4-Amino-5-vinyl-3(2*H*)-pyridazinones and Related Compounds: Synthesis and Evaluation of Antinociceptive Activity

Vittorio Dal Piaz* [a], Stefano Pieretti [b], Claudia Vergelli [a], Maria Carla Castellana [a] and Maria Paola Giovannoni [a]

[a] Dipartimento di Scienze Farmaceutiche, via G.Capponi 9, 50121 Firenze, Italy
[b] Istituto Superiore di Sanità, Laboratorio di Farmacologia, Viale Regina Elena 299, Roma , Italy

J. Heterocyclic Chem., **39**, 523 (2002).

Introduction.

It is necessary to go back many years to find the first examples of pyridazine derivatives endowed with analgesic activity. In fact from 1949 to 1951 Gregory, Haworth and Wiggings, working at Birmingham University, published the first paper on this topic and claimed the corresponding patents [1,2] on compounds of general structure **1** (Figure 1).

the amino group at position 4 for analgesic activity. Indeed the replacement of this substituent with different groups like OH, OR, Cl, which display similar electronic effects, resulted in a complete loss of activity.

During the fifties Druey at the Ciba laboratories claimed the 4-cyano-2,5,6-trimethylpyridazinone (**triladine**) as an analgesic 3-5 fold more potent than **aminophenazone** in animal models [4].

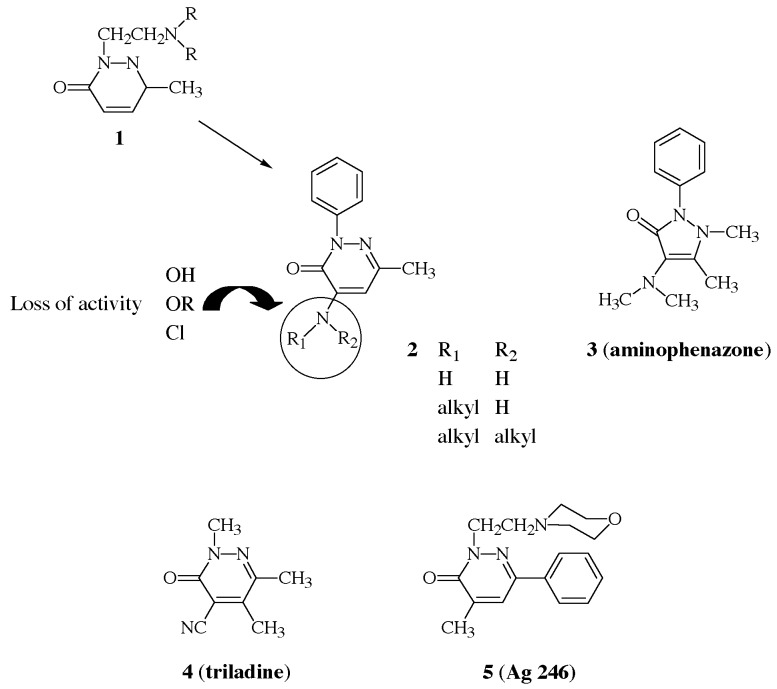
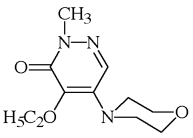


Figure 1. The first examples of 3(2H)-pyridazinones with analgesic activity.

In the same period these Authors also reported on a series of 4-amino and 4-alkyl(dialkylamino)-6-methyl-2-phenylpyridazinones **2**, which were designed with the idea to reproduce in the pyridazine class the structure of the **aminophenazone 3** [3]. This compound, both alone and in association, was at that time one of the most largely prescribed drugs worldwide, and remained an analgesic antiinflammatory and antipyretic drug of great success until the mid sixties. During their pioniering studies Gregory and coworkers stressed the crucial role played by

In the mid sixties Wermuth and Laborit [5,6] reported on compound **Ag 246**, as representative of a series of analogues. This potent analgesic-antiinflammatory agent is characterized by the presence of an aromatic system at position 6 and by morpholine, as tertiary amino group, at the end of a two-carbon side-chain linked at position 2. None of these compounds was commercially exploited.

It was necessary to wait until the mid eighties to see the first and, till now the only analgesic drug bearing a pyridazine fragment to reach the market: the **emorfazone**



6 (emorfazone)

M. Takaya *et al. J. Med. Chem.*, **1979**, 22,53

The first analgesic-antiinflammatory drug based on pyridazine system.

