Arylpiperazinylalkylpyridazinones and Analogues as Potent and Orally Active Antinociceptive Agents: Synthesis and Studies on Mechanism of Action

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A number of arylpiperazinylalkylpyridazinones structurally related to the previously described lead **A** (5-{[4-(3-chlorophenyl)piperazin-1-yl]-propyl}-3-methyl-7-phenylisossazolo[4,5-*d*]pyridazin-4-(5*H*)-one) were synthesized and tested for their analgesic activity. Many of the tested molecules, at the dose of 20 mg kg^{-1} p.o., showed high antinociceptive activity, in particular, compounds **5a**, **11c**, **15a**, **21** and **22**, which were able to reduce the number of abdominal constrictions by more than 50% in writhing test. The pharmacological investigation of lead **A** led us to clarify the mechanism of action of this compound, showing that it carries out its analgesic action through the inhibition of reuptake of noradrenaline. The antinociception of some of the most interesting new molecules was completely prevented by pretreatment with α_2-antagonist yohimbine, suggesting the involvement of α_2-adrenoceptors, as with prototype **A**.

Introduction

The identification of compounds able to treat both acute and chronic pain states with limited side effects is one of the prominent goals in biomedical research. In fact, the clinical use of the two major classes of analgesic drugs, nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids, is associated with important side effects, which include gastrointestinal lesions and nefrotoxicity (NSAIDs),[1] respiratory depression, tolerance, and physical dependence (opioids).[2] The identification and characterization of an inducible form of cyclooxygenase (COX-2)[3] led to the development of more selective NSAIDs with fewer unwanted side effects.[4] Recent results demonstrated that these drugs represent a good choice for patients at high risk of serious gastrointestinal complications, but not for patients suffering from cardiovascular diseases.[5−7]

Finally, a particular type of pain, the neuropathic pain, is very difficult to treat since it is often refractory to conventional analgesic drugs, with the exception of particular opioids, which may be used with success in some cases.[8] Neuropathic pain is a very complex phenomenon that involves several mechanisms both in the peripheral and in the central nervous system, and it is characterized by burning pain together with hyperalgesia and allodynia. At present, the first-line treatment options for this pathology are represented by the so-called "analgesic adjuvants", such as antidepressants, anticonvulsivants, and local anaesthetics (gabapentin, lidocaine, tramadol, nortriptyline, doxepine).[9] Many drugs belonging to different therapeutic classes, such as NMDA receptor antagonists, α_2-agonists, nicotinic, and adenosine receptor agonists, are in development as promising agents for the treatment of neuropathic pain.[10,11]

Previous studies in the field of antinociceptive agents[12−16] have led us to identify a group of potent antinociceptive compounds that are active in the hot-plate test, with an efficacy comparable to that of morphine in the same test and showing a very good MNTD/MAD ratio (MNTD = maximal nontoxic dose; MAD = minimal analgesic dose).[17] The most interesting term, the 5-{[4-(3-chlorophenyl)piperazin-1-yl]propyl}-3-methyl-7-phenylisoxazolo[4,5-*d*]pyridazinon-4-(5*H*)-one **A**, showed an MNTD/MAD ratio value of 200 in the hot plate test, an efficacy of 104% with respect to morphine, and it was able to almost completely abolish abdominal constrictions 15 and 30 min after administration at a dose of 20 mg/kg, sc (writhing test). Because a similar effect was observed after icv administration, it is reasonable to suppose that its site of action is within the central nervous system. Moreover, the analgesia induced by compound **A** (Figure 1) was completely prevented by pretreatment with reserpine, indicating that the antinociceptive effect is mediated by a partial or complete activation of the monoaminergic system.[17]

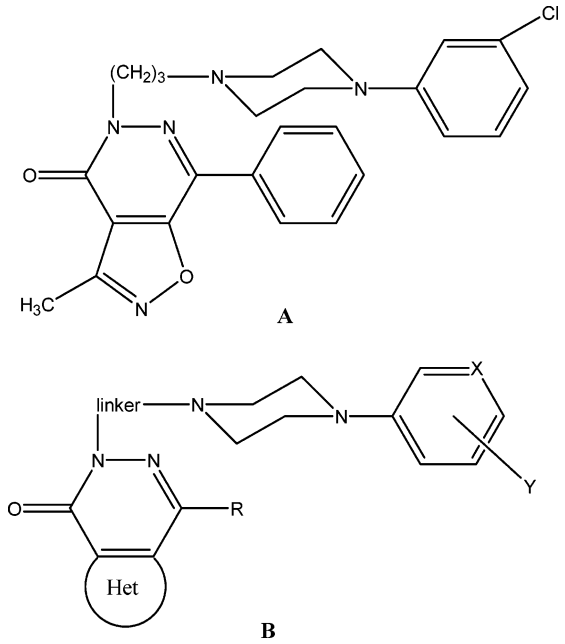

Figure 1. Lead **A** and general structure of compounds **B**.

On this basis, we designed and synthesized a new series of derivatives bearing an arylpiperazinyl moiety linked to different

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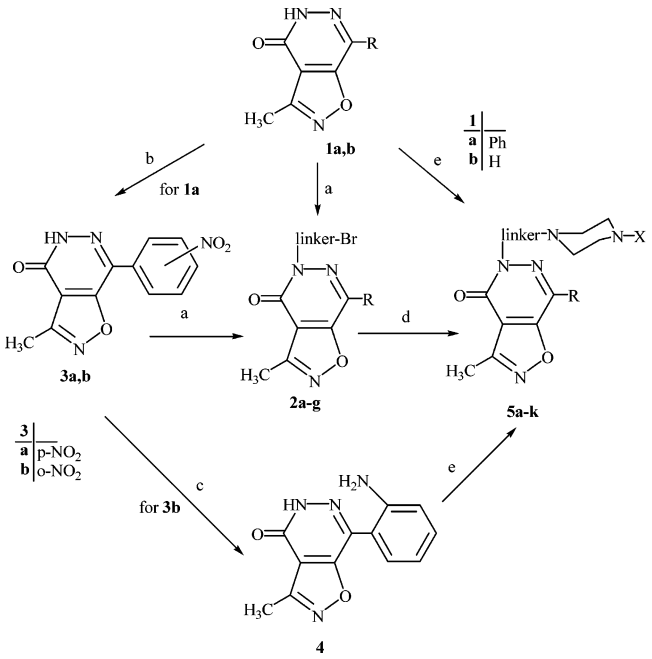
Scheme 1[a]



[a] Reagents and conditions: (a) Br-alkyl-Br, K_2CO_3, anhydrous DMF, T = 60 °C, 1−2 h; (b) H_2SO_4/HNO_3 1:1 v/v, 0 °C, 15 min; (c) $SnCl_2$, concd HCl, rt, 12h; (d) substituted arylpiperazine, K_2CO_3, anhydrous DMF, rt−60 °C, 5−15 h; (e) 1-(3-haloalkyl)-4-(3-chlorophenyl)-piperazine, K_2CO_3, anhydrous DMF, rt, 4h.

heterocyclic systems through (functionalized)alkyl chains (compounds **B**) and we evaluated all compounds for their ability to produce an analgesic effect. To define the role played by the different structural elements (arylpiperazinyl moiety, linker, and heterocyclic system) for the analgesic activity, we performed a relevant number of modifications. Thus, in addition to the previously investigated isoxazolo[4,5]pyridazinone, at the heterocyclic system level we explored phtalazinone, isoindole-1,3-dione, pyrazolo[3,4-*d*]pyridazinone, and pyrido[2,3-*d*]pyridazinone systems and, at the spacer level, we performed elongation, branching, and functionalization.

At the same time, we investigated in greater depth and elucidated the mechanism of action of lead compound **A**.

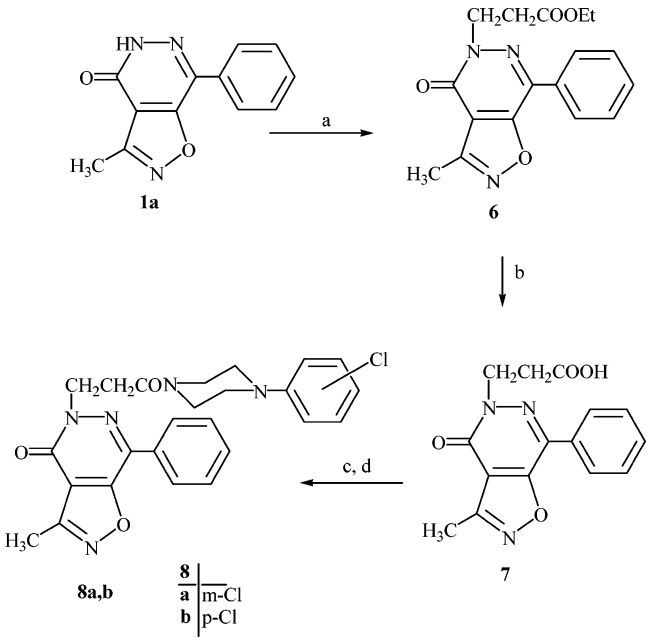
Chemistry

The synthetic pathways followed to obtain the final compounds are depicted in Schemes 1−7.

The synthesis of isoxazolo[4,5-*d*]pyridazinones **5a**−**k** is reported in Scheme 1 (for compounds **2** and **5** see Table 1 and Table 2), and it was performed starting from the precursors **1a**,**b**, previously described by Chantegrel and co-workers.[18] Compound **1b** (R═H), is fully characterized in the present work; the above paper reports its identification by ^{1}H NMR signals, but not its purification.

