[(3-chlorophenyl)piperazinylpropyl]pyridazinones and analogues as potent antinociceptive agents

[(3-Chlorophenyl)piperazinylpropyl]pyridazinones and Analogues as Potent Antinociceptive Agents

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A number of [(3-chlorophenyl)piperazinylpropyl]pyridazinones and the corresponding isoxazolopyridazinones, showing the arylpiperazinyl substructure present in very potent antinociceptive agents reported in the literature, were synthesized and tested for their analgesic activity. The investigated compounds showed antinociceptive properties in the mouse hot-plate test (thermal nociceptive stimulus) after systemic administration with an efficacy similar to that exerted by morphine. The increase of the pain threshold induced by the compounds labeled **5a**, **7**, **8**, and **11** was prevented by reserpine, suggesting the involvement of the noradrenergic and/or serotoninergic system in their mechanism of action. Among them, **7** and **11** showed the highest analgesic potency and efficacy together with a good ratio (133 and 200, respectively) of the minimal nontoxic dose (MNTD) to the minimal analgesic dose (MAD). Furthermore, they were also active after icv administration and in the presence of a chemical, painful stimulus (abdominal constriction test).

Introduction

Two major groups of drugs are currently used for treatment of pain: the traditional non-steroidal antiinflammatory drugs (NSAIDs) and opioids. The first class, whose effects are mediated by the peripheral inhibition of cyclooxygenase (COX), the enzyme responsible for prostaglandins synthesis,[1] is generally used in the treatment of mild to moderate pain,[2] and the common side effects include gastrointestinal lesions, such as ulcerations and perforation, nephrotoxicity, and inhibition of platelet aggregation.[3] After the discovery of the two isoforms (COX1 and COX2),[1] together with the evidence that COX2 is induced by proinflammatory mediators,[4] potent and selective COX2 inhibitors were developed, showing that these compounds have comparable analgesic activity with respect to traditional NSAIDs but with very low incidence of unwanted side effects.[5]

The class of opioid drugs, which includes morphine and congeners, is used in moderate to severe pain and operates by activating central G-protein-coupled receptors μ, δ, and κ.[6] Side effects associated with the clinical use of opioids are very strong and use-limiting, the most important being respiratory depression, tolerance, physical dependence, and constipation.[7]

Finally, it is possible to define a third class of analgesic drugs, the so-called "analgesic adjuvants",[8] which are drugs with a different primary use, for example, antidepressants, anticonvulsants, and anesthetics, but able to control neuropathic pain.[8,9]

Current research in pain therapy looks at the discovery of new potent drugs devoid of the limiting side effects of the above-mentioned classes. It is necessary to take into account that pain is a very complex process in which many different neuromodulators are involved, such as glutamate, acetylcholine, GABA, adrenaline, and so on.[8,10−12]

Our experience in the pyridazine field,[13−16] together with the observation that some pyridazine derivatives such as emorfazone (**A**, Chart 1),[17] on the market in Japan, and compound **B**,[18] bearing an alkylpiperazinyl alkyl moiety, show interesting antinociceptive activity not related to effects on prostaglandins or opioid system, led us to design and synthesize a series of pyridazinone derivatives as potential analgesic drugs. In this study we identified compound **C**[14] as a promising lead, showing good antinociceptive activity in mouse abdominal constrictions model with an ED_{50} of 14.9 mg kg^{-1}, sc (quantal protection of 100% at 100 mg kg^{-1}), resulting in 7-fold more potent with respect to emorfazone. In the same investigation, another interesting compound (**D**)[14] emerged, showing 60% inhibition of abdominal constriction and 40% of quantal protection at 100 mg kg^{-1}. Taking into account these results, our research was focused on the synthesis of 4-amino-5-vinyl- and 4-amino-5-acetylpyridazinones bearing an arylpiperazinyl moiety at position 2 of the pyridazine ring. Very encouraging results were obtained by combining the vinyl and the arylpiperazinyl functions, in particular for compound **E**[19] which showed a dramatic increase of activity in the writhing test with respect to our previous lead **C** (ED_{50} = 2.5 mg kg^{-1}, sc and 100% of quantal protection at 100 mg kg^{-1}).