- Inhibition of vascular permeability
- Inhibition of bradykinin release
- Increase of β-endorphin release
- Pituitary-adrenocortical stimulation

M. Sato *et. al., Arzneim. Forsch. Drug Res.*, **31**, 1738 (1981).
M. Sato *et. al., Arzneim. Forsch. Drug Res.*, **32**, 379 (1982).

Figure 2. Emorfazone: structure and mechanism of action.

(Figure 2) which was synthesized by Takaya [7] and launched by Moroshita in Japan. This agent exhibits, attached at position 5, the same morpholine fragment which features **Ag 246**, whereas the main novelty versus the previous prototypes, is the presence of an alkoxy group linked to position 4 of the pyridazine backbone. The analgesic-antiinflammatory profile of **emorfazone**, which is a drug well tolerated in humans, appears particularly interesting. In fact its antinociceptive effect doesn't depend on prostaglandins system or opioid receptors affinity, but is mediated by a variety of mechanisms like inhibition of vascular permeability and bradikinin release, increase of β-endorphin release and, in part, by pituitary-adrenocortical stimulation.

In the last few years there has been a renewed interest for compounds with the pharmacological profile of **emorfazone** and in general for antinociceptive agents devoid of the well-known side effects of opiods, and those of non steroidal antiinflammatory drugs (NSAIDs). Many papers have been published in this field and, regarding the academic world, a French group directed by Viaud [8] at the Orleans University has been particularly active.

Interest in this area was emphasized by Williams [9] of the Abbott Laboratories (Figure 3) who recently published a perspective in which he reported that there are about 300 compounds currently in development with claims for use as analgesics. Among these, 70 are in clinical evaluation with a primary target of pain. Of these, 50% are alternative formulations, delivery technologies or combination of marketed analgesics and therefore are not new chemical entities; 20% are directed toward non opioid, non NSAID molecular targets and the remaining part is represented by opioids and cyclooxygenase 2 (COX_2**)** inhibitors.

Our interest in this area started at the beginning of the nineties, when among a large series of 4-amino-pyridazinones compound **7** (Figure 4) was identified as an agent displaying a promising pharmacological profile [10]. Indeed this compound exhibits a relevant antinflammatory activity p.o. in carrageenin rat paw edema and analgesic activity in the Randall Sellitto test (PAF-induced hyperalgesia) with ED_{50}=40 mg/Kg/po in both tests. On the contrary it was inactive in the writhing test. Taking into account its low acute toxicity (LD_{50}>1 g/Kg/po) **7** was considered an interesting lead for further synthetic development.

Further studies in this field [11] led us to find compound **8**, labeled $\mathbf{BB_3}$, as a more potent antinociceptive in the writhing test, with ED_{50}=15 mg/Kg/sc and being able to protect all the treated animals from the noxious stimulus at the dose of 100 mg/Kg (quantal protection 100%). On this basis BB_3 appears to be seven-fold more potent than **emorfazone**. Moreover, in the same test **emorfazone** failed to protect any treated animal at the dose of 100 mg/Kg.

In addition to the obvious different chemical, physicochemical and conformational properties of the acetyl and vinyl groups, replacement of $COCH_3$ with $CH{=}CH_2$ induces a significant improvement of the basic properties of the 4-amino group. In fact compound **7**, due to the strong electron-withdrawing effect of the conjugated

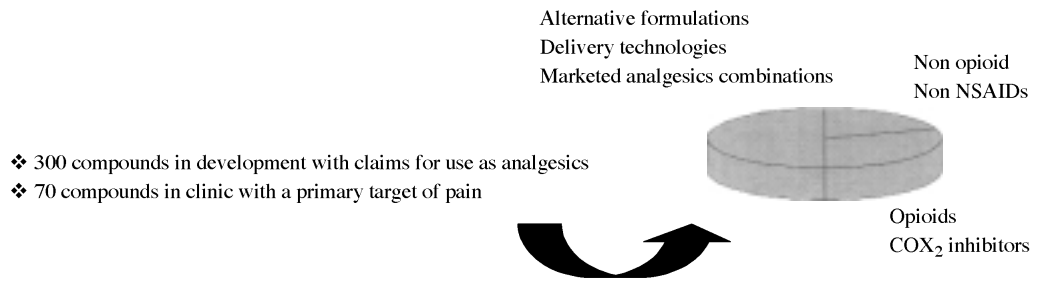
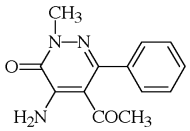
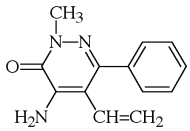


Figure 3. Compounds currently in development as analgesics.