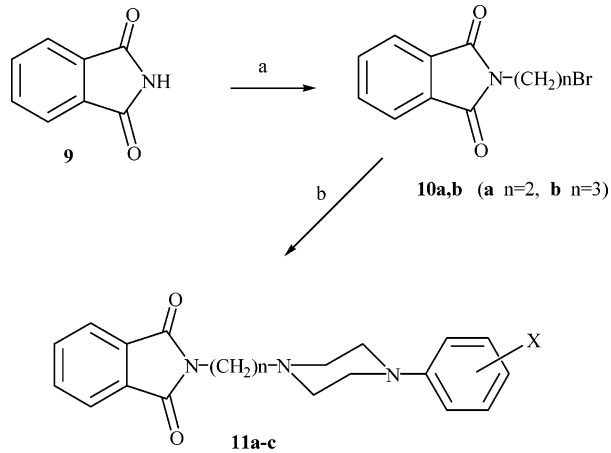
Treatment of **1a** with a mixture of H_2SO_4/HNO_3 1:1 v/v at 0 °C afforded a mixture of the intermediates **3a**,**b**, which were separated by column chromatography; compound **3b** was treated with $SnCl_2$ in concd HCl to give the *o*-amino derivative **4.** Starting from the above-described intermediates (**1**, **3**, and **4**), the introduction of the alkylpiperazinylaryl fragment was performed in two different ways: (1) by a direct alkylation with 1-(3-haloalkyl)-4-(3-chlorophenyl)-piperazine, prepared by condensing 3-chlorophenylpiperazine with 1-bromo-3-halopropane (route e for compounds **5g** and **5k**); and (2) by a two-step procedure (routes a and d), which consists of the synthesis of the *N*-alkylated intermediates **2a**−**g**, followed by condensation

Scheme 2[a]



[a] Reagents and conditions: (a) ethyl 3-bromopropionate, K_2CO_3, anhydrous DMF, rt, 15h; (b) 6 N NaOH, EtOH, rt, 15h; (c) $SOCl_2$, NEt_3, T = 60 °C, 2 h; (d) chlorophenylpiperazine, anhydrous THF, rt, 2 h.

Scheme 3[a]



11	n	X
a	2	p-F
b	3	m-Cl
c	3	p-F

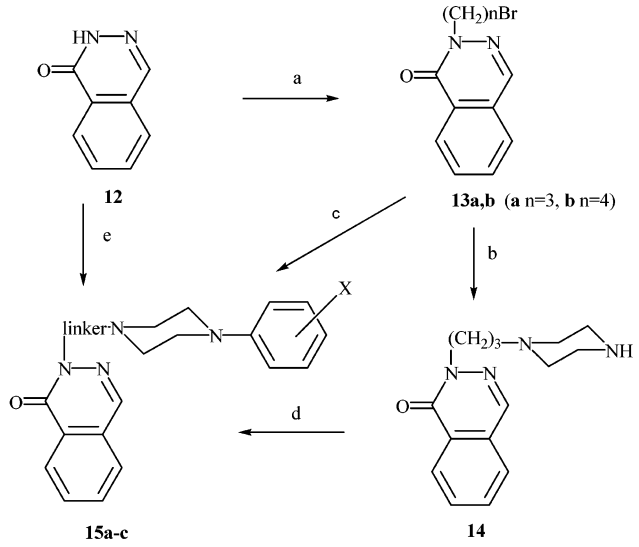
[a] Reagents and conditions: (a) Br-alkyl-Br, K_2CO_3, anhydrous DMF, rt, 90−120 min; (b) halophenylpiperazine, K_2CO_3, anhydrous DMF, rt, 15 h.

with the appropriate substituted arylpiperazine in anhydrous DMF.

Isoxazolo[4,5-*d*]pyridazinones **8a**,**b**, bearing a functionalized chain at N-5, were prepared as reported in Scheme 2, starting from the precursor **1a** and performing an alkylation with ethyl 3-bromopropionate. Intermediate **6** was converted into the corresponding carboxylic acid (compound **7**), which, by treatment with $SOCl_2$, followed by condensation with the appropriate chlorophenylpiperazine in anhydrous THF at room temperature, afforded the final **8a**,**b**.

In Scheme 3 is reported the synthesis of isoindole-1,3-dione derivatives **11a**−**c**, which were prepared starting from commercially available compound **9**. This precursor was alkylated

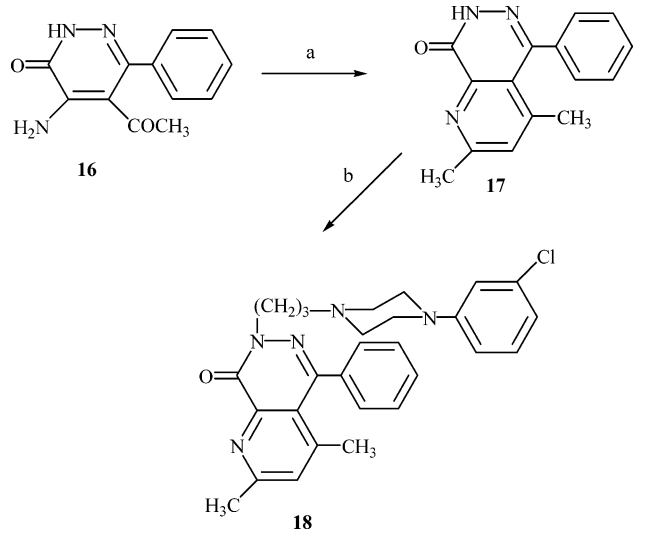
Scheme 4[a]



15	linker	X	route
a	$(CH_2)_3$	m-Cl	e
b	$CH_2CH(CH_3)CH_2$	m-Cl	e
c	$(CH_2)_3$	p-Cl	a, c
d	$(CH_2)_4$	m-Cl	a, c
e	$(CH_2)_3$	m-CNPh	a, b, d

[a] Reagents and conditions: (a) $Br(CH_2)_nBr$, K_2CO_3, anhydrous DMF, rt, 2 h; (b) piperazine, K_2CO_3, anhydrous DMF, rt, 15 h; (c) *m*- and *p*-chlorophenylpiperazine, K_2CO_3, anhydrous DMF, rt, 15 h; (c) *m*- and *p*-chlorophenylpiperazine, K_2CO_3, anhydrous DMF, rt, 2 h; (d) *m*-CN-phenylboronic acid, $Cu(AcO)_2$, anhydrous CH_2Cl_2, NEt_3; (e) appropriate 1-(3-haloalkyl)-4-(chlorophenyl)piperazine, K_2CO_3, anhydrous DMF, rt, 2 h.

Scheme 5[a]

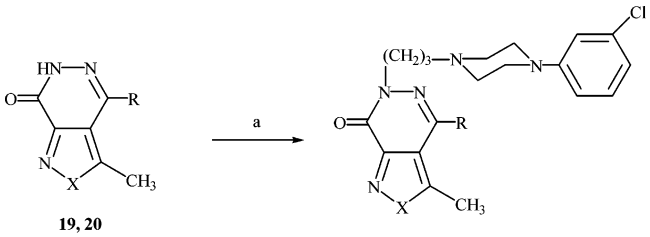


[a] Reagents and conditions: (a) anhydrous acetone, EtONa, abs EtOH, T = 90 °C, 12 h; (b) 1-(3-bromopropyl)-4-(3-chlorophenyl)-piperazine, K_2CO_3, anhydrous DMF, rt, 15 h.

to afford **10a**,**b** which, in turn, were condensed with the appropriate arylpiperazine in standard conditions to give **11a**−**c**.

The final compounds **15a**−**e** (Scheme 4) were obtained from the commercially available 2*H*-phtalazin-1-one **12**. For compounds **15a**,**b**, the alkylpiperazinylaryl substructure, prepared by condensing the appropriate arylpiperazine with the opportune alkyl halide, was inserted in one step (route e). The synthesis of compounds **15c**−**e** was performed through the intermediates **13a**,**b**, which were transformed into the final compounds (**15c**,**d**)

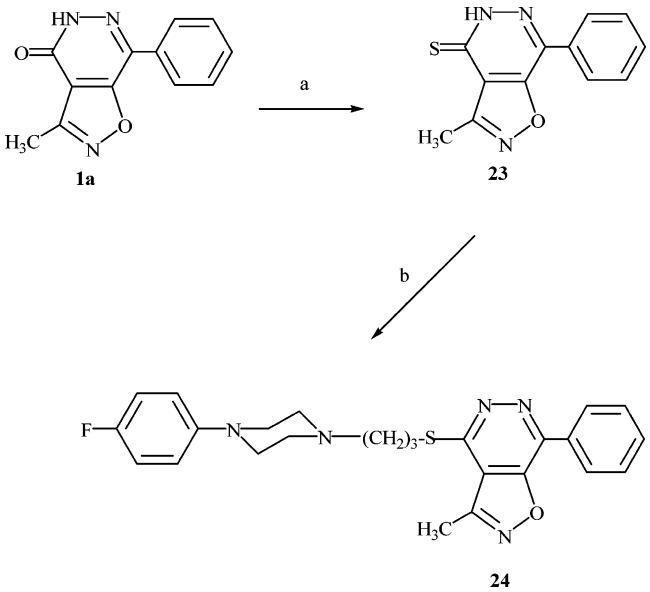
Scheme 6[a]



comp.	R	X
19, 21	2-thienyl	O
20,22	CH_3	N-Ph

[a] Reagents and conditions: (a) 1-(3-bromopropyl)-4-(3-chlorophenyl)-piperazine, K_2CO_3, anhydrous DMF, T = 60−90 °C, 1.5-5 h.