In the present paper, we report the effect of the elongation of the methylenic spacer in compound **E** and that of insertion of a chlorine at the meta position of the arylpiperazinyl moiety; in this way, we inserted into our nucleus a substructure present in very potent antinociceptive agents, like compound **F**.[20] At the same time, we synthesized and tested bicyclic derivatives

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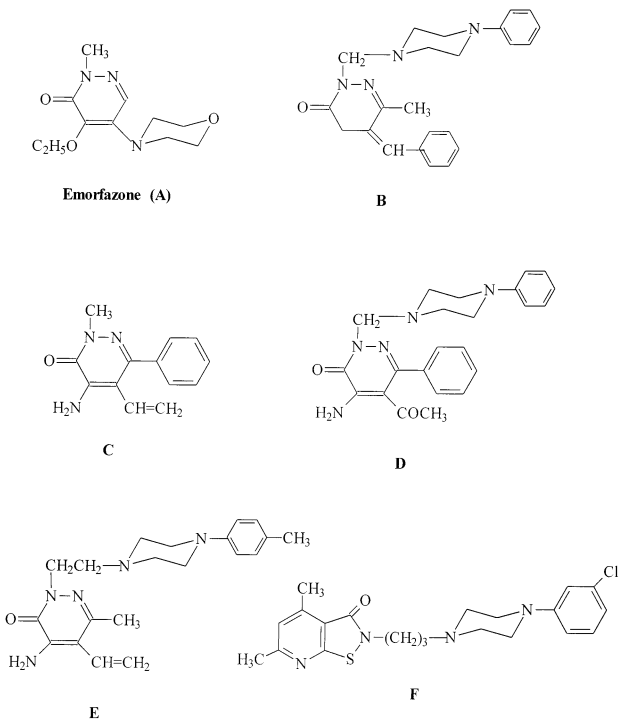
Chart 1



bearing the same fragment, in which the pyridazinone ring is [4,5-*d*]- and [3,4-*d*]-fused with an isoxazole.

Chemistry

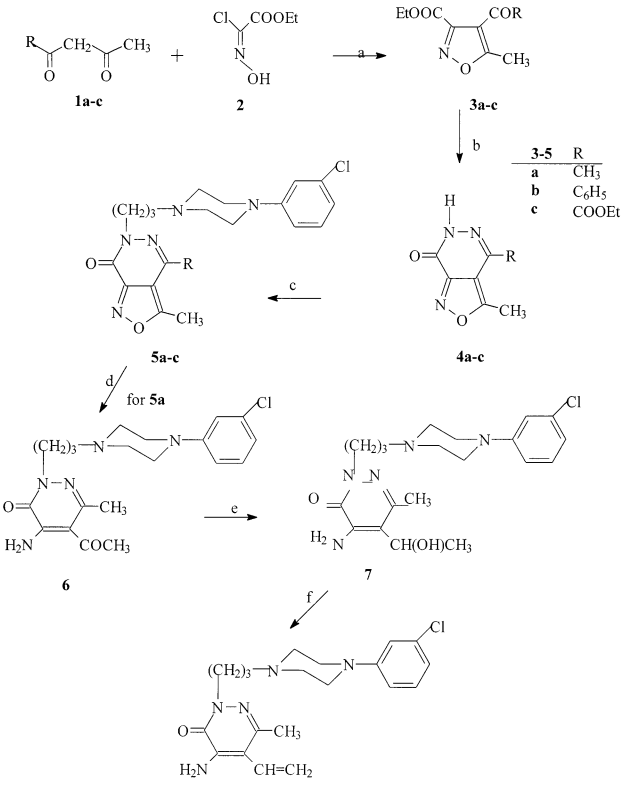
The synthetic pathway affording the final **5**−**8** is depicted in Scheme 1. With the exception of **3c** and **4c**, the isoxazoles **3** and the isoxazolo[3,4-*d*]pyridazinones **4** were previously described.[21−23] 1-(3-Bromopropyl)-4-(3-chlorophenyl)piperazine was prepared by condensing 3-chlorophenylpiperazine with 1,3-dibromopropane in DMF and in the presence of K_2CO_3 at room temperature. The crude reaction product was reacted with compounds **4** under the same reaction conditions to afford **5**. Compound **5c** was transformed into the corresponding 5-acetyl-4-amino derivative **6** by reductive cleavage with ammonium formate in ethanol and then in the corresponding 5-hydroxyethyl **7** by using sodium borohydride in anhydrous methanol. Finally, the dehydratation of **7** with polyphosphoric acid (PPA) afforded the vinyl derivative **8**.

