; mp = 172−174 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.35 (s, 3H, 6-CH_3), 2.80−3.20 (m, 6H: 4H, piperazine and 2H, $NCH_2\underline{CH_2}N$), 3.25−3.45 (m, 4H, piperazine), 4.40−4.55 (m, 2H, $N\underline{CH_2}CH_2N$), 6.35 (exch br s, 2H, NH_2), 6.55 (d, 1H, Ar), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar), 7.75 (d, 1H, Ar). Anal. ($C_{21}H_{27}N_7O$) C, H, N.

General Procedure for 25a−c. Compounds **25a**−**c** were obtained from **24a**−**c**[24−26] following the general procedure described for **4a**−**d**. For compound **25b**, the suspension was heated for 2 h at 60 °C. Differently from compounds **4a**−**d**, compounds **25a**−**c** were not recovered by suction, but after dilution, the reaction mixture was extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded **25a**−**c**, which were purified by column chromatography using CH_2Cl_2/MeOH 9:1 as eluent.

6-Methyl-2-[3-(4-*p*-tolylpiperazin-1-yl)propyl]pyridazin-3(2*H*)-one 25a. Yield = 57%; mp = 93−94 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.00−2.10 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.25 (s, 3H, 6-$CH_3$), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.50 (t, 2H, $CONCH_2CH_2\underline{CH_2}N$), 2.65 (m, 4H, piperazine), 3.15 (m, 4H, piperazine), 4.20 (m, 2H, $CON\underline{CH_2}CH_2CH_2N$), 6.80−6.90 (m, 3H, Ar), 7.00−7.10 (m, 3H, Ar). Anal. ($C_{19}H_{26}N_4O$) C, H, N.

4-Amino-6-methyl-2-[3-(4-*p*-tolylpiperazin-1-yl)propyl]pyridazin-3(2*H*)-one 25b. Yield = 52%; mp = 165−168 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.00−2.20 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.20 (s, 3H, 6-$CH_3$), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.60−3.05 (m, 6H: 4H, piperazine and 2H, $CONCH_2CH_2\underline{CH_2}N$), 3.20−3.45 (m, 4H, piperazine), 4.20 (m, 2H, $CON\underline{CH_2}CH_2CH_2N$), 4.90 (exch br s, 2H, NH_2), 6.15 (s, 1H, Ar), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{19}H_{27}N_5O$) C, H, N.

5-Amino-1-[3-(4-*p*-tolylpiperazin-1-yl)-propyl]-1*H*-[3,4′]bipyridinyl-6-one 25c. Yield = 30%; mp = 65−67 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.10−2.20 (m, 2H, $CONCH_2\underline{CH_2}CH_2N$), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.50−2.60 (m, 2H, $CONCH_2CH_2\underline{CH_2}N$), 2.70−2.80 (m, 4H, piperazine), 3.20−3.30 (m, 4H, piperazine), 4.20 (t, 2H, $CON\underline{CH_2}CH_2CH_2N$), 4.40 (exch br s, 2H, NH_2), 6.85 (m, 3H, Ar), 7.10 (d, 2H, Ar), 7.25 (s, 1H, Ar), 7.35 (d, 2H, Ar), 8.60 (d, 2H, Ar). Anal. ($C_{24}H_{29}N_5O$) C, H, N.

5-Acetyl-2,6-dimethyl-4-[2-(4-*p*-tolylpiperazin-1-yl)ethylamino]-pyridazin-3(2*H*)-one 28. A mixture of 165 mg (0.77 mmol) of **26**[27] and 170 mg (0.77 mmol) of **27**[28] in anhydrous ethanol was stirred for 12 h at room temperature. After evaporation of the solvent, the mixture was diluted with cold water (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded the final compound **29**, which was purified by column chromatography using cyclohexane/ethyl acetate 1:1 as eluent.