Scheme 7[a]



[a] Reagents and conditions: (a) Lawesson's reagent, toluene; (b) 1-(3-bromopropyl)-4-(4-fluororophenyl)-piperazine, K_2CO_3, anhydrous acetone, rt, 4 h.

Table 1. Legend for Compounds **2**

2	R	linker
a	H	$(CH_2)_3$
b	H	$(CH_2)_4$
c	Ph	$(CH_2)_2$
d	Ph	$(CH_2)_3$
e	Ph	$(CH_2)_4$
f	*o*-NO_2Ph	$(CH_2)_3$
g	*p*-NO_2Ph	$(CH_2)_3$

by condensation with the appropriate substituted phenylpiperazine in anhydrous DMF (routes a and c). To obtain compound **15e**, the intermediate **13a** was condensed with piperazine leading compound **14**, which, in turn, was treated with *m*-CN-phenylboronic acid, $Cu(AcO)_2$ in anhydrous CH_2Cl_2 (routes a, b, and d).

In Schemes 5 and 6, the synthetic pathways affording products **18**, **21**, and **22** are described.[19−21] These final compounds exhibit the same propyl-4-(3-chlorophenyl)-piperazine fragment of the lead **A**, but they differ from this latter for the heterocondensed system, which is a pyrido[2,3-*d*]pyridazin-8-one (compound **18**), an isoxazolo[3,4-*d*]pyridazin-7(6*H*)-one (compound **21**) or a pyrazolo[3,4-*d*]pyridazin-7-one (compound **22**). For all compounds, the starting products have been previously described in literature,[19−21] with the exception of intermediate **17**, which

Table 2. Legend for Compounds **5**

5	R	linker	X
a	H	$(CH_2)_3$	*m*-ClPh
b	H	$(CH_2)_4$	*m*-ClPh
c	Ph	$(CH_2)_3$	*p*-FPh
d	Ph	$(CH_2)_3$	2-Py
e	Ph	$(CH_2)_3$	*p*-ClPh
f	Ph	$(CH_2)_4$	*m*-ClPh
g	Ph	$CH_2CH(CH_3)CH_2$	*m*-ClPh
h	Ph	$(CH_2)_2$	*m*-ClPh
i	*o*-NO_2Ph	$(CH_2)_3$	*m*-ClPh
j	*p*-NO_2Ph	$(CH_2)_3$	*m*-ClPh
k	*o*-NH_2Ph	$(CH_2)_3$	*m*-ClPh

was obtained by treatment of **16**[19] with anhydrous acetone and EtONa in absolute ethanol in the sense of Friedlander's synthesis; the condensation of **17**, **19**,[20] and **20**[21] with the piperazinyl substructure was performed in standard conditions using K_2CO_3 in anhydrous DMF, as depicted in Scheme 1.

Finally, to define the importance of the lactamic function for the antinociceptive activity, we synthesized compound **24** (Scheme 7), bearing the alkylpiperazinyl chain linked at position 4 of the pyridazine ring. Starting compound **1a** was transformed into the corresponding 4-thione derivative (**23**), using Lawesson's reagent in toluene. Treatment of **23** with 1-(3-bromopropyl)-4-(fluorophenyl)-piperazine[22] regioselectivity afforded the final compound **24**.

Results and Discussion

In the present study, the potential antinociceptive activity of the investigated compounds was tested by two different experimental models: the abdominal constriction test, in which a painful chemical stimulus was applied, and the hot plate test, in which an acute thermal stimulus was used.

Compounds **5a**, **5h**, **8a**, **11a**, **11c**, **15a**, **15c**, **15e**, **18**, **21**, and **22** were able to reduce the number of abdominal constrictions, in particular **15a** which, tested at 20 mh kg^{-1} p.o., was the most potent in this series (Table 3). Compound **15e** induced a good antinociceptive effect at 10 mg kg^{-1} p.o. but, unfortunately, when tested at higher doses, it induced a reduced spontaneous mobility in animals. The same effect was observed for compound **5b**, which was tested only at 5 mg kg^{-1} p.o. Compounds that showed the highest intensity of antinociceptive activity were **5a**, **11c**, **15a**, **18**, **21**, and **22** being able to reduce by more than 50% the number of abdominal constrictions. Conversely, compounds **5b**, **5c**, **5d**, **5e**, **5f**, **5g**, **5i**, **5j**, **5k**, **6**, **11b**, **15b**, **15d**, and **24** were devoid of any effect in the abdominal constriction test.

From the inactive and less-active compounds in the abdominal constriction test, we choose several products (**5c**, **5d**, **5k**, **11b**, **6**, **5h**, **8b**), and we also performed the hot plate test to unmask a potential antinociceptive activity against an acute thermal stimulus. Compounds **5c**, **5d**, **5h**, **5k**, **6**, **8b**, and **11** were all unable to increase the pain threshold in the mouse hot plate test (Table 3). At the same time, to further confirm the antinociceptive profile of some promising molecules, we also tested compounds **5a**, **11c**, and **15c** in the hot plate test. They showed an antinociceptive activity comparable to that observed in the writhing test.

Previously we demonstrated that the analgesia induced by compound **A**, which was the prototype of this series, was completely prevented by pretreatment with reserpine, indicating that the antinociceptive effect is mediated by a partial or complete activation of the monoaminergic system.[17] To clarify whether the noradrenergic and/or serotoninergic systems were involved in the mechanism of action of compound **A**, we

Table 3. Antinociceptive Effect of Final Compounds in the Writhing Test and Hot Plate Test[a]

treatment	dose (mg kg^{-1})	no. of writhes in the absence of yohimbine	no. of writhes in the presence of yohimbine	licking latency
CMC		34.5 ± 2.2		15.3 ± 1.7
yohimbine	3		35.9 ± 3.7	15.8 ± 1.5
5a	20	11.9 ± 3.4[b]	27.4 ± 3.5	27.7 ± 1.9[b]
5b	5	27.2 ± 3.5		
5c	30	39.2 ± 5.0		17.6 ± 1.8
5d	30	30.2 ± 5.1		15.1 ± 2.1
5e	20	27.1 ± 2.4		
5f	20	30.6 ± 3.6		
5g	20	35.5 ± 2.7		
5h	20	23.6 ± 2.8[b]	37.2 ± 4.3	15.4 ± 2.0
5i	20	31.7 ± 4.4		
5J	20	26.8 ± 2.5		
5k	20	28.0 ± 3.1		16.5 ± 2.2
6	20	32.7 ± 5.3		17.4 ± 1.5
8a	20	22.1 ± 4.2[b]	41.3 ± 4.4	
8b	20	23.5 ± 3.4[b]	45.4 ± 3.8	17.2 ± 2.5
11a	20	22.6 ± 3.2[b]	37.9 ± 4.1	
11b	20	34.9 ± 4.4		16.2 ± 2.0
11c	20	19.4 ± 3.8[b]		36.5 ± 3.6[b]
15a	20	8.2 ± 2.5[b]	31.8 ± 4.2	28.0 ± 2.2[b]
15b	20	36.7 ± 4.0		
15c	20	23.9 ± 3.2[b]		
15d	20	28.5 ± 4.1		
15e	10	17.2 ± 2.9[b]		
18	20	18.0 ± 2.6[b]		
21	20	16.2 ± 3.4[b]		
22	20	10.3 ± 3.9[b]		
24	20	28.6 ± 4.6		

[a] All drugs were administered per os 30 min before test. [b] $P < 0.01$ versus CMC treated-mice. Each value represents the mean of at least two experiments.

Table 4. Effect of Yohimbine, BRL-44408, and ARC-239 on Antinociception Induced by Compound **A** in the Abdominal Constriction Test

treatment[a]	No. of mice	abdominal constrictions
saline/CMC	15	31.8 ± 3.2
yohimbine + CMC	12	28.5 ± 3.6
BRL-44408 + CMC	11	32.9 ± 2.7
saline + **A**	10	11.6 ± 3.3[b]
yohimbine + **A**	12	27.8 ± 4.1
BRL−44408 + **A**	12	33.4 ± 4.6
ARC−239 + **A**	10	9.6 ± 3.8*

[a] Yohimbine was tested at 3 mg/kg i.p., BRL-44408 was tested at 1 mg/kg i.p., ARC-239 was tested at 10 mg/kg i.p., and compound **A** was tested at 1 mg/kg s.c. in the abdominal constriction test. [b] $P < 0.05$ in comparison with saline/CMC-treated mice

pretreated animals with the α_2-adrenoceptor antagonist yohimbine. The dose of yohimbine employed to prevent the analgesia induced by the above-reported compounds is the minimal dose able to antagonize antinociception induced by activation of α_2-adreniceptor since opioid, muscarinic, and GABAergic antinociception is not modified.[23] A complete reversal of the antinociceptive effect of compound **A** was evidenced in the mouse abdominal constriction test (Table 4). We demonstrated that the α_2-adrenoceptor subtype involved in the modulation of pain perception is the α_{2A}, whereas the subtype α_{2C} is devoid of any effect.[23,24] For this reason, we evaluated the effect produced by the administration of the selective α_{2A}-adrenoceptor antagonist BRL44408 and of the selective α_{2C}-adrenoceptor antagonist ARC239 on compound **A** analgesia. As illustrated in Table 4, only BRL44408 was able to completely antagonize compound **A** antinociception. Pretreatment with the α_1-adrenoceptor antagonist prazosin did not modify the increased pain

