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Synthesis of five and six-membered heterocycles bearing an arylpiperazinylalkyl side chain as orally active antinociceptive agents

Claudia Vergelli [a], Giovanna Ciciani [a], Agostino Cilibrizzi [b], Letizia Crocetti [a], Lorenzo Di Cesare Mannelli [c], Carla Ghelardini [c], Gabriella Guerrini [a], Antonella Iacovone [a], Maria Paola Giovannoni [a,*]

[a] NEUROFARBA, Sezione di Farmaceutica e Nutraceutica, Università degli Studi di Firenze, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy
[b] Department of Chemistry, Imperial College London, South Kensington, London SW7 2AZ, UK
[c] NEUROFARBA, Sezione di Farmacologia e Tossicologia, Università degli Studi di Firenze, Viale Pieraccini 6, 50139 Firenze, Italy

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ABSTRACT

A number of heterocycles bearing an arylpiperazinylalkyl side chain and structurally related to the previously described lead **ET1** (4-amino-6-methyl-2-[3-(4-*p*-tolylpiperazin-1-yl)propyl]-5-vinylpyridazin-3(2*H*)-one) was synthesized and tested for their antinociceptive activity in Writhing Test. Many compounds, tested at doses of 20–40 mg/kg po were able to reduce the number of abdominal constrictions by more than 47% and, in same cases, the potency is comparable to lead **ET1** as for **5e**, **24a**, **27b** and **27c**. The analgesia induced by the active compounds was completely prevented by pretreatment with α_2-antagonist yohimbine, confirming the involvement of the adrenergic system in the mechanism of action for these new compounds.

1. Introduction

Pain relief continues to be an important medical and community problem and millions of people worldwide use drugs for different pain intensity. The identification of new analgesic agents with limited side effects, as for acute and chronic pain, represents an important research field for pharmaceutical industry and academic. In fact the two major classes of analgesic drugs, the traditional non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, showed severe side effects.

NSAIDs, primarily used for the treatment of mild to moderate inflammatory pain[1] induce gastrointestinal lesions, such as ulcerations and perforations, nephrotoxicity and inhibition of platelet aggregation.[2] Development of potent and selective COX-2 inhibitors[3] only partially solved the problem, since recent studies correlated the use of these inhibitors with an elevated risk of acute myocardial infarction.[4,5] On the other hand, the clinical use of opioid, for moderate to severe pain, is associated with very strong and use-limiting side effects, including respiratory depression, constipation, tolerance and physical dependence.[6]

A particular search field regards compounds active on neuropatic pain, which is often resistant to conventional analgesic drugs.[7] Recently defined by the International Association for the Study of Pain (IASP) as 'pain caused by a lesion or disease of the somatosensory system',[8] neuropathic pain is a complex phenomenon characterized by burning pain coupled with hyperalgesia and allodynia involving both the peripheral and central nervous system.[9] At present, first-line drugs recommended for this pathology include anticonvulsant, as gabapentin and pregabalin,[10,11] antidepressants, as amitriptyline and nortriptyline[12,13] and serotonin-norepinephrine reuptake inhibitor antidepressant as duloxetine[14,15] and milnacipran[16] as well as compounds belonging to different therapeutic classes.[17,18]

Our studies in the field of analgesic agents let us to identify a large number of potent compounds, with pyridazine scaffold[19–28] and the most interesting term is **ET1** (Fig. 1), belonging to the series of arylpyperazinylalkyl pyridazinones.[28] It results a potent and orally active antinociceptive agent showing an ED_{50} = 0.5 mg/kg in the hot plate test and a comparable activity in the tail flick test (ED_{50} = 0.8 mg/kg). The adrenergic system is involved in the analgesic activity of **ET1** as demonstrated by its ability to act as α_2AR agonist.[28] Recent studies show its activity in a model of peripheral neuropathy (data not shown).

We report here the synthesis and the antinociceptive evaluation of a series of pyridazinones derivatives as elaboration of the lead **ET1**. At the same time, we designed and synthesized new compounds bearing an arylpiperazinyl moiety linked to different heterocyclic system through alkyl chains.

* Corresponding author. Tel.: +39 055 4573682.
E-mail address: mariapaola.giovannoni@unifi.it (M.P. Giovannoni).

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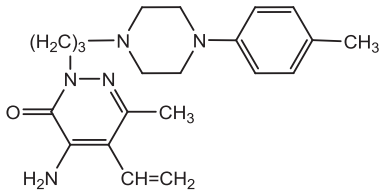
0968-0896/



ET$_1$

4-Amino-6-methyl-2-[3-(4-*p*-tolylpiperazin-1-yl)propyl]-5-vinylpyridazin-3(2H)-one

Figure 1.