Yield = 30%; oil. ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, 6-CH_3), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.55 (s, 3H, CO$\underline{CH_3}$), 2.65−2.80 (m, 6H: 4H, piperazine and 2H, $NHCH_2\underline{CH_2}N$), 3.20−3.40 (m, 6H: 4H, piperazine and 2H, $NH\underline{CH_2}CH_2N$), 3.75 (s, 3H, $N\underline{CH_3}$), 6.55 (exch br s, 1H, NH), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{21}H_{29}N_5O_2$) C, H, N.

5-(1-Hydroxyethyl)-2,6-dimethyl-4-[2-(4-*p*-tolylpiperazin-1-yl)-ethylamino]pyridazin-3(2*H*)-one 29. Compound **29** was obtained from compound **28** following the procedure described for **5a**−**i**.

Yield = 90%; mp = 90−92 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 3H, CH(OH)$\underline{CH_3}$), 2.20 (s, 3H, 6-CH_3), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.65−2.75 (m, 2H, $NHCH_2\underline{CH_2}N$), 2.75−2.90 (m, 4H, piperazine), 3.25 (m, 4H, piperazine), 3.70 (s, 3H, NCH_3), 3.70−3.80 (m, 2H, $NH\underline{CH_2}CH_2N$), 4.20−4.30 (exch br s, 1H, OH), 5.00 (q, 1H, $\underline{CH}(OH)CH_3$), 6.50 (exch br s, 1H, NH), 6.55 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{21}H_{31}N_5O_2$) C, H, N.

2,6-Dimethyl-4-[2-(4-*p*-tolylpiperazin-1-yl)ethylamino]-5-vinyl-pyridazin-3(2*H*)-one 30. To a solution of 70 mg (0.182 mmol) of **29** in 3 mL of toluene, 1.5 g of H_2SO_4 on silica gel, prepared following the procedure previously described in literature,[30] was added portionwise over 2 h. The mixture was refluxed for 4 h. After cooling, silica gel was removed by suction and washed with K_2CO_3 saturated solution (100 mL) first and then with ethyl acetate (60 mL). The aqueous layer was eliminated, and the organic layer was evaporated in vacuo to afford the vinyl derivative, which was purified by column chromatography using CH_2Cl_2/CH_3OH 9.5:0.5 as eluent.

Yield = 45%; mp = 114−115 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, 6-CH_3), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.60−2.70 (m, 6H: 4H, piperazine and 2H, $NHCH_2\underline{CH_2}N$), 3.20 (m, 4H, piperazine), 3.40 (q, 2H, $NH\underline{CH_2}CH_2N$), 3.75 (s, 3H, NCH_3), 5.25 (dd, 1H, CH=$\underline{CH_2}$), 5.65 (dd, 1H, CH=$\underline{CH_2}$), 6.40 (exch br s, 1H, NH), 6.65 (dd, 1H, $\underline{CH}$=CH_2), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{21}H_{29}N_5O$) C, H, N.

5-Acetyl-4-methyl-3-[3-(4-*p*-tolylpiperazin-1-yl)-propyl]-3*H*-thiazol-2-one, 33. Compound **33** was obtained starting from **31**[29] following the general procedure described for **4a**−**d**. For compound **33**, the mixture was stirred at 40 °C for 2 h and the final compound was purified by column chromatography using cyclohexane/ethyl acetate 1:2 as eluent.

Yield = 30%; mp = 106−107 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.95 (m, 2H, $NCH_2\underline{CH_2}CH_2N$), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.35 (s,

3H, 4-CH_3), 2.50 (t, 2H, $NCH_2CH_2\underline{CH_2}N$), 2.60−2.70 (m, 7H: 4H, piperazine and 3H, $COCH_3$), 3.20 (m, 4H, piperazine), 3.90 (t, 2H, $N\underline{CH_2}CH_2CH_2N$), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{20}H_{27}N_3O_2S$) C, H, N.