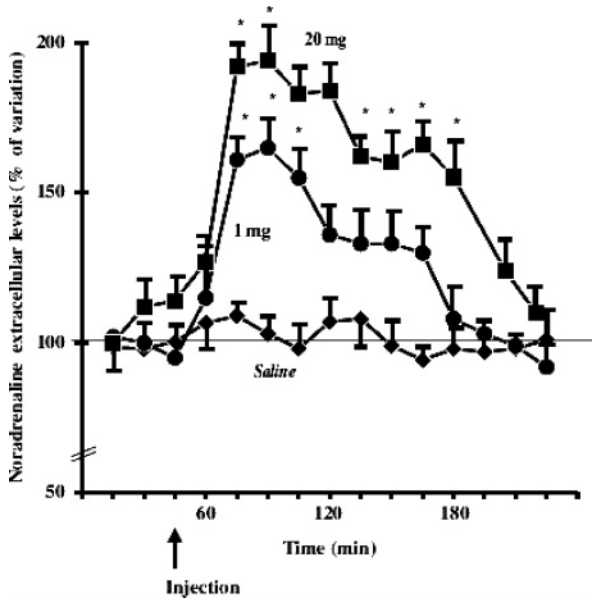


Figure 2. Effect of compound **A** (i.p.) on noradrenaline extracellular levels from rat cerebral cortex. The doses are expressed as mg kg^{-1}. *$P < 0.01$ in comparison with saline treated rats. Each point represents the mean of at least three rats.

threshold produced by compound **A**, further confirming, among the alpha subtypes, an α_2-mediated mechanism of action. The selective involvement of the α_{2A}-adrenoceptor subtype was also evidenced by the complete lack of antagonism exerted by some other receptor antagonists, like the opioid antagonist naloxone, the muscarinic antagonist atropine, and the GABA antagonist CGP35348 (data not shown).

In these experimental conditions, yohimbine, BRL 44408, and ACR 239 did not modify the pain threshold of mice in comparison with control animals. The lack of effect of these antagonists agrees with the results of studies in which these compounds did not modify the nociceptive threshold against either thermal (hot plate) or chemical (writhing) noxious stimuli.[25] Therefore, we can exclude the hypothesis that the prevention of compound **A** analgesia was due to a hyperalgesic effect of the α_1-adrenoceptor antagonist used.

The antinociceptive doses of compound **A** did not provoke any visible change in normal behavior of mice as demonstrated in rota-rod experiments in which no impairment of mouse rota-rod performance was observed (data not shown). Moreover, compound **A** was endowed with a good potency and efficacy, comparable to that exhibited by some well-known analgesic drugs clinically employed, such as diphenhidramine, baclofen, amitriptiline, and so on.

The α_{2A}-mediated analgesia can be induced by a direct α_{2A}-adrenoceptor activation or by an indirect α_{2A}-adrenoceptor system activation, such as an increase of noradrenaline release and/or inhibition of noradrenaline reuptake. To elucidate whether compound **A** is endowed with a direct or indirect noradrenergic mechanism, we performed α_2 binding experiments. In the rat cortical membranes, compound **A** showed a $K_i > 1$ μM, indicating the absence of affinity for α_2-adrenoceptors and ruling out a direct noradrenergic mechanism.

Noradrenaline extracellular levels from rat cerebral cortex were determined after administration of compound **A** at the doses of 1 and 20 mg kg^{-1} i.p.; a dose-dependent increase of noradrenaline contents was revealed (Figure 2). Also, an inhibition of the synaptosomal [^{3}H]-noradrenaline uptake in the presence of concentrations of compound **A** was observed ($IC_{50} = 1.3 \times 10^{-7}$ M). These results indicate that the analgesic action of compound **A** underlies an indirect activation of the noradrenergic system through an inhibition of the noradrenaline reuptake.

Because all the new molecules tested in the present study were derivatives of compound **A**, we choose as representative some of the most potent compounds to evaluate if their analgesic effect was mediated through an activation of the noradrenergic system. The antinociception of **5a**, **5h**, **8a**, **8b**, **11a**, and **15a** was completely prevented by pretreatment with the α_2-antagonist yohimbine (3 mg kg^{-1} i.p.), suggesting the involvement of α_2-adrenoceptors in the mechanism of analgesic action of the above-mentioned compounds (Table 3).

The highest doses investigated did not produce any alteration of the animals' gross behavior nor modify motor coordination, as revealed by the rota rod test, or spontaneous motility, as revealed by the hole board test (data not shown).

All these data clearly indicate that a relevant part of the synthesized compounds are endowed with potent antinociceptive effect by oral route, with a maximum of activity for **15a**, which was able to reduce by 75% the writhes number at 20 mg/kg. Moreover, the same compound, tested in the hot plate, exhibited a licking latency of 83% at the same dose. Thus, this compound appears to be a very interesting lead for further development, especially when taking into account the absence of behavioral effects at the maximum tested doses. Because the therapeutic use of the amitriptiline, a mechanism-related drug used in the management of neurophatic pain, is associated with several unwanted effects, **15a** may have some possibilities for further pharmacological evaluation.

Taking into account the fact that the tested compounds were administered by oral route and, therefore, that ADME properties may influence the obtained results for all of them, some SARs can be inferred. For the series of isoxazolopyridazinones **5a**−**k**, which are structurally related to the prototype **A**, the obtained data indicated that both the three carbon chain of the spacer and the *m*-chlorophenyl moiety placed at the end of the side chain are essential requirements for antinociceptive activity. In fact, when a phenyl group is appended in position 7 of the heterocyclic core, as in compound **A**, elongation of (**5f**) and branching (**5g**) of the spacer are detrimental for the activity. The same result is associated with some modifications of the phenyl at position 7, being the *ortho*-and *para*-nitro derivatives **5i** and **5j** and the *o*-amino derivative **5k** devoid of activity. Moreover, when in the prototype **A** the chlorine at Ar is shifted to position para (**5e**), a reduced activity was observed; furthermore, by replacing the chlorine with a fluorine (**5c**), a complete loss of activity was evidenced. Replacement of 3-chlorophenyl with 3-pyridyl (**5d**) led to the same result. The only tolerated modification with respect to **A** was, quite surprisingly, the replacement of the 7-phenyl with hydrogen, which led to one of the most active compounds (**5a**).

When the tertiary amino group of the piperazine was transformed into an amidic nitrogen, the activity was maintained (compounds **8a** and **8b**). This finding suggests that the three-carbon chain plays an essential role as spacer and that the piperazine nitrogen does not interact with the biological target in a protonated form. It is interesting to observe that, in this series, when moving the chlorine from the position meta to the position para, the antinociceptive activity remained unmodified.

In the series of phthalimide derivatives, different SARs can be evidenced, with the *m*-chlorophenyl derivative ($n = 3$; **11b**) being the only inactive term and the *p*-fluorophenyl analogue being active both with $n = 2$ (**11a**) and $n = 3$ (**11c**).

In the series of the phthalazinone derivatives, the classical SARs are partially restored, with *m*-chlorophenyl and $n = 3$ (**15a**) being important requirements for the activity. Also, in this series, the shift of the chlorine to the para position (**15c**) or its replacement with a pseudo halogen (**15e**) maintained the activity.

Finally, the interesting antinociceptive activity found for compounds **18**, **21**, and **22** seems to confirm that, when the lactamic nitrogen is substituted with the 3-chlorophenylpiperazinylpropyl chain, both the fused system (pyridine, pyrazole, and isoxazole) and the group linked to the pyridazine (methyl, 2-thienyl, and phenyl) can be extensively modified without significant loss of activity.

Conclusion

The present results indicate the involvement of α_2-adrenoceptor and, in particular, of the subtype α_{2A}. Compound **A** increases the pain threshold, and this effect is imputable to an amplification of adrenergic neurotransmission due to a inhibition of noradrenaline uptake.

Experimental Section

Chemistry. All melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Avance 400 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 **9** and 1**2** were commercially available.

3-Methyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 1b. To a cooled and stirred solution of 5-formyl-3-methylisoxazole-4-carboxylic acid ethyl ester[18] (2.7 mmol) in EtOH (3 mL), hydrazine hydrate (26 mmol) was added. The mixture was stirred for 1 h at room temperature, and the crude precipitate was recovered by suction. Yield = 53%; mp = 214−217 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 3H, CH_3), 8.35 (s, 1H, 7-H), 11.3−11.5 (exch br s, 1H, NH).

General Procedure for 2a−g. A mixture of isoxazolo[4,5-*d*]-pyridazinones **1a**,**b**[18] or **3a**,**b** (0.1 mmol), anhydrous K_2CO_3 (0.2 mmol), and the appropriate alkyl dibromide (0.20 mmol) in anhydrous DMF (2 mL) was heated under stirring for 0.5−2 h at room temperature. After dilution with cold water (20−30 mL), the mixture was extracted with CH_2Cl_2 (3 × 15 mL), and the solvent was evaporated in vacuo to afford compounds **2a**−**g** (compound **2d** was previously described[17]), which were purified by column chromatography. (eluents: cyclohexane/ethyl acetate 2:1 for compounds **2a**,**c**,**f**; cyclohexane/ethyl acetate 1:1 for **2g**; and cycloexane/ethyl acetate 1:3 for **2b)**.