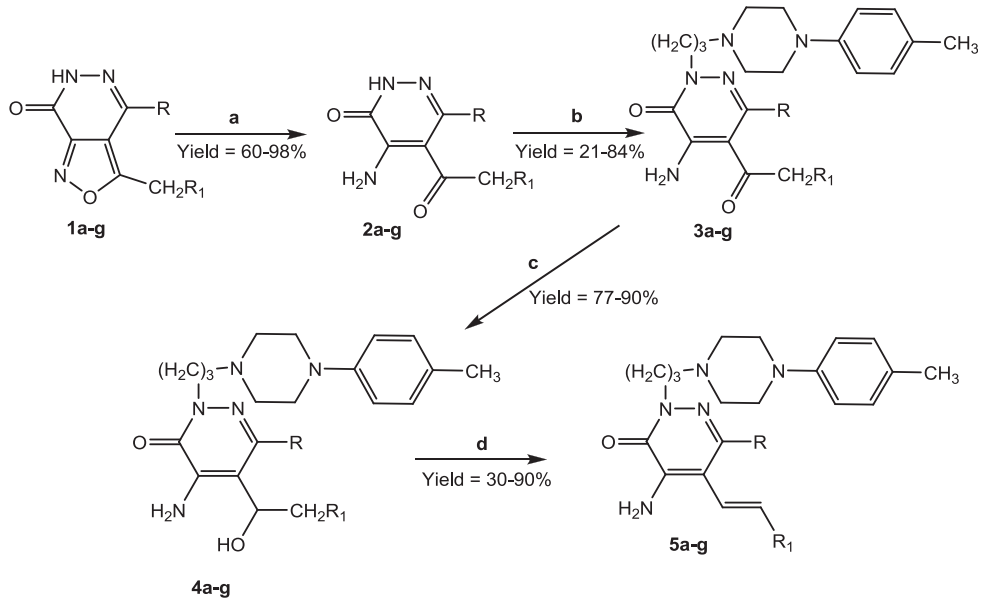
2. Chemistry

All new compounds were synthesized as reported in Schemes 1–4 and the structures were confirmed on the basis of the analytical and spectral data.

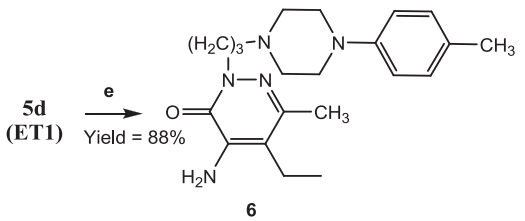
Scheme 1 shows the synthetic pathway affording the final compounds **5a–g** (**5d**[28], alias **ET1**) and **6**, in which we reported modifications at position 5 or 6 of lead **ET1**. The 4-amino-5-acyl derivatives **2a–g** (**2d–g**[29,30]) were obtained starting from isoxazolo[3,4-*d*]pyridazinones **1a–g**[30–32] by reductive cleavage with ammonium formate and Pd/C and represent the key intermediates of the reported synthetic pathway. The alkylation of **2a–g** with 1-(3-bromopropyl)-4-(*p*-tolyl)-piperazine[28] under standard conditions afforded compounds **3a–g** (**3d**[28]) which were reduced with sodium borohydride in methanol to give the corresponding secondary alcohols (**4a–g**, **4d**[28]), which finally were transformed into the final 4-amino-5-vinyl derivatives **5a–g** (**5d**[28]) with polyphoshoric acid (PPA) (**5a–e**, **5g**) or with sulfuric acid adsorbed on silica gel (**5f**). The vinyl group of **5d** was further reduced with Parr instrument to afford compound **6**.

In Schemes 2 and 3 are depicted the synthesis of **ET1** analogues bearing a different N-2 basic side chain with respect to lead compound.