7



8 (BB_3)

	Carrageenin rat paw edema	Randall Selitto	Writing test	Acute toxicity
ED_{50} mg/Kg/po	42	40	>100	
LD_{50} mg/Kg/po				>1000

	Writing test	Acute toxicity
ED_{50} mg/Kg/sc	14.9*	
LD_{50} mg/Kg/sc		>600

* Quantal protection = 100% at 100 mg/Kg s.c.

Figure 4. Pharmacological profile of compounds **7** and **8**.

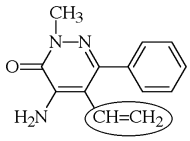
$COCH_3$, behaves as a very weak base. The major basicity of **BB_3** influences, of course, its possibility to exist, at least in part in a protonated form at physiological pH. Moreover the major basicity of **BB_3** could allow NH_2 to work as a hydrogen bond acceptor.

The discovery of **BB_3** represents the starting point for two different lines of research: a) studies on the mechanism of action, b) lead evolution.

a) Pharmacological investigations allowed us to establish that **BB_3** is able to induce antinociception in different animal models at relatively low doses [12]. This effect can be elicited regardless of the noxious stimulus applied: both in the hot-plate and tail-flick tests, which are based on termal stimuli, **BB_3** was able to induce antinociception. Similar results were obtained in writhing and formalin tests, where nociception was induced by chemicals (Figure 5).

A central mechanism can be hypothesized, since the pain threshold was enhanced both in hot plate and tail flick after in i.c.v. injection. At doses where **BB_3** induces a relevant antinociception in all the four tests, using both i.p. and i.c.v. administration, the treated animals retained their motor coordination and normal exploratory behaviour as evidenced in experiments performed with rotarod and activity cage respectively.

Involvement of opioid, α_2 adrenergic, GABA B and cholinergic nicotinic receptors could be ruled out on the basis of lack of reduction of antinociceptive activity by specific antagonists (naloxone, yohimbine, GCP 35348 and mecamilamine respectively). Involvement of the central muscarinic receptor system was suggested by the block of antinociceptive effect induced by atropine and also confirmed by the moderate affinity for central muscarinic receptors evidenced in binding experiments.



8 (BB_3)

BB_3 showed antinociceptive activity in hot plate and tail flick (termal stimulus) and in writhing test and formalin test (chemical stimulus)

CENTRAL MECHANISM

BB_3 enhanced pain treshold by icv injection

- Specific opioid, α_2 adrenergic, $GABA_B$, nicotinic antagonists did not reduce antinociceptive effect of BB_3
- Atropine blocked antinociceptive effect of BB_3 suggesting involvement of central muscarinic receptors

CH_2CH_3
$CH(OH)CH_3$
$CH(OC_2H_5)CH_3$
$COOCH_3$
$COCH_3$
CN

Loss of Activity

Figure 5. Studies on BB_3.

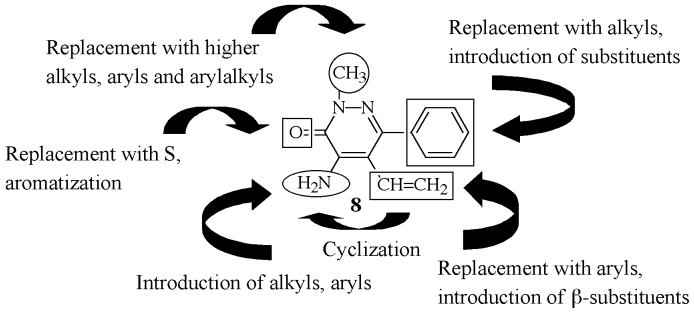


Figure 6. Chemical development of BB_3.

b) From this point of view our interest was greatly stimulated by the presence in **BB_3** of a vinyl group, which is unusual both in the previously reported pyridazines, and in other antinociceptive agents belonging to different chemical classes. Furthermore the vinyl group in **BB_3** appeared to be an essential requirement for antinociceptive activity measured in the writhing test since its replacement with a variety of functional groups like $COCH_3$, $COOCH_3$, $CH(OH)CH_3$, $CH(OR)CH_3$ and CN, as well as the saturation of the double bond resulted in a complete loss of activity. These groups are characterized, like $CH=CH_2$, by the presence of a 2 carbon chain or by 2 atoms linked by a multiple bond, as in the case of the cyano derivative.