5-(3-Bromopropyl)-3-methyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 2a. Yield = 31%; mp = 59−62 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.40 (m, 2H, $CH_2CH_2CH_2$), 2.70 (s, 3H, CH_3), 3.45 (t, 2H, CH_2CH_2Br), 4.40 (t, 2H, $CONCH_2$), 8.30 (s, 1H, Ar).

5-(4-Bromobutyl)-3-methyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 2b. Yield = 38%; oil; ^{1}H NMR ($CDCl_3$) δ 1.90−2.10 (m, 4H, $NCH_2CH_2CH_2CH_2$), 2.70 (s, 3H, CH_3), 3.45 (t, 2H, CH_2CH_2Br), 4.30 (t, 2H, $CONCH_2$), 8.30 (s, 1H, Ar).

5-(2-Bromoethyl)-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 2c. Yield = 71%; mp = 145−147 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, CH_3), 3.85 (t, 2H, CH_2CH_2Br), 4.75 (t, 2H, $CONCH_2$), 7.50 (m, 3H, Ar), 8.15 (m, 2H, Ar).

5-(4-Bromobutyl)-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 2e. Yield = 44%; mp = 82−84 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.95−2.15 (m, 4H, $NCH_2CH_2CH_2CH_2$), 2.75 (s, 3H, CH_3), 3.50 (t, 2H, CH_2CH_2Br), 4.40 (t, 2H, $CONCH_2$), 7.50−7.60 (m, 3H, Ar), 8.20 (m, 2H, Ar).

5-(3-Bromopropyl)-3-methyl-7-(2-nitrophenyl)-5*H*-isoxazolo-[4,5-*d*]pyridazin-4-one 2f. Yield = 35%; oil; ^{1}H NMR ($CDCl_3$) δ 2.40−2.50 (m, 2H, $CH_2CH_2CH_2$), 2.75 (s, 3H, CH_3), 3.45 (t, 2H, CH_2CH_2Br), 4.45 (t, 2H, $CONCH_2$), 7.75 (m, 1H, Ar), 7.80 (m, 2H, Ar), 8.20 (d, 1H, Ar).

5-(3-Bromopropyl)-3-methyl-7-(4-nitrophenyl)-5*H*-isoxazolo-[4,5-*d*]pyridazin-4-one 2g. Yield = 21%; oil; ^{1}H NMR ($CDCl_3$) δ 2.45−2.55 (m, 2H, $CH_2CH_2CH_2$), 2.75 (s, 3H, CH_3), 3.50 (t, 2H, CH_2CH_2Br), 4.55 (t, 2H, $CONCH_2$), 8.40 (m, 4H, Ar).

General Procedure for 3a,b. To a cooled ($T = 0$ °C) and stirred mixture of concd HNO_3 (1 mL) and concd H_2SO_4 (1 mL), 0.7 mmol of compound **1a** was added. The reaction was carried out at room temperature for 15 min and then the mixture was poured into ice water (40 mL) drop by drop to afford a mixture of 4-nitrophenyl and 2-nitrophenyl derivatives. Final compounds **3a** and **3b** were separated by column chromatography using WE7 as eluent. (WE7 = EtOH, 4.5 mL; NH_4OH, 0.25 mL; $CHCl_3$, 18 mL; Et_2O, 18 mL; and petroleum ether, 45 mL)

3-Methyl-7-(4-nitrophenyl)-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 3a. Yield = 22%; mp = >250 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 2.65 (s, 3H, CH_3), 8.30 (d, 2H, Ar), 8.40 (d, 2H, Ar), 10.15 (exch br s, 1H, NH).

3-Methyl-7-(2-nitrophenyl)-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 3b. Yield = 24%; mp = >250 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 3H, CH_3), 7.75 (t, 2H, Ar), 8,25 (d, 1H, Ar), 8.85 (t, 1H, Ar), 10.35 (exch br s, 1H, NH).

7-(2-Aminophenyl)-3-methyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 4. To a solution of **3b** (0.4 mmol) in concd HCl (0.5 mL), $SnCl_2$ (1.9 mmol) dissolved in concd HCl (0.5 mL) was added, and the reaction was carried out at room temperature for 12 h. The mixture was treated with ice water, basified with 6 N NaOH, and extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded compound **4**, which was purified by column chromatography using toluene/ethyl acetate 1:1 as eluent. Yield = 40%; mp = 246−250 °C; ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, CH_3), 6.85 (d, 1H, Ar), 6.90 (t, 1H, Ar), 7.30 (m, 1H, Ar), 8.05 (d, 1H, Ar), 10.50 (exch br s, 1H, NH).

General Procedure for 5a−f and 5h−j. A mixture of isoxazolo-[4,5-*d*]pyridazinones **2a**−**g** (0.4 mmol), anhydrous K_2CO_3 (0.8 mmol), and the appropriate (substituted)arylpiperazine (0.8 mmol), commercially available, in anhydrous DMF (4 mL) was stirred for 3−15 h at rt to 60 °C. After dilution with cold water (20−30 mL), the mixture was extracted with CH_2Cl_2 (3 × 15 mL) and the solvent was evaporated in vacuo to afford final compounds that were purified by column chromatography using cyclohexane/ethyl acetate 2:1 (**5a**,**h**), cyclohexane/ethyl acetate 1:3 (**5b**,**f**), cyclohexane/ethyl acetate 1:2 (**5c**−**e**), and cyclohexane/ethyl acetate 1:1 (**5i**,**j**) as eluents.

5-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3-methyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5a. Yield = 64%; mp = 104−106 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.05−2.20 (m, 2H, $CH_2CH_2CH_2$), 2.55−2.70 (m, 6H: 2H, CH_2CH_2N; 4H, piperazine), 2.70 (s, 3H, CH_3), 3.15−3.30 (m, 4H, piperazine), 4.35 (t, 2H, $CONCH_2$), 6.75−6.85 (m, 2H, Ar), 6.90 (s, 1H, Ar), 7.20 (t, 1H, Ar), 8.30 (s, 1H, Ar).

5-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-3-methyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5b. Yield = 52%; mp = 98−100 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.50−1.70 (m, 2H, $NCH_2CH_2CH_2CH_2$), 1.85−1.95 (m, 2H, $NCH_2CH_2CH_2CH_2$), 2.70−2.90 (m, 6H: 2H, CH_2CH_2N; 4H, piperazine), 2.70 (s, 3H, CH_3), 3.20−3.35 (m, 4H, piperazine), 4.30 (t, 2H, $CONCH_2$), 6.75−6.85 (m, 2H, Ar), 6.85 (s, 1H, Ar), 7.20 (t, 1H, Ar), 8.25 (s, 1H, Ar).

5-{3-[4-(4-Fluorophenyl)piperazin-1-yl]propyl}-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5c. Yield = 90%; mp = 117−120 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.10−2.20 (m, 2H, $CH_2CH_2CH_2$), 2.60 (m, 2H, CH_2N), 2.65 (m, 4H, piperazine), 2.75 (s, 3H, CH_3), 3.10−3.20 (m, 4H, piperazine), 4.45 (t, 2H, $CONCH_2$), 6.80−6.90 (m, 2H, Ar), 6.90−7.00 (m, 2H, Ar), 7.55 (m, 3H, Ar), 8.10−8.20 (m, 2H, Ar).

3-Methyl-7-phenyl-5-[3-(4-pyridin-2-yl-piperazin-1-yl)propyl]-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5d. Yield = 80%; mp = 110−

113 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.20−2.35 (m, 2H, $CH_2CH_2CH_2$), 2.55−2.70 (m, 6H: 2H, CH_2N; 4H, piperazine), 2.75 (s, 3H, CH_3), 3.50−3.70 (m, 4H, piperazine), 4.95 (t, 2H, $CONCH_2$), 6.60−6.70 (m, 2H, Ar), 7.50−7.60 (m, 4H, Ar), 8.15−8.25 (m, 3H, Ar).

5-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5e. Yield = 80%; mp = 120−123 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.15−2.25 (m, 2H, $CH_2CH_2CH_2$), 2.60−2.70 (m, 6H: 2H, CH_2N; 4H, piperazine), 2.75 (s, 3H, CH_3), 3.15−3.25 (m, 4H, piperazine), 4.45 (t, 2H, $CONCH_2$), 6.80 (d, 2H, Ar), 7.20 (d, 2H, Ar), 7.50−7.60 (m, 3H, Ar), 8.20 (m, 2H, Ar).

5-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5f. Yield = 41%; mp = 124−127 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.65−1.80 (m, 2H, $NCH_2CH_2CH_2CH_2$), 1.90−2.00 (m, 2H, $NCH_2CH_2CH_2CH_2$), 2.50−2.75 (m, 9H: 3H, CH_3; 2H, CH_2CH_2N; 4H, piperazine), 3.20−3.40 (m, 4H, piperazine), 4.40 (m, 2H, $CONCH_2$), 6.75−6.90 (m, 3H, Ar), 7.20 (m, 1H, Ar), 7.50−7.60 (m, 3H, Ar), 8.15 (d, 2H, Ar).