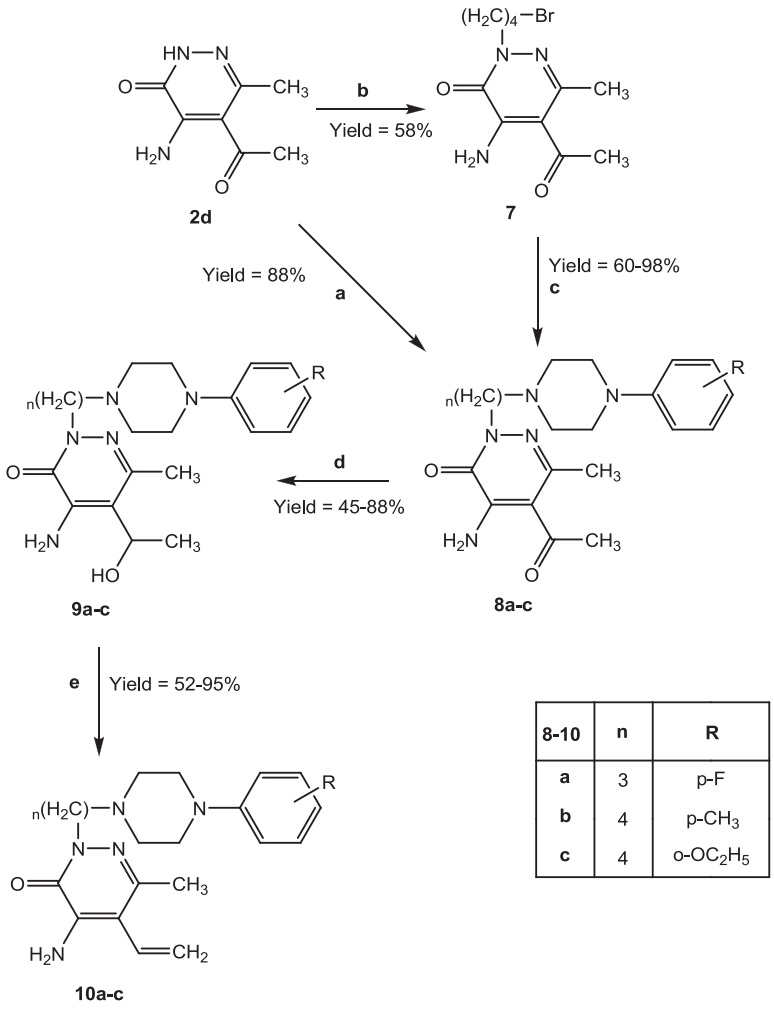
In Scheme 2 compounds **8a–c** were obtained from 4-amino-5-acetylpyridazinone **2d**,[29] following two different procedures: a direct alkylation of **2d** with 1-(3-bromopropyl)-4-(4-fluorophenyl)piperazine[24] in anhydrous DMF and K_2CO_3 at room temperature, for **8a**, or through the synthesis of *N*-butylbromide **7** followed by condensation with the appropriate R-arylpiperazine for **8b**,**c**. Final compounds **10a–c** were then obtained following the same synthetic procedure described in Scheme 1.



1-5	R	R_1
a	C_2H_5	H
b	nC_3H_7	H
c	nC_4H_9	H
d	CH_3	H
e	CH_3	CH_3
f	CH_3	C_2H_5
g	CH_3	nC_3H_7



Scheme 1. Reagents and conditions: (a) 10% Pd/C, $HCOONH_4$, EtOH abs, reflux, 2 h; (b) 1-(3-bromopropyl)-4-(*p*-tolyl)-piperazine, K_2CO_3, anhydrous DMF, rt, 16 h; (c) $NaBH_4$, anhydrous MeOH, rt, 1 h; (d) for **5a–e** and **5g**: PPA, 90 °C, 5 h; for **5f**: H_2SO_4 on silica gel, anhydrous toluene, 40–50 °C, 5 h, then rt, 16 h; (e) 10% Pd/C, EtOH abs, H_2, Parr, 60 PSI, 5 h.



8-10	n	R
a	3	p-F
b	4	p-CH_3
c	4	o-OC_2H_5

Scheme 2. Reagents and conditions: (a) 1-(3-bromopropyl)-4-(4-fluorophenyl)piperazine, K_2CO_3, anhydrous DMF, rt, 12 h; (b) 1,4-bromobutane, K_2CO_3, anhydrous, DMF, rt, 3 h; (c) appropriate R-arylpiperazine, K_2CO_3, anhydrous DMF, rt, 18 h; (d) $NaBH_4$, anhydrous MeOH, rt, 5–20 min; (e) PPA, 70 °C, 3 h.

Further modifications of the side chain are reported in Scheme 3. The basic fragment **13**, **16** and **18** were prepared by condensing 1,3-dibromopropane or 1-bromo-3-chloro-2-methylpropane with appropriate substituted-arylpiperazine **12**, **15** and **17** in anhydrous acetone and K_2CO_3 at room temperature (part 1). Compounds **11**, **12**, **14** and **15** are commercially available, while compound **17** was synthesized following the procedure reported in leterature.[33] Fragment **13**, **16** and **18** were then used for the synthesis of the final compounds **21a–c** in the same conditions described in Scheme 1 (part 2).

Finally, in Scheme 4 is represented the synthetic pathway affording the final compounds **24a–e**, **27a–e** and **30a–c**, characterized by a different heterocyclic scaffolds. The starting compounds **22**[34], **25**, commercially available, and **28**[35] undergo to the usual synthetic route to give the desired compounds of type **24**, **27** and **30**.