The chemical development of the lead **BB_3** was performed following these criteria (Figure 6): -nature of the substituents at positions 2,4,6; -introduction of substituents on the vinyl function;. -modification of the carbonyl dipole;. -embodiment of the amino and vinyl groups into a heteroaromatic system; -replacement of the vinyl group with an aromatic system.

The effect of the introduction of different groups at position 2 on the antinociceptive activity (writhing test) is shown in Figure 7. Higher alkyls, arylalkyl, as well as functional groups are all compatible with antinociceptive activity, the ED_{50} ranging from 12 to 29 mg/Kg. Thus a considerable tolerance exists for this position, the least potent compound showing a 2 fold higher value of ED_{50} versus the most potent.

Looking at the structural modifications of NH_2 (Figure 8) a considerably higher range of ED_{50} was evidenced. The best results were obtained with the introduction of low alkylamino groups (compounds **14**, **15**). On the contrary introduction of dimethylamino (**18**), cycloalkylamino (**21**), arylamino (**17**), arylalkyl-amino (**19**) residues were associated with a dramatic

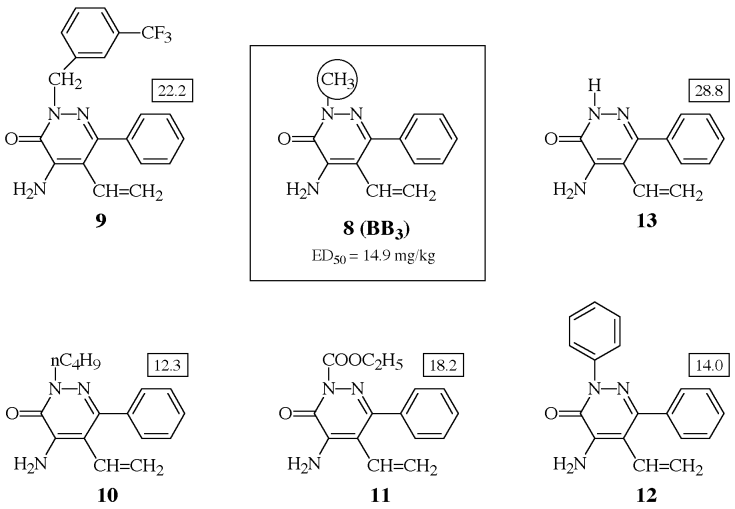


Figure 7. Antinociceptive activity: effect of structural modifications at positions 2.

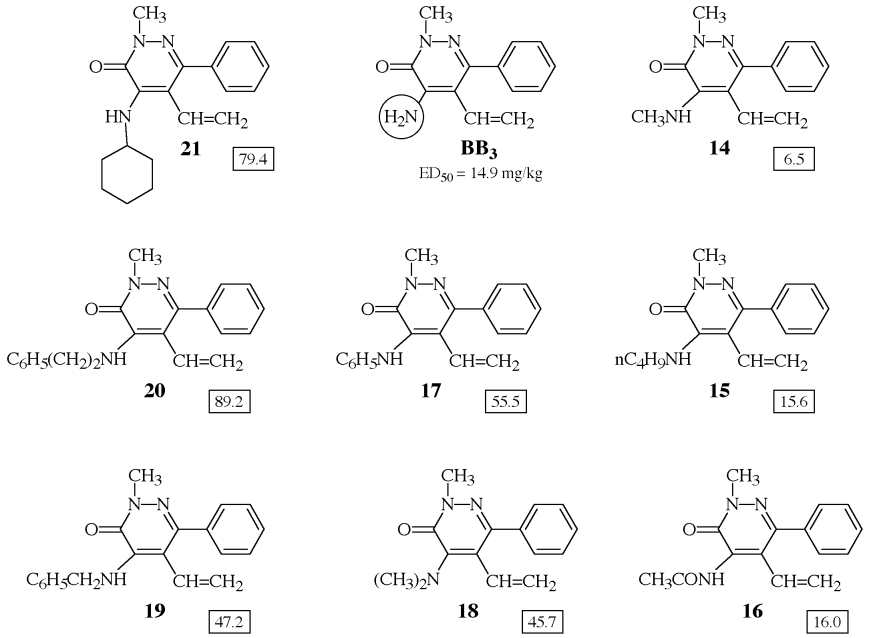


Figure 8. Antinociceptive activity: effect of structural modifications at position 4.