5-{2-[4-(3-Chlorophenyl)piperazin-1-yl]ethyl}-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5h. Yield = 51%; mp = 155−158 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.70−2.85 (m, 5H: 3H, CH_3; 2H, CH_2CH_2N), 2.90−3.10 (m, 4H, piperazine), 3.15−3.30 (m, 4H, piperazine), 4.50−5.60 (m, 2H, $CONCH_2$), 6.75−6.85 (m, 2H, Ar), 6.90 (s, 1H, Ar), 7.15 (s, 1H, Ar), 7.55 (m, 3H, Ar), 8.15 (d, 2H, Ar).

5-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3-methyl-7-(2-nitrophenyl)-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5i. Yield = 26%; oil; ^{1}H NMR ($CDCl_3$) δ 2.00−2.15 (m, 2H, $CH_2CH_2CH_2$), 2.50−2.70 (m, 6H: 2H, CH_2N; 4H, piperazine), 2.75 (s, 3H, CH_3), 3.10−3.30 (m, 4H, piperazine), 4.40 (t, 2H, $CONCH_2$), 6.75−6.85 (m, 2H, Ar), 6.90 (s, 1H, Ar), 7.20 (t, 1H, Ar), 7.70−7.80 (m, 1H, Ar), 7.75−7.85 (m, 2H, Ar), 8.15 (d, 1H, Ar).

5-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3-methyl-7-(4-nitrophenyl)-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5j. Yield = 52%; mp = 142−145 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.15−2.30 (m, 2H, $CH_2CH_2CH_2$), 2.55−2.75 (m, 6H: 2H, CH_2N; 4H, piperazine), 2.80 (s, 3H, CH_3), 3.15−3.25 (m, 4H, piperazine), 4.90 (t, 2H, $CONCH_2$), 6.75−6.85 (m, 2H, Ar), 6.90 (s, 1H, Ar), 7.20 (t, 1H, Ar), 8.35−8.45 (m, 4H, Ar).

General Procedure for 5g,k. The isoxazolopyridazinone **1a** or **4** (0.1 mmol) was condensed with the appropriate 1-(3-haloalkyl)-4-(3-chlorophenyl)piperazine (0.1−0.2 mmol)[17,27] in anhydrous DMF (1−3 mL) and K_2CO_3 (0.2 mmol). The reaction was carried out at room temperature for 48 h for compound **4** and at 80 °C for 6 h for compound **1a**. After dilution with cold water (10−15 mL), the mixture was extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the desiderate **5g** and **5k**, which were purified by column chromatography using cyclohexane/ethyl acetate 2:1 as eluent for compound **5g** and WE2 for compound **5k** (WE2: EtOH, 1.8 mL; NH_4OH, 0.1 mL; $CHCl_3$, 3.6 mL; Et_2O, 3.6 mL; and petroleum ether, 9 mL).

5-{3-[4-(3-Chlorophenyl)piperazin-1-yl]-2-methylpropyl}-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5g. Yield = 35%; mp = 112−113 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.00 (d, 3H, $CH(CH_3)$), 2.30−2.75 (m, 7H: 1H, $CH(CH_3)$; 2H, $CHCH_2N$; 4H, piperazine), 2.75 (s, 3H, CH_3), 3.00−3.20 (m, 4H, piperazine), 4.05−4.20 (m, 1H, $CONCH_2$), 4.50−4.65 (m, 1H, $CONCH_2$), 6.70−6.90 (m, 3H, Ar), 7.15 (m, 1H, Ar), 7.50−7.60 (m, 3H, Ar), 8.15 (d, 2H, Ar).

7-(2-Aminophenyl)-5-{3-[4-(3-chlorophenyl)piperazin-1-yl]-propyl}-3-methyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 5k. Yield = 50%; oil; ^{1}H NMR ($CDCl_3$) δ 2.05−2.15 (m, 2H, $CH_2CH_2CH_2$), 2.55 (t, 3H, CH_2N), 2.55−2.65 (m, 4H, piperazine), 2.75 (s, 3H, CH_3), 3.15 (m, 4H, piperazine), 4.40 (t, 2H, $CONCH_2$), 5.45 (exc br s, 2H, NH_2), 6.75−6.95 (m, 5H, Ar), 6.90 (t, 1H, Ar), 7.20−7.40 (m, 1H, Ar), 8.05 (d, 1H, Ar).

3-(3-Methyl-4-oxo-7-phenyl-4*H*-isoxazolo[4,5-*d*]pyridazin-5-yl)propionic Acid Ethyl Ester 6. To a solution of compound **1a** (0.4 mmol) in anhydrous DMF (1.5 mL), 0.6 mmol of K_2CO_3 and 0.4 mmol of 3-bromopropionic acid ethyl ester were added. After stirring for 15 h at room temperature, the mixture was diluted with cold water (15−20 mL) and extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded a crude precipitate, which was purified by column chromatography using cyclohexane/ethyl acetate 2:1 as eluent. Yield = 70%; mp = 89−91 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, $COOCH_2CH_3$), 2.75 (s, 3H, CH_3), 2.95 (t, 2H, CH_2COO), 4.15 (q, 2H, $COOCH_2CH_3$), 4.65 (t, 2H, $CONCH_2$), 7.50−7.60 (m, 3H, Ar), 8.10−8.20 (m, 2H, Ar).

3-(3-Methyl-4-oxo-7-phenyl-4*H*-isoxazolo[4,5-*d*]pyridazin-5-yl)propionic Acid 7. Compound **6** (0.7 mmol) in ethanol (2−3 mL) was treated with 6 N NaOH (8 mL) at room temperature for 15 h, to afford the corresponding carboxylic derivative **7**. The mixture was concentrated, diluted with water (10 mL), and acidified with 6 N HCl. The crude compound **7** was recovered by suction. Yield = 80%; mp = 195−198 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, CH_3), 3.00 (t, 2H, CH_2COOH), 4.65 (t, 2H, $CONCH_2$), 7.40−7.60 (m, 3H, Ar), 8.10−8.20 (m, 2H, Ar).

General Procedure for 8a,b. To a cooled (T = 0 °C) and stirred solution of compound **7** (0.4 mmol) in $SOCl_2$ (2 mL), 0.05 mL of Et_3N was slowly added. The mixture was heated to 60 °C and stirred for 2 h. The excess of $SOCl_2$ was removed in vacuo, and the residue was dissolved in anhydrous THF (1−2 mL) and added portionwise to a cooled and stirred solution of appropriate commercially available piperazine (0.8 mmol) in anhydrous THF (1−2 mL). The mixture was stirred at room temperature for 2 h. The solid residue was filtered off, and the solution was concentrated in vacuo, diluted with cold water (10−15 mL), and extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded compounds **8a**,**b**, which were purified by column chromatography using cyclohexane/ethyl acetate 1:3 as eluent.

5-{3-[4-(3-Chlorophenyl)piperazin-1-yl]-3-oxopropyl}-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 8a. Yield = 58%; mp = 153−156 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, CH_3), 3.05 (t, 2H, CH_2CON), 3.25 (m, 2H, piperazine), 3.30 (m, 2H, piperazine), 3.70−3.80 (m, 2H, piperazine), 3.85−3.95 (m, 2H, piperazine), 4.70 (t, 2H, $CONCH_2$), 6.95 (m, 2H, Ar), 7.00 (s, 1H, Ar), 7.25 (m, 1H, Ar), 7.55 (m, 3H, Ar), 8.15 (m, 2H, Ar).

5-{3-[4-(4-Chlorophenyl)piperazin-1-yl]-3-oxo-propyl}-3-methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one 8b. Yield = 57%; mp = 160−163 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, CH_3), 3.05 (t, 2H, CH_2CON), 3.10−3.20 (m, 4H, piperazine), 3.60−3.75 (m, 2H, piperazine), 3.80−3.90 (m, 2H, piperazine), 4.70 (t, 2H, $CONCH_2$), 6.85−7.00 (m, 2H, Ar), 7.20−7.30 (m, 2H, Ar), 7.55 (m, 3H, Ar), 8.15 (m, 2H, Ar).

General Procedure for 10a,b. To a suspension of the appropriate dibromoalkane (2.0 mmol) and K_2CO_3 (2.7 mmol) in anhydrous DMF (2−3 mL), a solution of the phtalimmide **9** commercially available (1.35 mmol) in anhydrous DMF (0.5 mL) was added dropwise. The suspension was stirred at room temperature for 90 min. After dilution with cold water, the mixture was extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded compounds **10a**,**b**, which were purified by column chromatography using cyclohexane/ethyl acetate 2:1 as eluent.

2-(2-Bromoethyl)isoindole-1,3-dione 10a. Yield = 73%; mp = 150−153 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.65 (t, 2H, CH_2CH_2Br), 4.15 (t, 2H, NCH_2CH_2), 7.70−7.80 (m, 2H, Ar), 7.85−7.95 (m, 2H, Ar).

2-(3-Bromopropyl)isoindole-1,3-dione 10b. Yield = 45%; mp = 72−75 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.30 (m, 2H, $CH_2CH_2CH_2$), 3.45 (t, 2H, CH_2CH_2Br), 3.85 (t, 2H, NCH_2CH_2), 7.75 (m, 2H, Ar), 7.80−7.90 (m, 2H, Ar).