5-(1-Hydroxyethyl)-4-methyl-3-[3-(4-*p*-tolylpiperazin-1-yl)-propyl]-3*H*-thiazol-2-one 34. To a cooled (0 °C) and stirred solution of **33** (120 mg, 0.107 mmol) in CH_3OH (4 mL), 30 mg of sodium borohydride (0.214 mmol) were added. The mixture was stirred for 5 min at room temperature, concentrated in vacuo, diluted with cold water, and extracted with $CHCl_3$ (3 × 20 mL). Evaporation of the solvent afforded final compound **34**.

Yield = 98%; mp = 116−117 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 3H, CH(OH)$\underline{CH_3}$), 1.90−2.00 (m, 2H, $NCH_2\underline{CH_2}CH_2N$), 2.20 (s, 3H, 4-$\underline{CH_3}$), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.50−2.60 (m, 2H, $NCH_2CH_2\underline{CH_2}N$), 2.70−2.80 (m, 4H, piperazine), 3.15−3.25 (m, 4H, piperazine), 3.70−3.80 (m, 2H, $N\underline{CH_2}CH_2CH_2N$), 5.05 (q, 1H, $\underline{CH}(OH)CH_3$), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{20}H_{29}N_3O_2S$) C, H, N.

4-Methyl-3-[3-(4-*p*-tolylpiperazin-1-yl)-propyl]-5-vinyl-3*H*-thiazol-2-one 35. Compound **35** was obtained starting from **34** following the procedure[30] described for compound **30**.

Yield = 15%; mp = 166−168 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.95 (m, 2H, $NCH_2\underline{CH_2}CH_2N$), 2.20 (s, 3H, 4-$CH_3$), 2.30 (s, 3H, Ph-$\underline{CH_3}$), 2.55 (t, 2H, $NCH_2CH_2\underline{CH_2}N$), 2.65−2.75 (m, 4H, piperazine), 3.20 (m, 4H, piperazine), 3.80 (t, 2H, $N\underline{CH_2}CH_2CH_2N$), 5.05−5.15 (m, 2H, CH=$\underline{CH_2}$), 6.65 (dd, 1H, $\underline{CH}$=CH_2), 6.85 (d, 2H, Ar), 7.10 (d, 2H, Ar). Anal. ($C_{20}H_{27}N_3OS$) C, H, N.

Biological Assays. Animals. Male CD-1 mice (Harlan, Italy) weighing 25−30 g were used for all experiments. Mice were housed for at least 1 week before experimental sessions in colony cages (seven mice in each cage) under standard light (light on from 7.00 a.m. to 7.00 p.m.), temperature (21 ± 1 °C), relative humidity (60 ± 10%) with food and water available ad libitum. The guidelines of the European Community Council (86/609/EEC) for animal care and use were followed.

Tail Flick Test. The tail flick latency was obtained using a commercial unit (Ugo Basile, Italy), consisting of an infrared radiant light source (100 W, 15 V bulb) focused onto a photocell utilizing an aluminum parabolic mirror. During the trials, the mice were gently hand-restrained with a glove. Radiant heat was focused 3−4 cm from the tip of the tail, and the latency (s) of the tail withdrawal recorded. The measurement was interrupted if the latency exceeded the cut off time (15 s at 15 V). Also in this case, the baseline was calculated as mean of three readings recorded before testing at intervals of 15 min and the time course of latency determined at 15, 30, 45, 60, 90, and 120 min after treatment.[36]

Hot Plate Test. In the hot plate test, the thermal nociception was assessed with a commercially available apparatus consisting in a metal plate 25 cm × 25 cm (Ugo Basile, Italy) heated to a constant temperature of 48.5 ± 0.1 °C, on which a plastic cylinder (20 cm diameter, 18 cm high) was placed. The time of latency (s) was recorded from the moment the animal was inserted inside the cylinder up to when it licked its paws or jerked them off the hot plate or jumped off the hot plate; the latency exceeded the cutoff time of 60 s. The baseline was calculated as mean of three readings recorded before testing at intervals of 15 min. The time course of latency was then determined at 15, 30, 45, 60, 72, and 90 min after treatment.[36]