Under the same conditions described above for **5**, compound **11** was obtained starting from **9**[24] (Scheme 2) through the bromoderivative **10**.

Results and Discussion

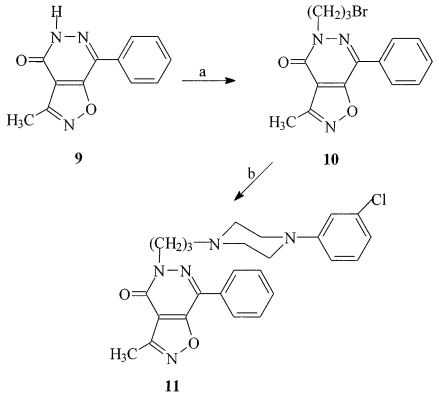
Subcutaneous injection of **5a** (20 mg kg^{-1}), **11** (20 mg kg^{-1}), **6** (3 mg kg^{-1}), **7** (6 mg kg^{-1}), **8** (1 mg kg^{-1}), and **5c** (20 mg kg^{-1}) and per os administration of **5b** (10 mg kg^{-1}) induced antinociception in the mouse hot-plate test. The effect appeared 15 min after administration, peaked after 30 min, and then slowly diminished (Table 1). Analgesic efficacy of the investigated compounds was comparable to that exerted by morphine (8 mg kg^{-1}), used as a reference drug, since 71−75% of the morphine effect was reproduced by the less active compounds **5a**−**c** of the series (Table 2). All compounds showed a good ratio of the minimal nontoxic dose (MNTD) to the minimal analgesic dose (MAD). The range of MNTD/MAD values is from 4 (**5a**, **5c**) to 200 (**6**, **8**, **11**). All the compounds, at the highest effective doses, neither produced any alteration of the animals' gross behavior nor modified the spontaneous motility and inspection activity, as revealed by the hole board test (data not shown). The analgesia induced by **5a**, **5b**, **7**, and **11** was completely prevented by pretreatment with the monoamine store depletor reserpine (2 mg kg^{-1} ip, administered 48 and 24 h before the test), **8** was partially prevented, whereas **5c** and **6** were unmodified by reserpine pretreatment (Table 2). The dose and administration schedule of reserpine was able to selectively prevent antinociception induced by the antidepressant drugs

Scheme 1[a]



[a] (a) EtOH, EtONa; (b) hydrazine hydrate, EtOH, room temp; (c) 1-(3-bromopropyl)-4-(3-chlorophenyl)piperazine, DMF, anhydrous K_2CO_3, 60−70 °C; (d) ammonium formate, 10% Pd/C, EtOH, reflux; (e) $NaBH_4$, MeOH, room temp; (f) PPA, room temp.

Scheme 2[a]



[a] (a) 1,3-Dibromopropane, K_2CO_3, anhydrous DMF, 60 °C; (b) 3-chlorophenylpiperazine, K_2CO_3, anhydrous DMF, 70 °C.