loss of antinociceptive activity. Since the acetamido (**16**) analogue proved to be a potent antinociceptive, it seems that a hydrogen bond donor group is an essential requirement for activity in this test. The inactivity of the more hindered aryl, cycloalkyl and arylalkylamino analogues could be explained by a limited steric tolerance for the substituent in this position. The first hypothesis was confirmed by the lack of activity found both for the methoxy analogue (**22**), which is the isoster of the most potent methylamino (**14**), as well as by the inactivity of the more hindered phenoxy analogue (**23**) (Figure 9).

The structural modifications of the carbonyl dipole, combined or not with the aromatization of the pyridazinone system (Figure 10), gave significant, but only preliminar results: the thiopyridazinone (**24**) was less potent than the oxo analogue (**27**), the 3-methoxy-pyridazine (**26**) was more than 3 fold less potent with respect to the isomeric reference compound (**8**), whereas the methylthio, quite surprisingly, was equipotent with **BB3**. Much work still must be done in this class to gain further data for structure-activity relationships (SARs).

Introduction of substituents like CH_3 and Br at the β-position of the vinyl group led to compounds with a comparable level of antinociceptive activity (Figure 11). The combination in the same molecule of a bromine atom on the vinyl group and an *n*-butyl at position 2 (**29**) led to a loss of activity.

A series of compounds characterized by the presence of a phenyl group instead of the vinyl, and bearing different carbon chains at position 2, was synthesized in order to verify if the vinyl group could be embodied into an aromatic system maintaining the antinociceptive activity

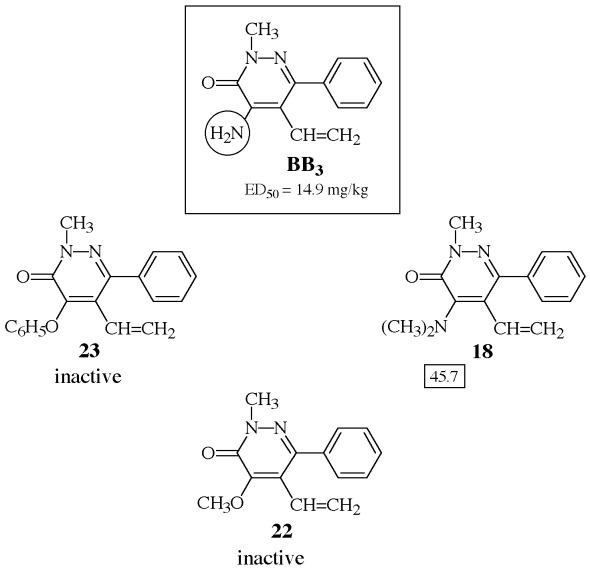


Figure 9. Antinociceptive activity: effect of further modifications at position 4.

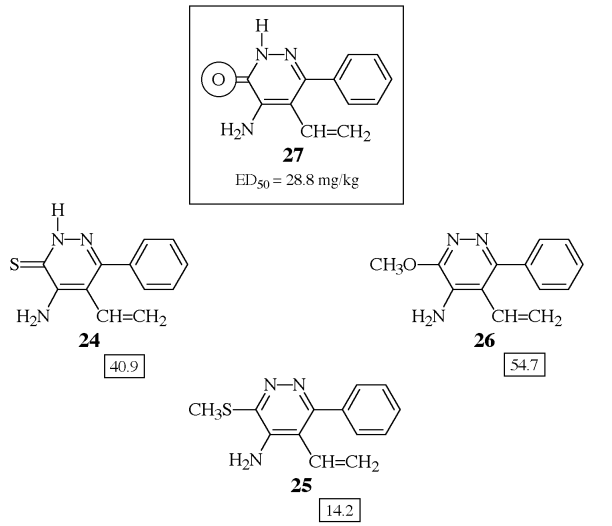


Figure 10. Antinociceptive activity: effect of structural modifications at position 3 and 2-3.