Surgery for Central Injections. Mice were anesthetized with ketamine-xylazine (80 + 10 mg kg^{-1}, ip), and a small incision was made on the scalp to expose the reference point bregma. The position of the right lateral ventricle was stereotaxically located at +2 mm ML and −0.3 mm AP to bregma. Intracerebral ventricular injection was performed by using a Hamilton microsyringe fitted with a 26 gauge needle that was directly inserted into the skull to a depth of 2 mm.[36]

Drug and Treatments. In the first series of experiments, morphine hydrochloride and compounds under investigation were administered in the right cerebral ventricle (icv) and the mice performed the tail flick test. Then, the relative ED_{50} were calculated. In the second series of experiments, morphine hydrochloride and the compound **6a** showing the lowest ED_{50}, was administered po in the hot plate and the tail flick test and the relative ED_{50} was calculated. Antagonism studies were performed administering the putative antagonist ip 30 min before **6a** po administration. The following antagonists were used: naloxone hydrochloride (1 mg/kg), CGP 35348 (50 mg/kg), mecamylamine hydrocholoride (2 mg/kg), and yohimbine hydrochloride (2 mg/kg).

In some experiments, prazosin (0.1 and 1 mg/kg) was ip administered 15 min before **6a** (administered po at the dose of 1 mg/kg).

Radioligand Binding Assays. All the procedures were performed according to standard radioligand binding technique. In particular, the α_{1A}-adrenoceptor and α_{1B}-adrenoceptor binding assays[37,38] were performed on Wistar rat submaxillary gland and Wistar rat liver membrane extracts, respectively, using 0.25 nM [^{3}H]-Prazosin as radioligand. Nonspecific binding was measured in the presence of 10 μM of phentolamine in incubation buffer containing 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA.

α_{2A}-Adrenoceptor binding assay[39] was performed on human recombinant insect Sf9 cell extracts using 1 nM of [^{3}H]-MK-912 as radioligand. Nonspecific binding was measured in the presence of 10 μM of WB-4101 in incubation buffer containing 50 mM Tris-HCl, pH 7.4, 12.5 mM $MgCl_2$, 2 mM EDTA

After 60 min of incubation at 25 °C, membranes were harvested into glass fiber (GF/B) filters (presoaked in 0.5% polyethylenimine) using Filtermate cell harvester (PerkinElmer). Subsequently, the filters were washed with 2.5 mL of ice-cold buffer and dried for 30 min in an oven at 45 °C. Then 50 μM of Microscint 20 (Packard) were added to each well, incubated 15 min on an orbital shaker, and counted with a TopCount for 1 min/well.

The compound was dissolved in dimethyl sulfoxide (DMSO) and diluted in incubation buffer, then tested in duplicate at 1 μM concentration (1% vehicle DMSO final concentration).

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) were determined by nonlinear regression analysis of five 10-fold serially diluted concentrations (ranging from 10 to 0.001 μM) in duplicate competition curves with constant top = 100, bottom = 0, and Hill coefficients (nH) = 1 using GraphPad 3.0 software.

Statistical Analysis. Data obtained from hot plate and tail flick experiments were expressed as time course of the percentage of maximum effect (% MPE) = (post drug latency − baseline latency)/(cutoff time − baseline latency) × 100. Data obtained from the hot plate and the tail flick test were first evaluated with the analysis of variance (one-way ANOVA), followed by Student's *t*-test or Bonferroni's posthoc comparisons using the statistical software SPSS. Then, data collected at the peak time after compound **6a**−**25c** administration were made quantal by designating as a positive an increase of % MPE up to 50%. At least four groups of six mice were used to generate dose−response curves and to estimate ED_{50} values.[40] ED_{50s} and their significant differences were calculated with the aid of a computer program.[41] Statistical significance was assumed at $P < 0.05$.

Supporting Information Available: Elemental analyses for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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