Table 1. Antinociceptive Effect of Compounds **5a**−**c**, **6**−**8**, and **11** in the Mouse Hot-Plate Test

	licking latency (s)				
		after treatment			
treatment	before treatment	15 min	30 min	45 min	60 min
saline, 10 mL kg^{-1}, sc	14.1 ± 0.9	15.7 ± 2.4	14.4 ± 1.0	15.5 ± 1.9	14.7 ± 1.6
vehicle, 10 mL kg^{-1}, sc	14.2 ± 1.2	14.9 ± 1.9	15.3 ± 1.5	16.0 ± 2.1	15.3 ± 1.8
morphine, 8 mg kg^{-1}, sc	14.6 ± 1.3	24.5 ± 1.3[b]	28.7 ± 1.9[b]	26.4 ± 1.8[b]	23.8 ± 1.6[b]
5a, 20 mg kg^{-1}, sc	16.6 ± 0.7	18.0 ± 2.0	24.0 ± 1.6[b]	22.6 ± 1.5[b]	17.6 ± 1.9
5b, 10 mg kg^{-1}, po	15.1 ± 1.3	20.8 ± 1.7[a]	25.1 ± 2.0[b]	19.1 ± 1.8[a]	16.6 ± 1.8
5c, 20 mg kg^{-1}, sc	16.0 ± 0.9	22.2 ± 1.4[b]	23.8 ± 2.6[b]	23.2 ± 2.1[b]	17.8 ± 2.0
6, 3 mg kg^{-1}, sc	15.5 ± 0.8	23.7 ± 1.4[b]	33.2 ± 2.0[b]	34.3 ± 2.4[b]	32.7 ± 2.3[b]
7, 6 mg kg^{-1}, sc	14.5 ± 0.9	22.4 ± 1.8[b]	33.7 ± 1.9[b]	29.3 ± 1.8[b]	23.3 ± 1.5[b]
8, 1 mg kg^{-1}, sc	14.2 ± 0.8	21.3 ± 1.5[a]	33.5 ± 2.3[b]	28.2 ± 2.5[b]	22.6 ± 2.1[b]
11, 20 mg kg^{-1}, sc	14.6 ± 0.9	19.6 ± 1.8	34.5 ± 1.6[b]	31.2 ± 1.6[b]	29.3 ± 1.6[b]

[a] $P < 0.05$ in comparison with vehicle-treated animals. Vehicle is represented by saline + DMSO 2:1. [b] $P < 0.01$ in comparison with vehicle-treated animals. Vehicle is represented by saline + DMSO 2:1.

Table 2. Comparison among the Minimal Analgesic Dose (MAD), the Maximal Nontoxic Dose (MNTD), and the Efficacy of the Tested Compounds and Morphine and Effect of Reserpine on Antinociception Induced by the Same Compounds

compd	MAD,[a] mg kg^{-1}	MNTD,[b] mg kg^{-1}	% analgesic efficacy compared to morphine[c]	% of antagonism exerted by reserpine[d]
morphine	0.2 sc	30 sc	100	0
5a	10 sc	40 sc	72	92
5b	1 po	50 sc	75	94
5c	10 sc	40 sc	71	0
6	0.1 sc	20 sc	100	0
7	0.3 sc	40 sc	101	100
8	0.1 sc	20 sc	100	61
11	0.3 sc	60 sc	104	100

[a] Antinociceptive effect was evaluated on the mouse hot-plate test. The percent of analgesic efficacy was evaluated at the maximal analgesic dose. The maximal analgesic effect of morphine is indicated as 100%. [b] Minimal dose able to induce a statistically significant increase of the pain threshold. [c] Tested at 8 mg kg^{-1}. [d] Tested at 2 mg kg^{-1}.

clomipramine and amitriptyline,[25] indicating that it is ideal for preventing the increase of pain threshold induced by activation of the catecholaminergic and serotoninergic systems. On the basis of these data, we can exclude that **5c** and **6** exert their antinociceptive effect through a catecholaminergic mechanism. Among the compounds completely prevented by reserpine, **7** and **11** were further investigated to elucidate their pharmacological profile because they showed the best MNTD/MAD ratio. **7** (6 mg kg^{-1} sc) and **11** (20 mg kg^{-1} sc) also showed antinociceptive properties in the abdominal constriction test in which a chemical nociceptive stimulus was applied (Figure 1). Both compounds were able to almost completely abolish the number of abdominal constriction 15 and 30 min after administration. Their analgesic activity persisted almost unchanged up to 60−75 min, and then it progressively diminished, disappearing at 120 min. Furthermore, **7** and **11** induced analgesia after icv administration with an efficacy and time course similar to those observed after systemic administration (Figure 2). These results indicate that their site of action is within the central nervous system. It was, in fact, possible to reach the same intensity of analgesia by injecting directly into the cerebral ventricles doses of both compounds that were lower than those needed parenterally. That the antinociception depends on a retrodiffusion of the drug from the cerebral ventricles to the periphery can thus be ruled out. In conclusion, all the investigated compounds