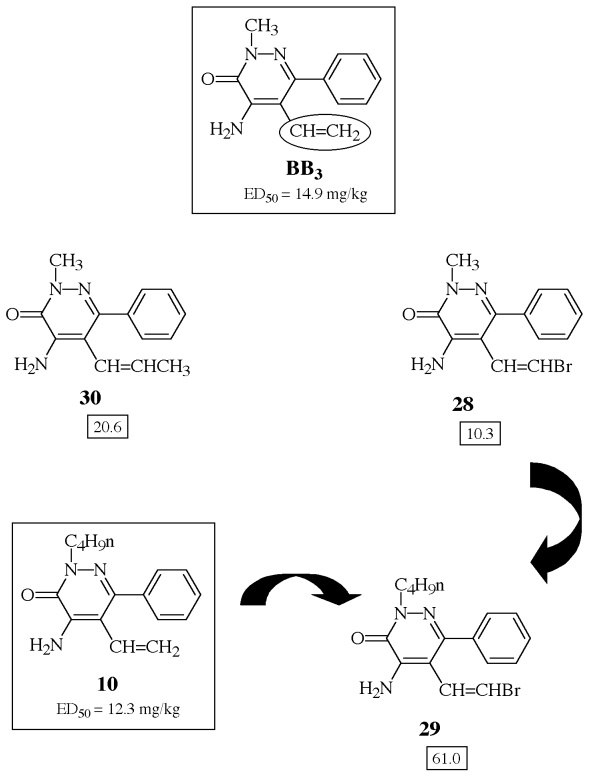


Figure 11. Antinociceptive activity: effect of structural modifications of the vinyl group.

(Figure 12). Furthermore we were stimulated to explore this structural modification also by the high activity reported in the literature by Nannini *et al*., [13] for compounds bearing the same 5,6-diphenylpyridazine fragment (**35**). The ED_{50} of these **BB_3** analogues range from 14 to 162 mg/Kg as a function of the substituent at position 2. Thus an order of magnitude difference between the most potent and the least potent was observed. This finding clearly suggests that there is an optimum length for the carbon chain (2 carbons). Probably for this series of 5,6-diphenylpyridazinones a different type of interaction with the target and/or a different mechanism of action could operate since in the vinyl series we found very high tolerance for this position.

The results obtained with a limited number of **BB_3** analogues differently substituted at position 6 seem to indicate that a wide tolerance exists for this substituent (Figure 13). It is worth noting the high level of activity evidenced for the 4-amino-2,6-dimethyl-5-vinylpyridazinone (**38**). The pyrazolopyridazinone (**39**) was synthesized as a rigid analogue of **BB_3** and tested as antinociceptive. An interesting level of activity was found for this compound. This finding opens promising perspectives for future developments.

In Figure 14 the idea which generated the synthesis of a further series of pyridazinones potentially useful as antinociceptives is depicted. In the literature there are many examples of non opioid, non NSAIDs characterized by the presence of an arypiperazinyl alkyl moiety linked to the nitrogen of a lactamic system. Among them a group of compounds described by Viaud *et al*., [8] (**40**), as well as the well-known antidepressant **trazodone** (**41**) [14], show ED_{50} values in writhing test in the range 5-10 mg/Kg. Moreover compounds (**42**), previously synthesized in our laboratory showed promising activity in writhing test [10]. Thus we have combined in the same molecule the previous structural fragment and the vinyl function, using the 6-phenylpyridazinone as spacer (**43**). The obtained results were quite disappointing and seem to indicate that a similar combination is not associated with a relevant antinociceptive activity. Only two compounds (n=2, R_1=H, R_2=CH_3 and n=3, R_1=OC_2H_5, R_2=H) emerged, showing 53 and 76% reduction of writhes at 100 mg/Kg respectively. Concomitant sedative effects evidenced for the last one make void the obtained result. The 53% inhibition (at 100 mg/Kg) obtained with the first one is comparable with that of Emorfazone (60% at the same dose). Moreover our compound was able to completely protect 20% of the treated animals from the noxious stimulus, while Emorfazone does not. The synthesis of further analogues is in progress.