3. Biological results and discussion

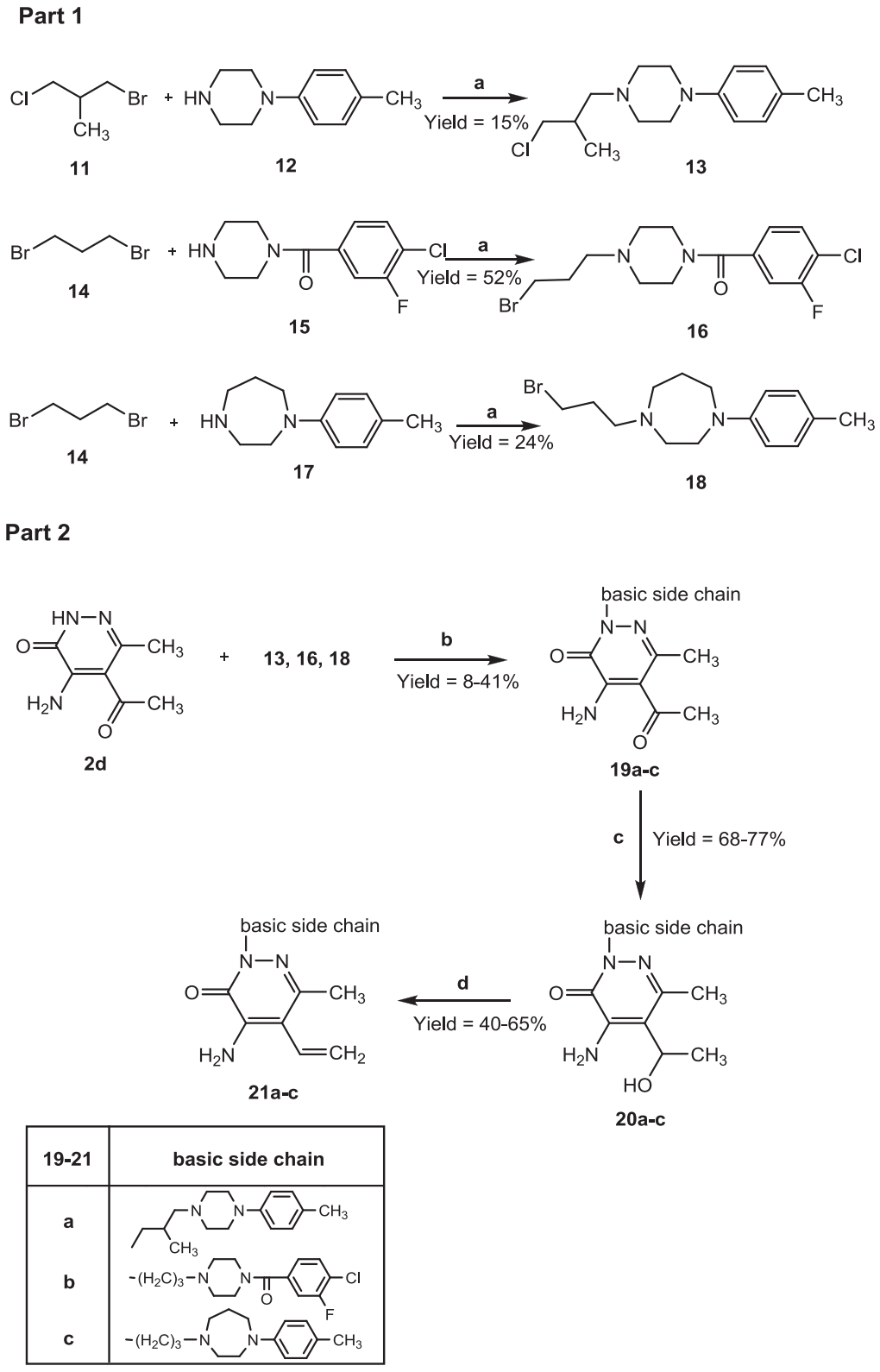
The antinociceptive activity of the new products was evaluated per os in the experimental model of the abdominal constriction test[36] and data are reported in Tables 1–3 in comparison with that of lead, compound **ET1**.

Starting from the modification at position 6 of **ET1** (Table 1, compounds **5a–c**) the replacement of CH_3 with C_2H_5 (**5a**) or C_3H_7 (**5b**) was associated with complete a loss of activity, whereas the presence of a butyl group (**5c**) unexpectedly determined an interesting antinociceptive effect with an appreciable reduction of constriction at 20 and 40 mg/kg.

The effects of the elongation of the vinyl group at position 5 was also studied: substitution of a hydrogen at β-carbon with a methyl group (compound **5e**) afforded a potent antinociceptive agent able to reduce for 44% and 63% the abdominal constrictions at 20 and 40 mg/kg, respectively therefore with an activity comparable to lead **ET1**. Further lengthening (compounds **5f** and **5g**) clearly suggested that position 5 shows specific requirements of steric hindrance as demonstrated by the progressive decrease of potency of compounds **5f** and **5g**.