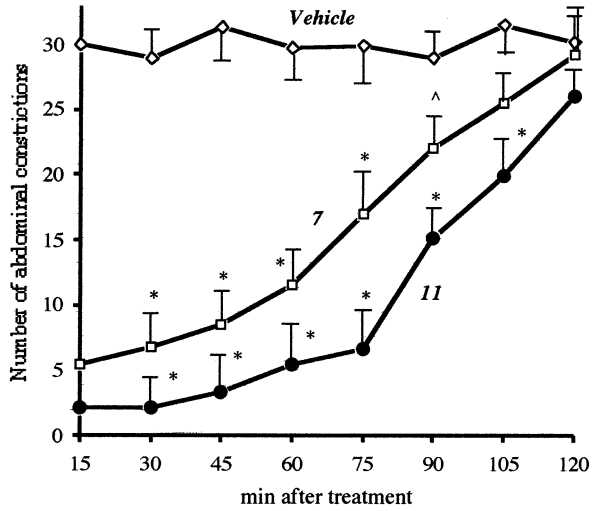


Figure 1. Antinociceptive effect of **7** (6 mg kg^{-1}, sc) and **11** (20 mg kg^{-1}, sc) in the mouse abdominal constriction test. Both compounds were administered 30 min before the test. Vertical lines show SEM, and the asterisk (*) represents $P < 0.01$ in comparison with vehicle controls. Each point represents the mean of at least eight mice.

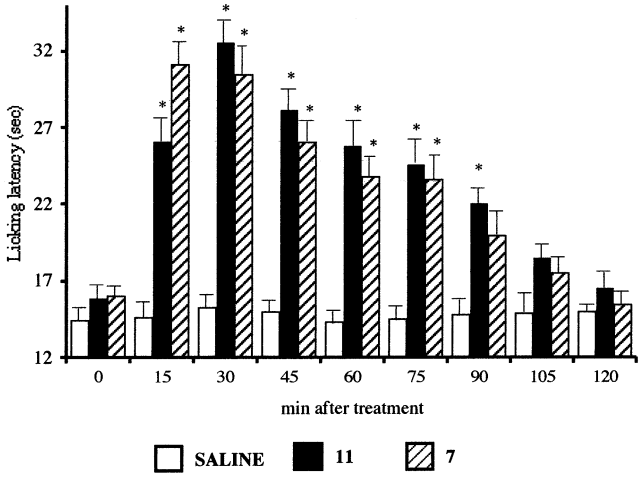


Figure 2. Antinociceptive effect of **7** (10 μg per mouse icv) and **11** (20 μg per mouse icv) in the mouse hot-plate test. Vertical lines give SEM. Each point is the mean of at least 12 mice. The asterisk (*) represents $P < 0.01$ in comparison with saline-treated mice.

are endowed with good analgesic activity, comparable to that exerted by morphine. Furthermore, with the exception of **5c** and **6**, these derivatives exert their

analgesic activity through a partial or complete activation of the monoaminergic system.

For the structure−activity relationships (SARs), the data obtained in the hot-plate test clearly suggest that the activity of this series does not depend on the presence of an isoxazolo[3,4-*d*]- (**5a**−**c**) or [4,5-*d*]-fused system (**11**). Likewise, when the substructure arylpiperazinylpropyl is linked to a functionalized pyridazinone, good results were obtained with different functional groups at position 5 (compounds **6**−**8**).

Thus, it seems that the side chain plays a prominent role in determining the antinociceptive activity of the present series. Since compound **F** is the most potent antinociceptive agent in a large series of isothiazolo-[5,4-*b*]pyridines, it seems to confirm this hypothesis.