General Procedure for 11a−c. A mixture of derivative **10a**,**b** (0.6 mmol), anhydrous K_2CO_3 (1.20 mmol), and opportune piperazine (0.9 mmol) in anhydrous DMF (3−4 mL) was stirred at room temperature for 15 h. After cooling, ice water was added and the solution was extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the final compounds **11a−c**, which were purified by column chromatography using cyclohexane/ethyl acetate 3:1 as eluent for **11a** and cycloexane/ethyl acetate 2:1 for **11b**,**c**.

2-{2-[4-(4-Fluorophenyl)piperazin-1-yl]ethyl}isoindole-1,3-dione 11a. Yield = 40%; oil; ^{1}H NMR ($CDCl_3$) δ 2.70−2.90 (m, 6H: 2H, CH_2CH_2N; 4H piperazine), 3.05−3.20 (m, 4H, piperazine), 3.90 (t, 2H, NCH_2CH_2), 6.80−6.90 (m, 2H, Ar), 6.90−7.00 (m, 2H, Ar), 7.70−7.80 (m, 2H, Ar), 7.85 (m, 2H, Ar).

2-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}isoindole-1,3-dione 11b. Yield = 39%; oil; ^{1}H NMR ($CDCl_3$) δ 2.00−2.10 (m, 2H, $CH_2CH_2CH_2$), 2.60−2.70 (m, 6H: 2H, CH_2CH_2N; 4H, piperazine), 3.10−3.20 (m, 4H, piperazine), 3.85 (t, 2H, NCH_2CH_2), 6.75−6.85 (m, 3H, Ar), 7.15 (t, 1H, Ar), 7.75 (m, 2H, Ar), 7.80−7.90 (m, 2H, Ar).

2-{3-[4-(4-Fluorophenyl)piperazin-1-yl]propyl}isoindole-1,3-dione 11c. Yield = 73%; mp = 92−94 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.90−2.05 (m, 2H, $CH_2CH_2CH_2$), 2.55 (t, 2H, CH_2CH_2N), 2.60−2.75 (m, 4H, piperazine), 2.95−3.10 (m, 4H, piperazine), 3.85 (t, 2H, NCH_2CH_2), 6.80 (m, 2H, Ar), 6.90−7.00 (m, 2H, Ar), 7.65−7.75 (m, 2H, Ar), 7.85 (m, 2H, Ar).

General Procedure for 13a,b. Compounds **13a**,**b** were obtained from the commercially available **12**, following the general procedure described for **2a**−**g**. For these compounds, the reaction was carried out at 50 °C for 4 h. The final compounds were purified by column chromatography using cyclohexane/ethyl acetate 1:1 as eluent.

2-(3-Bromopropyl)-2*H*-phthalazin-1-one 13a. Yield = 24%; oil; ^{1}H NMR ($CDCl_3$) δ 2.45−2.55 (m, 2H, $CH_2CH_2CH_2$), 3.50 (t, 2H, CH_2CH_2Br), 4.40 (t, 2H, $CONCH_2$), 7.75 (d, 1H, Ar), 6.85−7.80 (m, 2H, Ar), 8.20 (s, 1H, Ar), 8.45 (d, 1H, Ar).

2-(4-Bromobutyl)-2*H*-phthalazin-1-one 13b. Yield = 32%; mp = 87−88 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.95−2.10 (m, 4H, $CH_2CH_2CH_2CH_2$), 3.45−3.55 (m, 2H, CH_2CH_2Br), 4.25−4.35 (m, 2H, $CONCH_2$), 7.65−7.90 (m, 3H, Ar), 8.15 (s, 1H, Ar), 8.45 (d, 1H, Ar).

2-(3-Piperazin-1-yl-propyl)-2H-phthalazin-1-one 14. A mixture of **13a** (0.5 mmol), anhydrous K_2CO_3 (0.7 mmol), and piperazine (0.5 mmol) in anhydrous DMF (3 mL), was stirred for 15 h at room temperature. After dilution with cold water, the mixture was extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded **14**, which was purified by column chromatography using CH_2Cl_2/MeOH 8:2 as eluent. Yield = 76%; oil; ^{1}H NMR ($CDCl_3$) δ 2.00−2.10 (m, 2H, $CH_2CH_2CH_2$), 2.30−2.55 (m, 6H: 2H, CH_2CH_2N; 4H, piperazine), 2.80−2.95 (m, 4H, piperazine), 4.30 (t, 2H, $CONCH_2$), 7.70 (d, 1H, Ar), 7.75−7.85 (m, 2H, Ar), 8.20 (s, 1H, Ar), 8.45 (d, 1H, Ar).

General Procedure for 15a,b. A mixture of **12** (0.6 mmol), anhydrous K_2CO_3 (1.2 mmol), and appropriate piperazine[17,26] (0.8 mmol) in anhydrous DMF (2−3 mL) was stirred at 70 °C for 4 h. After cooling, the mixture was diluted with ice water (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded the final compounds **15a**,**b**, which were purified by column chromatography using $CHCl_3$/MeOH 9:1 as eluent for compound **15a** and cyclohexane/ethyl acetate 1:2 for compound **15b**.

2-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-2*H*-phthalazin-1-one 15a. Yield = 40%; mp = 83−84 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.00−2.20 (m, 2H, $CH_2CH_2CH_2$), 2.55−2.75 (m, 6H: 2H, CH_2CH_2N; 4H, piperazine), 3.10−3.30 (m, 4H, piperazine), 4.35 (t, 2H, $CONCH_2$), 6.75−6.90 (m, 3H, Ar), 7.20 (t, 1H, Ar), 7.70−7.90 (m, 3H, Ar), 8.20 (s, 1H, Ar), 8.45 (d, 1H, Ar).

2-{3-[4-(3-Chlorophenyl)piperazin-1-yl]-2-methylpropyl}-2*H*-phthalazin-1-one 15b. Yield = 24%; oil; ^{1}H NMR ($CDCl_3$) δ 1.00 (d, 3H, $CH(CH_3)$), 2.30−2.80 (m, 7H: 2H, CH_2CH_2N; 4H, piperazine; 1H, $CH(CH_3)$), 3.05−3.20 (m, 4H, piperazine), 3.95−4.05 (m, 1H, $CONCH_2$), 4.45 (m, 1H, $CONCH_2$), 6.70−6.80 (m, 3H, Ar), 7.15 (t, 1H, Ar), 7.70−7.85 (m, 3H, Ar), 8.20 (s, 1H, Ar), 8.45 (d, 1H, Ar).

General Procedure for 15c,d. Compounds **15c** and **15d** were obtained from compounds **13a**,**b**, following the procedure described for **5a**−**f** and **5h**−**j.** For these compounds, dilution with cold water (20−30 mL) of the reaction mixture afforded a crude precipitate that was recovered by suction. Purification of the final compounds was performed by column chromatography using cyclohexane/ethyl acetate 1:2 as eluent for compound **15c** and cyclohexane/ethyl acetate 1:3 for compound **15d**.

2-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}-2*H*-phthalazin-1-one 15c. Yield = 90%; mp = 110−113 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.20−2.35 (m, 2H, $CH_2CH_2CH_2$), 2.70−2.90 (m, 6H: 2H, CH_2CH_2N; 4H, piperazine), 3.25−3.40 (m, 4H, piperazine), 4.35 (t, 2H, $CONCH_2$), 6.85 (d, 2H, Ar), 7.25 (d, 2H, Ar), 7.70 (d, 1H, Ar), 7.75−7.90 (m, 2H, Ar), 8.20 (s, 1H, Ar), 8.45 (d, 1H, Ar).

2-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-2*H*-phthalazin-1-one 15d. Yield = 41%; mp = 127−129 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.65−1.80 (m, 2H, $NCH_2CH_2CH_2CH_2$), 1.90−2.00 (m, 2H, $NCH_2CH_2CH_2CH_2$), 2.50−2.90 (m, 6H: 2H, CH_2CH_2N; 4H, piperazine), 3.20−3.45 (m, 4H, piperazine), 4.30 (t, 2H, $CONCH_2$), 6.80 (m, 2H, Ar), 7.00 (s, 1H, Ar), 7.15 (t, 1H, Ar), 7.70 (d, 1H, Ar), 7.75−7.85 (m, 2H, Ar), 8.20 (s, 1H, Ar), 8.45 (d, 1H, Ar).

3-{4-[3-(1-Oxo-1*H*-phthalazin-2-yl)propyl]piperazin-1-yl}-benzonitrile 15e. Molecular sieves (4Å) were heated under stirring for 30 min at 200 °C in a N_2 atmosphere. After cooling a mixture of compound **14** (0.4 mmol) in anhydrous CH_2Cl_2 (8−10 mL), 3-cyanophenylboronic acid (0.8 mmol), $Cu(Ac)_2$ (0.6 mmol), and Et_3N (0.8 mmol) was added to molecular sieves. The reaction was carried out at room temperature for 15 h. Anhydrous CH_2Cl_2 (15 mL) was added, the residue was filtered off, and the organic layer was washed with 33% NH_4OH (2 × 15 mL). CH_2Cl_2 was evaporated, and the final compound **15b** was purified by column chromatography using cyclohexane/ethyl acetate 1:1 as eluent. Yield = 28%; oil; ^{1}H NMR ($CDCl_3$) 2.05−2.20 (m, 2H, $CH_2CH_2CH_2$), 2.55 (t, 2H, CH_2CH_2N), 2.60−2.70 (m, 4H, piperazine), 3.15−3.25 (m, 4H, piperazine), 4.35 (t, 2H, $CONCH_2$), 7.10 (m, 2H, Ar), 7.25−7.35 (d, 2H, Ar), 7.70 (d, 1H, Ar), 7.75−7.85 (m, 2H, Ar), 8.20 (s, 1H, Ar), 8.45 (d, 1H, Ar).