Finally, the 5-acyl **3a**, **3d–g** and 5-ethyl derivatives **6** were synthesized as metabolically more stable homologues of lead **ET1**. In any case, these modifications led to compounds less potent then **ET1** and in the series of 4-acyl derivatives, it is possible to observe a trend similar to vinyl derivatives since antinociceptive activity decreases moving from **3d** (5-$COCH_3$) to **3g** (5-COC_4H_9).

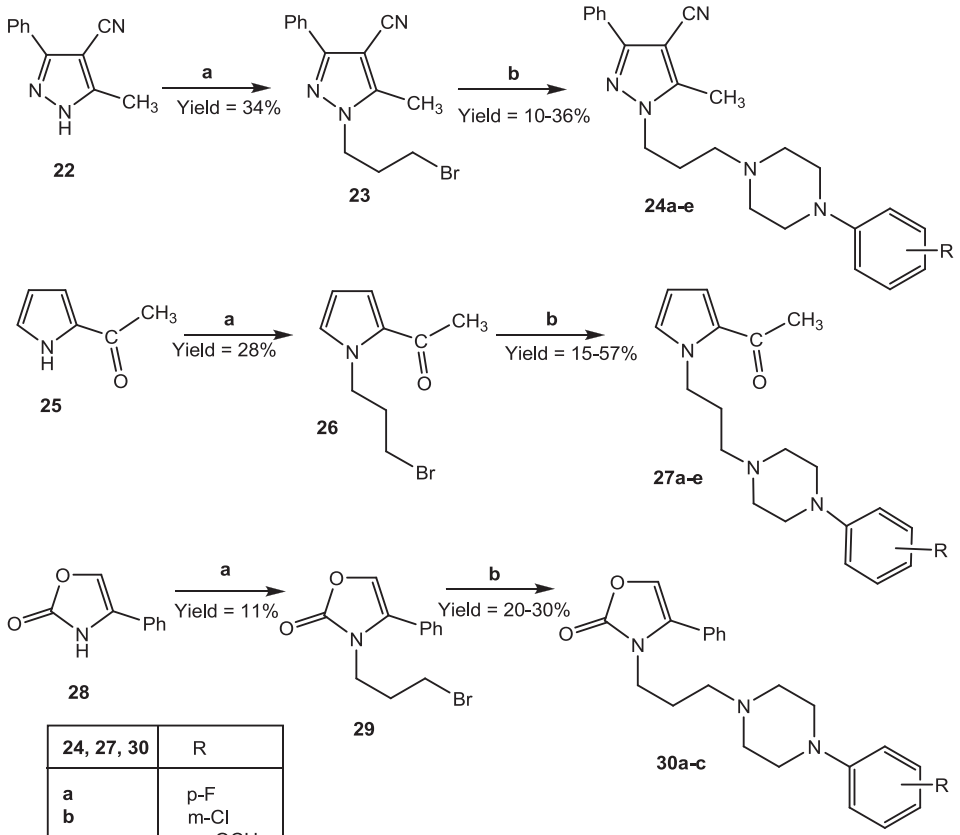
In Table 2 was reported the antinociceptive activity of **ET1** derivatives modified at the arylpiperazinylalkyl chain at position 2. All compounds were less active (**10a** and **21c**) or completely inactive (**10c** and **21b**) than the reference compound. Only derivatives **10b** and **21a**, at 30 mg/kg per os showed an appreciable antinociceptive activity reducing by 42% and 47%, respectively, the number of abdominal constrictions. These data indicated that the best arrangement for the basic side chain is represented by



Scheme 3. Reagents and conditions: (a) K_2CO_3, anhydrous acetone, rt, 3–16 h; (b) for **19a**: K_2CO_3, anhydrous DMF, 70 °C, 12 h; for **19b**,**c**: K_2CO_3, anhydrous DMF, rt, 16 h; (c) $NaBH_4$, anhydrous MeOH, rt, 10 min–1 h; (d) PPA, 70–90 °C, 3–5 h.

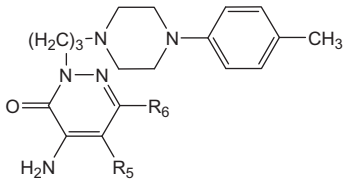
the sequence *p*-tolyl-pyperazinyl-alkyl where the alkyl linker $-(CH_2)_3-$ (**ET1**) is the best, but can be homologated to $-(CH_2)_4-$ (**10b**) or branched (**21**). All other modifications such as substitution of the p-CH_3 group with a fluorine (**10a**) or the enlargement of piperazine to homopiperazine (**21c**) was detrimental for activity.