In conclusion, in this study we identified a group of potent antinociceptive agents that are active in the hot-plate test with an efficacy comparable to or higher than that of morphine. Interestingly, these compounds showed a good MNTA/MAD ratio, which in some cases achieved a value of 200.

Experimental Section

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. 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. Reagents and starting materials **1a**−**c** and **2** were commercially available.

Ethyl 4-ethyloxoacetate-5-methylisoxazole-3-carboxylate, 3c. To a cooled (0 °C) and stirred solution of sodium ethoxide, obtained from sodium (15 mmol) and anhydrous ethanol (30 mL), a solution of ethyl 2,4-dioxopentanoate **1c** (15 mmol) in the same solvent (15 mL) was slowly added. A solution of ethyl chloro(hydroximino)acetate **2** (15 mmol) in anhydrous EtOH (10 mL) was added dropwise. The mixture, neutralized with 6 N HCl, was evaporated to afford **3c**, which was purified by column chromatography using cyclohexane/ethyl acetate 1:2 as eluent. Yield = 73%; oil; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 6H, $2CH_2CH_3$), 2.70 (s, 3H, CCH_3), 4.40 (q, 4H, $2CH_2CH_3$).

Ethyl [(6,7-dihydro-3-methyl-7-oxoisoxazole[3,4-*d*]pyridazinyl]-4-carboxylate, 4c. To a solution of **3c** (0.2 mmol) in EtOH (2 mL), hydrazine hydrate (0.4 mmol) was added, and the mixture was stirred at room temperature for 10 min. The precipitate **4c** was recovered by suction. Yield = 65%; mp = 189−190°C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.50 (t, 3H, CH_2CH_3), 3.05 (s, 3H, CCH_3), 4.50 (q, 2H, CH_2CH_3).

General Procedure for 5a−c. A mixture of isoxazolopyridazinones **4a**−**c**[22,23] (0.1 mmol), anhydrous K_2CO_3 (0.5 mmol), and 1-(3-bromopropyl)-4-(3-chlorophenyl)piperazine (0.4 mmol) in anhydrous DMF (2 mL) was heated under stirring for 2−6 h at 60−70 °C. After dilution with cold water (20−30 mL), compounds **5a**,**b** were recovered by suction. For compound **5a**, the suspension was extracted with CH_2Cl_2 (3 × 15 mL) and the solvent was evaporated in vacuo to afford a crude precipitate.

6-{[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3,4-dimethylisoxazolo[3,4-*d*]pyridazin-7-(6*H*)-one, 5a: yield = 51%; mp = 100−102 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.15 (m, 2H, $CONCH_2CH_2CH_2N$), 2.55 (s, 3H, 4-CH_3), 2.65 (m, 6H, $CONCH_2CH_2CH_2N$ and 4H piperazine), 2.85 (s, 3H, 3-CH_3), 3.15 (m, 4H, piperazine), 4.20 (t, 2H, $CONCH_2CH_2CH_2N$), 6.80 (m, 3H, Ar), 7.15 (m, 1H, Ar). Anal. ($C_{20}H_{24}N_5O_2Cl$) C, H, N.

6-{[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3-methyl-4-phenylisoxazolo[3,4-*d*]pyridazin-7-(6*H*)-one, 5b: yield = 88%; mp = 112−115 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.15 (m, 2H, $CONCH_2CH_2CH_2N$), 2.55 (s, 3H, 4-CH_3), 2.60 (m, 6H, piperazine), 3.20 (m, 4H, $CONCH_2CH_2CH_2N$ and 2H piperazine), 4.30 (t, 2H, $CONCH_2CH_2CH_2N$), 6.80 (m, 3H, Ar), 7.25 (m, 1H, Ar), 7.60 (s, 5H, Ar). Anal. ($C_{25}H_{26}N_5O_2Cl$) C, H, N.