2,4-Dimethyl-5-phenyl-7*H*-pyrido[2,3-*d*]pyridazin-8-one 17. To a stirred solution of sodium ethoxide (1 mmol) in anhydrous ethanol (3 mL), a solution of compound **16**[19] (0.5 mmol) in anhydrous acetone (0.8 mL) was added, and the reaction was carried out at 90 °C for 12 h. The mixture was concentrated in vacuo, diluted with water (10−15 mL), and extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded compound **17**, which was purified by flash chromatography using as eluent $CHCl_3$/MeOH 9:1. Yield = 22%; mp > 250 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 7.35 (s, 1H, Ar), 7.40 (m, 2H, Ar), 7.25 (s, 1H, Ar), 7.40 (m, 2H, Ar), 7.50 (m, 3H, Ar), 10.15 (exc br s, 1H, NH).

7-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-2,4-dimethyl-5-phenyl-7*H*-pyrido[2,3-*d*]pyridazin-8-one 18. Compound **18** was obtained from compound **17**, following the general procedure described for **15c**,**e**. The mixture was stirred at room temperature for 15 h, and the final compound **18** was purified by flash chromatography using WE7 as eluent. Yield = 52%; oil; ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3H, CH_3), 2.10−2.20 (m, 2H, $CH_2CH_2CH_2$), 2.55 (t, 2H, CH_2CH_2N), 2.60 (m, 4H, piperazine), 2.80 (s, 3H, CH_3), 3.10−3.20 (m, 4H, piperazine), 4.40 (t, 2H, $CONCH_2$), 6.70−6.80 (m, 2H, Ar), 6.85 (s, 1H, Ar), 7.15 (t, 1H, Ar), 7.30 (s, 1H, Ar), 7.30−7.40 (m, 2H, Ar), 7.45−7.55 (m, 3H, Ar).

General Procedure for 21 and 22. Compounds **21** and **22** were obtained starting from compounds **19**[20] and **20**,[21] following the general procedure described for **15c**,**e**. The mixture was stirred at 60−80 °C for 2−5 h. After cooling, cold water was added, and compound **21** was recovered by suction and recrystallized by ethanol. Compound **22** was recovered after extraction with CH_2Cl_2 (3 × 15 mL), evaporation in vacuo of the solvent, and it was purified by column chromatography using cyclohexane/ethyl acetate 1:1 as eluent.

6-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3-methyl-4-thiophen-2-yl-6*H*-isoxazolo[3,4-*d*]pyridazin-7-one 21. Yield = 24%; mp = 85−87 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.00−2.30 (m, 2H, $CH_2CH_2CH_2$), 2.50−2.70 (m, 6H: 2H, CH_2CH_2N; 4H, piperazine), 2.75 (s, 3H, CH_3), 3.10−3.20 (m, 4H, piperazine), 4.30 (t, 2H, $CONCH_2$), 6.70−6.85 (m, 3H, Ar), 7.10−7.20 (m, 2H, Ar), 7.30−7.50 (m, 2H, Ar).

6-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3,4-dimethyl-2-phenyl-2,6-dihydropyrazolo[3,4-*d*]pyridazin-7-one 22. Yield =

28%; mp = 115−118 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.90−2.15 (m, 2H, $CH_2CH_2CH_2$), 2.45−2.70 (m, 12H: 2H, CH_2CH_2N; 4H, piperazine; 3H, 3C-CH_3; 3H, 4C-CH_3), 3.15 (m, 4H, piperazine), 4.30 (t, 2H, $CONCH_2$), 6.70−6.90 (m, 3H, Ar), 7.15 (t, 1H, Ar), 7.50 (s, 5H, Ar).

3-Methyl-7-phenyl-5*H*-isoxazolo[4,5-*d*]pyridazine-4-thione 23. Isoxazolopyridazinone **1a** (0.9 mmol) was treated with Lawesson's reagent (0.8 mmol) in toluene (10 mL) and heated under stirring for 2 h at 110 °C. The precipitate **23** was recovered by suction. Yield = 84%; mp = 241−243 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.85 (s, 3H, CH_3), 3.85 (exch br s, 1H, NH), 7.50−7.60 (m, 3H, Ar), 8.10−8.20 (m, 2H, Ar).

4-{3-[4-(4-Fluorophenyl)piperazin-1-yl]propylsulfanyl}-3-methyl-7-phenylisoxazolo[4,5-*d*]pyridazine 24. A mixture of compound **23** (0.8 mmol), anhydrous K_2CO_3 (1.6 mmol), and 1-(3-bromopropyl)-4-(4-fluorophenyl)-piperazine[22] (0.8 mmol) in anhydrous acetone (4 mL) was stirred at room temperature for 4 h. The mixture was concentrated, diluted with cold water (15 mL), and extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the desiderated **24**, which was purified by column chromatography using cyclohexane/ethyl acetate 1:2 as eluent. Yield = 24%; mp = 111−113 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.20−2.40 (m, 2H, $CH_2CH_2CH_2$), 2.50−2.80 (m, 6H: 4H, piperazine; 2H, $CONCH_2$), 2.85 (s, 3H, CH_3), 3.10−3.20 (m, 4H, piperazine), 4.95 (t, 2H, SCH_2), 6.80−7.05 (m, 4H, Ar), 7.45−7.60 (m, 3H, Ar), 8.10−8.25 (m, 2H. Ar).

Biological Assays. Animals. Male Swiss albino mice (23−30 g) from Morini breeding farm (Italy) and male Sprague−Dawley (200−250) rats from Harlan Laboratories (Italy) were used. Fifteen mice and four rats 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.[27] 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 stop-watch 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 i.p. with a 0.6% solution of acetic acid (10 mL kg^{-1}) according to Koster et al.[28] The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.

Statistical Analysis. All experimental results are given as the mean ± S.E.M. Analysis of variance, followed by Fisher's protected least significant difference (PLSD) procedure for post-hoc comparison, was used to verify the significance between two means. Data were analyzed with the StatView software for the Macintosh (1992). *P* values of less than 0.05 were considered significant.

Drugs. The following drugs were used: yohimbine hydrochloride, naloxone hydrochloride, atropine sulfate and prazosin (Sigma), BRL-44408 (Tocris), ARC-239 (Tocris), and CGP-35358 (Ciba Geigy). Other chemicals were of the highest quality commercially available. All drugs were dissolved in isotonic (NaCl 0.9%) saline solution or dispersed in sodium carboxymethylcellulose 1%.

Noradrenaline Release from Rat Cerebral Cortex Evaluated by Microdialysis Technique. Experiments were performed following the methods described by Oropeza et al.[29]

Rats were anesthetized with 8% chloral hydrate (400 mg/kg) and placed in a stereotaxic apparatus with the skull flat. A small burr hole was made in the skull centered at 3.2 mm anterior and ±0.9 mM $MgCl_2$ and 4 mM KCl was continuously perfused through the probe by a microliter infusion pump. Approximately 18 h following surgery, dialysate samples were collected every 20 min. Dialisate samples were stored at −80 °C for subsequent analysis by HPLC-ED. Vertical concentric microdialysis probes were used.

The amount of NE in the dialysate samples was determined with HPLC-ED. Dialysate samples were injected into the HPLC system. The detection system consisted of an ESA Coulochem II electrochemical detector. The baseline value against which drug administration was compared to was derived from the average of three samples just prior to manipulation. The neurochemical data were expressed as the mean ± SEM. All statistics were performed using StatView software.

Synaptosomal [^{3}H]-Noradrenaline Uptake. Synaptosomal preparation brain synaptosomes were isolated according to the method described by Franceschini et al.[30] Rats were decapitated, the brains rapidly excised, and the frontal cortex dissected out on ice. The tissue was homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose and then centrifuged at 1800 g for 10 min (4 °C). The pellet was discarded and the supernatant was centrifuged at 17 000 g for 60 min (4 °C). The pellet was resuspended in a small volume of 0.32 M sucrose, layered on a 0.8−1.2 M sucrose gradient, and centrifuged at 37 000 g for 60 min (4 °C). The synaptosomal fraction at the interface between 0.8 and 1.2 M sucrose was collected and further centrifuged at 17 000 g for 30 min. The pellet was resuspended in buffer (composition in mM: NaCl, 115; KCl, 4.97; $CaC1_2$, 1; $MgSO_4$, 1.22; KH_2P0_4, 1.2; $NaHC0_3$, 25; and glucose, 11.1, pH 7.4, plus 0.01 mM pargyline) and immediately used. Synaptosomes were preincubated for 5 min at 37 °C in the absence or presence of different concentrations of drug. Uptake was started by addition of [^{3}H]-NE, and incubation continued for 6 min. Control samples were incubated at 0 °C to evaluate membrane diffusion. The reaction was stopped by cooling the tubes in ice. The samples were then filtered through Whatman GF/C glass fiber filters (Whatman, Inc., Clifton, NJ) and washed twice with 150 mM Tris HCl, pH 7.4. Filter-bound radioactivity was counted by liquid scintillation spectrometry with Filter Count (Packard). The difference in [^{3}H]-NE accumulation at 37 °C and 0 °C was taken as a measure of active uptake.

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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