Finally, in order to evaluate the role of the pyridazinone scaffold, we synthesized a new series of compounds in which appropriate propylpiperazinylaryl chains were inserted in different heterocycles (Table 3). In the subseries of pirazoles (**24a**–**e**), the most interesting compounds resulted derivatives **24a** and **24e**



Scheme 4. Reagents and conditions: (a) 1,3-dibromopropane, K_2CO_3, anhydrous DMF; for **23**, **29**: rt, 1–5 h; for **26**: rt, 20 h, then 60 °C, 3 h; (b) R-arylpiperazine, K_2CO_3, anhydrous DMF, rt–60 °C, 20–24 h.

Table 1
Antinociceptive effect of final compounds in the Writhing Test[a]

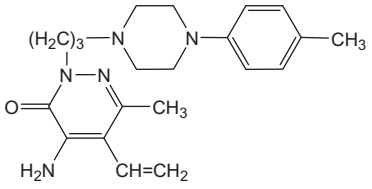


3a, 3d-g, 5a-c, 5e-g, 6

Compound	R_5	R_6	N° mice	Treatment (mg kg^{-1})	Abdominal constrictions	% rid n° constrictions
CMC			23		30.2 ± 1.3	
5a	$CH=CH_2$	C_2H_5	7	10	33.4 ± 2.1	0
			9	20	32.1 ± 3.1	0
			5	40	21.6 ± 3.0*	28
			10	40 + yohimbine	34.0 ± 2.6	
5b	$CH=CH_2$	C_3H_7	6	10	29.4 ± 3.5	3
			7	20	28.2 ± 3.7	7
			6	40	23.6 ± 3.1^	22
			7	40 + yohimbine	31.9 ± 3.4	
5c	$CH=CH_2$	C_4H_9	6	10	33.1 ± 2.2	0
			6	20	17.9 ± 2.6*	41
			7	40	15.6 ± 2.7*	48
			8	20 + yohimbine	29.8 ± 3.5	

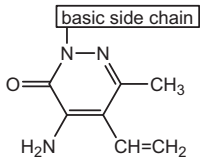
(*continued on next page*)

Table 1 (*continued*)

Compound	R_5	R_6	N° mice	Treatment (mg kg^{-1})	Abdominal constrictions	% rid n° constrictions
5e	CH = CH-CH_3	CH_3	5	10	33.1 ± 3.3	0
			6	20	16.8 ± 2.8*	44
			8	40	11.3 ± 2.5*	63
			8	20 + yohimbine	27.4 ± 3.0	
5f	CH = CH-C_2H_5	CH_3	5	10	28.8 ± 3.0	5
			6	20	19.6 ± 2.8*	35
			6	40	16.7 ± 2.6*	45
			6	20 + yohimbine	30.3 ± 3.0	
5g	CH = CH-C_3H_7	CH_3	5	10	31.6 ± 2.6	0
			6	20	25.3 ± 2.3	16
			6	40	19.3 ± 2.8*	36
			8	40 + yohimbine	29.1 ± 3.1§	
3a	$COCH_3$	C_2H_5	6	10	27.3 ± 3.1	10
			6	20	17.6 ± 2.5*	42
			6	40	17.9 ± 3.1*	41
			9	20 + yohimbine	28.5 ± 2.3	
3d[28]	$COCH_3$	CH_3	6	10	24.4 ± 3.1^	19
			6	30	16.2 ± 2.5*	46
			6	30 + yohimbine	29.7 ± 2.6	
3e	COC_2H_5	CH_3	8	10	27.5 ± 3.0	9
			8	20	18.2 ± 2.7*	40
			8	40	21.3 ± 2.5*	29
			10	20 + yohimbine	31.9 ± 3.3	
3f	COC_3H_7	CH_3	7	10	30.3 ± 2.8	0
			7	20	22.7 ± 3.3*	25
			6	40	20.5 ± 3.1*	32
			8	20 + yohimbine	28.9 ± 2.0	
3g	COC_4H_9	CH_3	6	10	27.9 ± 2.5	8
			6	20	23.2 ± 3.0*	23
			8	40	25.6 ± 2.9*	15
			9	20 + yohimbine	31.6 ± 2.2	
6	CH_2CH_3	CH_3	7	10	23.2 ± 2.9*	23
			6	30	16.7 ± 2.3*	45
			6	30 + yohimbine	30.5 ± 3.1	
 **ET_1**			6	10	20.2 ± 3.3^	33
			6	30	12.4 ± 3.1*	59
			6	40	10.6 ± 2.7*	65
			6	40 + yohimbine	29.1 ± 2.9	

[a] All drugs were administrated per os 30 min before test.
^ P <0.05.
* P <0.05 in comparison with CMC-treated mice.
§ Versus the corresponding Ai-compound.