Ethyl 6-{[4-(3-chlorophenyl)piperazin-1-yl]propyl}-3-methylisoxazolo[3,4-*d*]pyridazin-7-(6*H*)-one-4-carboxylate, 5c: yield = 72%; mp = 98−100 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, CH_2CH_3), 2.15 (m, 2H, $CONCH_2CH_2CH_2N$), 2.55 (m, 6H, piperazine), 3.05 (s, 3H, CCH_3), 3.10 (m, 4H, $CONCH_2CH_2CH_2N$ and 2H piperazine), 4.30 (t, 2H, $CONCH_2CH_2CH_2N$), 4.50 (q, 2H, CH_2CH_3), 6.80 (m, 3H, Ar), 7.20 (m, 1H, Ar). Anal. ($C_{22}H_{26}N_5O_4Cl$) C, H, N.

5-Acetyl-4-amino-2-{[4-(3-chlorophenyl)piperazin-1-yl]-propyl}-6-methylpyridazin-3(2*H*)-one, 6. A mixture of **5a** (0.4 mmol), 10% Pd/C (80 mg), and ammonium formate (2 mmol) in EtOH (3 mL) was refluxed for 1 h. After addition of CH_2Cl_2 (4 mL) and filtration of charcoal, crude **6** was recovered by suction. Yield = 75%; mp = 125−126 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.10 (m, 2H, $CONCH_2CH_2CH_2N$), 2.50 (s, 3H, CCH_3), 2.55 (s, 3H, $COCH_3$), 2.70 (m, 4H, piperazine), 3.25 (m, 6H, $CONCH_2CH_2CH_2N$ and 4H piperazine), 4.15 (t, 2H, $CONCH_2CH_2CH_2N$), 6.90 (m, 2H, Ar), 7.25 (m, 2H, Ar). Anal. ($C_{20}H_{26}N_5O_2Cl$) C, H, N.

4-Amino-2-{[4-(3-chlorophenyl)piperazin-1-yl]propyl}-5-hydroxyethyl-6-methylpyridazin-3(2*H*)-one, 7. To a solution of **6** (0.2 mmol) in methanol (3 mL), sodium borohydride (1.6 mmol) was added portionwise under stirring at room temperature. After 1 h, the mixture was concentrated, diluted with water (15 mL), and extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the desiderated **7**. Yield = 57%; mp = 132−134 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.55 (d, 3H, $CH(OH)CH_3$), 2.10 (m, 2H, $CONCH_2CH_2CH_2N$), 2.20 (s, 3H, CCH_3), 2.50 (t, 2H, $CONCH_2CH_2CH_2N$), 2.60 (m, 4H, piperazine), 3.20 (m, 4H, piperazine), 4.15 (t, 2H, $CONCH_2CH_2CH_2N$), 5.10 (q, 1H, $CH(OH)CH_3$), 5.85 (exch br s, 1H, OH), 6.50 (m, 3H, Ar), 7.30 (m, 1H, Ar). Anal. ($C_{20}H_{28}N_5O_2Cl$) C, H, N.

4-Amino-2-{[4-(3-chlorophenyl)piperazin-1-yl]propyl}-6-methyl-5-vinylpyridazin-3(2*H*)-one, 8. Compound **7** (0.5 mmol) was treated with PPA (50 mmol) at room temperature for 4 h. After dilution with water, the mixture was neutralized with 6 N NaOH and extracted with CH_2Cl_2 (3 × 20 mL). The residue was purified by column chromatography using $CHCl_3$/MeOH 9:1 as eluent.

Yield = 55%; mp = 86−88 °C; ^{1}H NMR ($CDCl_3$) δ 2.15 (m, 2H, $CONCH_2CH_2CH_2N$), 2.30 (s, 3H, CCH_3), 2.55 (s, 3H, $COCH_3$), 2.50 (t, 2H, $CONCH_2CH_2CH_2N$), 2.80 (m, 4H, piperazine), 3.20 (m, 4H, piperazine), 4.20 (t, 2H, $CONCH_2CH_2CH_2N$), 5.60 (d, 1H, J = 17.9 Hz, $CH{=}CH_2$), 5.70 (d, 1H, J = 12.1 Hz, $CH{=}CH_2$), 6.55 (dd, 1H, J = 12.1 Hz, J = 17.9 Hz, $CH{=}CH_2$), 6.90 (m, 2H, Ar), 7.30 (m, 2H, Ar). Anal. ($C_{20}H_{26}N_5OCl$) C, H, N.