Table 2
Antinociceptive effect of final compounds in the Writhing Test[a]



10a-c, 21a-c

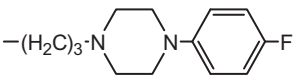
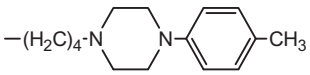
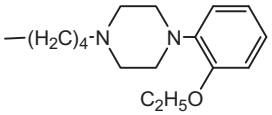
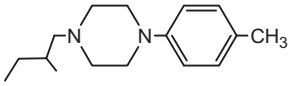
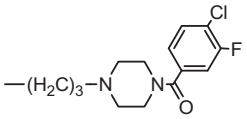
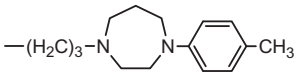
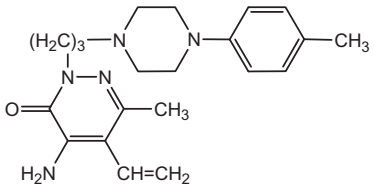
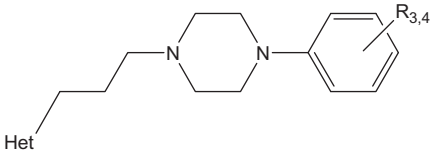
Compound	Basic side chain	N° mice	Treatment (mg kg^{-1})	Abdominal constrictions	% rid n° constrictions
CMC		23		30.2 ± 1.3	
10a		6	10	30.4 ± 2.5	
		6	30	21.8 ± 2.1*	0
		6	30 + yohimbine	29.1 ± 2.9	28

Table 2 (*continued*)

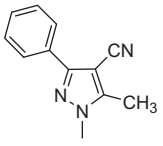
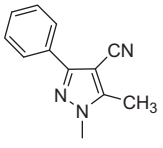
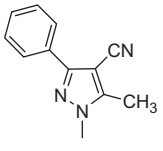
Compound	Basic side chain	N° mice	Treatment (mg kg^{-1})	Abdominal constrictions	% rid n° constrictions
10b		7 7 7	10 30 30 + yohimbine	29.3 ± 2.9 17.4 ± 2.8* 28.5 ± 2.6	3 42
10c		6 6	10 30	NA NA	
21a		5 6 6	10 30 30 + yohimbine	25.1 ± 2.6^ 15.9 ± 2.7* 28.8 ± 3.1	17 47
21b		6 6	10 30	NA NA	
21c		7 8 7 8	10 20 40 40 + yohimbine	31.3 ± 2.3 27.1 ± 2.5 20.8 ± 3.0* 30.5 ± 2.8	0 10 31
ET$_1$		6 6 6 6	10 30 40 40 + yohimbine	20.2 ± 3.3^ 12.4 ± 3.1* 10.6 ± 2.7* 29.1 ± 2.9	33 59 65

[a] All drugs were administrated per os 30 min before test.
^ P <0.05.
* P <0.05 in comparison with CMC-treated mice.

Table 3
Antinociceptive effect of final compounds in the Writhing Test[a]

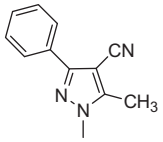
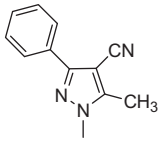
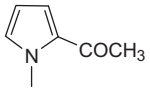
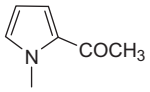
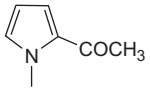
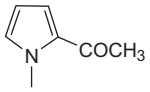
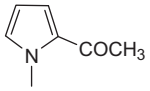
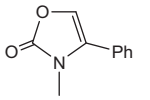
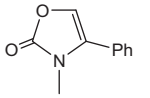
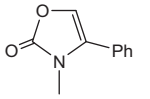
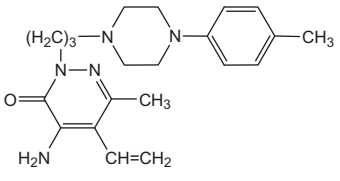


24a-e, 27a-e, 30a-c

Compound	Het	$R_{3,4}$	N° mice	Treatment (mg kg^{-1})	Abdominal constrictions	% rid n° constrictions
CMC			29		31.5 ± 1.8	
24a		4-F	7 9 8 10	10 20 40 40 + yohimbine	28.5 ± 2.5 22.6 ± 2.7* 15.4 ± 3.0* 28.7 ± 3.2§	9 28 51
24b		3-Cl	7 11 8	20 40 40 + yohimbine	32.5 ± 3.0 24.1 ± 2.7^ 32.6 ± 2.9§	0 23
24c		3-OCH$_3$	8 8 8 8	10 20 40 20 + yohimbine	31.6 ± 3.3 24.9 ± 3.2^ 20.8 ± 2.5* 27.8 ± 2.1§	0 21 34

(*continued on next page*)

Table 3 (*continued*)