5-(3-Bromopropyl)-3-methyl-7-phenylisoxazolo[4,5-*d*]-pyridazin-4(5*H*)-one, 10. A mixture of **9** (1.5 mmol), K_2CO_3 (7.2 mmol), and 1,3-dibromopropane (2.0 mmol) in anhydrous DMF (1 mL) was stirred at 60 °C for 1 h. After the mixture was cooled, water was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded **10**. Yield = 72%; mp = 108−110 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.45 (m, 2H, $CONCH_2CH_2CH_2N$), 2.75 (s, 3H, CCH_3), 3.50 (t, 2H, $CONCH_2CH_2CH_2Br$), 4.50 (t, 2H, $CONCH_2CH_2CH_2N$), 7.50 (m, 3H, Ar), 8.20 (m, 2H, Ar).

5-{[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3-methyl-7-phenylisoxazolo[4,5-*d*]pyridazin-4-(5*H*)-one, 11. A mixture of **10** (0.25 mmol), K_2CO_3 (1.3 mmol), and 3-chlorophenylpiperazine (0.6 mmol) in anhydrous DMF (1 mL) was stirred at 70 °C for 3 h. After dilution with cold water, the precipitate was recovered by suction and purified by column chromatography using cyclohexane/ethyl acetate 1:2 as eluent. Yield = 85%; mp = 102−103 °C (acetone); ^{1}H NMR ($CDCl_3$) δ 2.15 (m, 2H, $CONCH_2CH_2CH_2N$), 2.60 (m, 6H, $CONCH_2CH_2CH_2N$ and 4H piperazine), 2.75 (s, 3H, CCH_3), 3.20 (m, 4H, piperazine), 4.55 (t, 2H, $CONCH_2CH_2CH_2N$), 6.80 (m, 3H,

Ar), 7.15 (m, 1H, Ar), 7.55 (m, 3H, Ar), 8.10 (m, 2H, Ar). Anal. ($C_{25}H_{26}N_5O_2Cl$) C, H, N.

Biological Assays. Animals. Male Swiss albino mice (23−30 g) from Morini (San Polo d'Enza, Italy) breeding farm were used. Fifteen mice were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were kept at 23 ± 1 °C with a 12 h light/dark cycle, light at 7 a.m., with food and water ad libitum. All experiments were carried out according to the guidelines of the European Community Council.

Hot-Plate Test. The method adopted was described by O'Callaghan and Holzman.[26] Mice were placed inside a stainless steel container thermostatically set at 52.5 ± 0.1 °C in a precision water bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stopwatch before and at regular intervals up to a maximum of 60 min after treatment. The endpoint used was the licking of the fore or hind paws. Those mice scoring below 12 and over 18 s in the pretest were rejected (30%). An arbitrary cutoff time of 45 s was adopted.

Abdominal Constriction Test. Mice were injected ip with a 0.6% solution of acetic acid (10 mL kg^{-1}), according to the procedure of Koster et al.[27] The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.

Drugs. The following drugs were used: reserpine (Ciba-Geigy), morphine hydrochloride (SALARS). Other chemicals were of the highest quality commercially available. All drugs were dissolved in isotonic (0.9% NaCl) saline solution or dispersed in 1% sodium carboxymethylcellulose immediately before use except reserpine, which was dissolved in a 20% solution of ascorbic acid. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 mL kg^{-1} by the subcutaneous (sc) or per os (po) route or 5 μL by the intracerebroventricular (icv) route. The icv administration was performed under ether anaesthesia using isotonic saline as solvent, according to the method described by Haley and McCormick.[28] Briefly, during anaesthesia, mice were grasped firmly by the loose skin behind the head. A 0.4 mm external diameter hypodermic needle attached to a 10 μL syringe was inserted perpendicularly through the skull at a depth of no more than 2 mm into the brain of the mouse where an amount of 5 μL was then administered. The injection site was 1.5 mm from either side of the midline on a line drawn to the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice were icv-injected with 5 μL of diluted 1:10 India ink and their brains were examined macroscopically after sectioning.

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