Compound	Het	$R_{3,4}$	N° mice	Treatment (mg kg^{-1})	Abdominal constrictions	% rid n° constrictions
24d		4-CH_3	8 8 8 10	10 20 40 20 + yohimbine	33.2 ± 3.0 25.8 ± 3.0^ 21.9 ± 3.3* 29.3 ± 2.7§	0 8 30
24e		4-NO_2	8 7 8 10	10 20 40 40 + yohimbine	32.7 ± 2.5 24.9 ± 3.1 16.2 ± 3.2* 28.2 ± 2.6§	0 21 49
27a		4-F	8 7 12 9	10 20 40 40 + yohimbine	32.7 ± 2.6 26.3 ± 2.5 19.5 ± 2.1* 31.5 ± 2.7§	0 16 38
27b		3-Cl	8 8 9 10	10 20 40 20 + yohimbine	30.6 ± 2.7 18.7 ± 2.9* 14.5 ± 2.6* 32.1 ± 3.0§	3 41 54
27c		3-OCH_3	9 8 8 9	10 20 40 40 + yohimbine	31.6 ± 2.9 23.4 ± 3.1^ 15.2 ± 2.8* 30.8 ± 2.7§	0 26 52
27d		4-CH_3	8 8 8 10	10 20 40 80	27.8 ± 3.1 29.3 ± 2.5 28.6 ± 2.9 30.2 ± 3.5	12 7 9 4
27e		4-NO_2	9 8 10	20 40 80	27.9 ± 3.0 33.7 ± 2.5 28.8 ± 2.6	11 0 9
30a		4-F	8 8 8 8	10 20 40 40 + yohimbine	27.2 ± 3.1 27.8 ± 2.6 20.8 ± 3.0* 33.4 ± 3.5§	14 12 34
30b		3-Cl	6 8 8 8	10 20 40 20 + yohimbine	29.4 ± 3.0 21.8 ± 2.6* 19.5 ± 3.5* 29.3 ± 2.7§	7 31 38
30c		3-OCH_3	6 8 8 10	10 20 40 20 + yohimbine	29.1 ± 2.6 23.5 ± 2.8^ 19.3 ± 2.9* 29.6 ± 3.0§	8 25 39
ET$_1$			6 6 6 6	10 30 40 40 + yohimbine	20.2 ± 3.3^ 12.4 ± 3.1* 10.6 ± 2.7* 29.1 ± 2.9	33 59 65

[a] All drugs were administrated per os 30 min before test.
^ $P < 0.05$.
* $P < 0.05$ in comparison with CMC-treated mice.
§ Versus the corresponding Ai-compound.

bearing a fluorine and a nitro group in para position of the side chain: these compounds at 40 mg/kg reduced by 51% and 49%, respectively, the number of abdominal constriction and retain a weak antinociceptive activity at 20 mg/kg. On the contrary, the introduction of a methyl in para position of the basic side chain (compound **24d**) was associated with reduced activity (30% of inhibition at 40 mg/kg). In addition, compounds **24b** and 2**4c**, bearing a chlorine or a methoxy group in meta position, showed a very low activity.

In the series of pyrrolo derivatives **27a**–**e**, we observe an opposite trend of activity since the most active compounds resulted **27b** and **27c** (54% and 52% of inhibition at 40 mg/kg, respectively) bearing in the side chain respectively a 3-Cl and a 3-OCH_3 group. As in the pyrazole series, the insertion of a methyl in para position led to the loss of activity (compound **27d**) as well as compounds **27a** and **27e**.

Finally, the oxazolonic derivatives showed a weak antinociceptive activity (34–39% of inhibition at 40 mg/kg) regardless of the

nature and position of the substituent at the phenyl ring of the side chain. These data indicate that the replacement of the pyridazinone scaffold with pentatomic heterocyclic nucleus is detrimental for activity and only few compounds induced an appreciable reduction of abdominal constriction (about 50% at 40 mg/kg).

Finally, it's important to highlight that the antinociception of all tested compounds was completely prevented by pretreatment with the α_2-antagonist yohimbine, confirming the involvement of the adrenergic system in the mechanism of action also for these new products.

4. Conclusions

In conclusion, we synthesized new antinociceptive agents with an appreciable activity in the Writhing Test. The most interesting terms are able to reduce the abdominal constrictions by more than 50% when administered per os at the doses of 40 mg/kg (**5e**, **24a**, **27b** and **27c**). The chemical manipulation confirmed the importance of the basic side chain whose requirements seem to be different depending on the heterocyclic scaffold bearing it. As regard the heterocyclic nucleus, results demonstrated that the pyridazinone ring, widely investigated by us, could be replaced with 5-member nitrogen heterocycles, retaining a similar antinociceptive activity. For all new compounds, independently from the structure, the involvement of the adrenergic system in the mechanism of antinociception was proved, since the analgesia of tested products was completely reverted by pretreatment with α_2-antagonist yohimbine.

Supplementary data

Supplementary data (synthetic routes and spectroscopic data of all new compounds synthesized and biological procedures) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.08.043.

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