Among the newly synthesized compounds we selected the *n*-butyl analogue of **BB_3** (compound **10**) that was labeled **CV_1** and submitted it to a more in

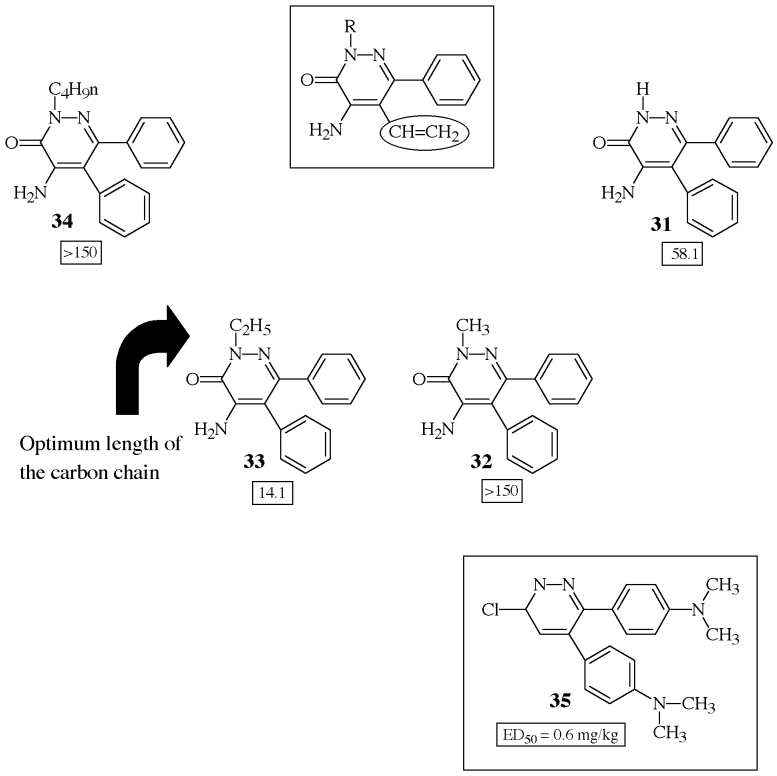


Figure 12. Antinociceptive activity: effect of the replacement of the vinyl group with a phenyl ring.

depth pharmacological study (Figure 15). Like the analogue **BB$_3$**, **CV$_1$** was devoid of affinity for a variety receptor systems like opioid, adenosine A_1/A_2, α_2 adrenergic, α_1 adrenergic, $5HT_{1A}$ receptors. Unlike **BB$_3$**, **CV$_1$** was devoid of affinity for central muscarinic receptors that we considered responsible, at least in part for the antinociceptive effect of **BB$_3$**.

Chemistry.

The final compounds **47** and **48** were synthesized following the procedure depicted in Scheme 1. Treatment of the appropriate isoxazolo[3,4-*d*]pyridazinone **44** with H_2/Pd/C afforded the 5-acyl-4-aminopyridazinones **45** which, in turn, by reduction with sodium borohydride gave the corresponding secondary alcohols **46**, which were converted into the 5-vinylpyridazinones **47** by treatment with PPA. Oxidative ring cleavage of the same precursor **44** with CAN in acetic acid afforded the 5-acyl-4-nitropyridazinones **51**. The nitro group of these intermediates was easily replaced by nitrogen and oxygen nucleophiles to afford the corresponding 4-amino and 4-alkoxy (aryloxy) derivatives **50**. The final compounds **48** were obtained using the same two steps procedure employed for the synthesis of **47**.

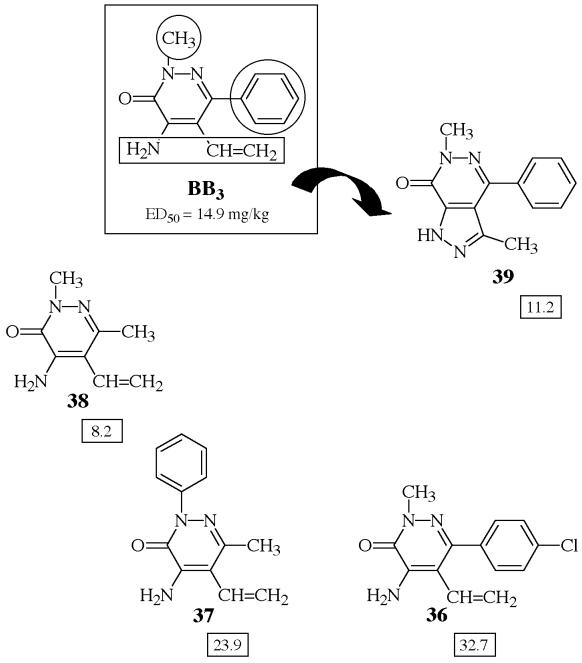


Figure 13. Antinociceptive activity: effect of structural modifications at position 2,6 and 4,5 (rigid analog).

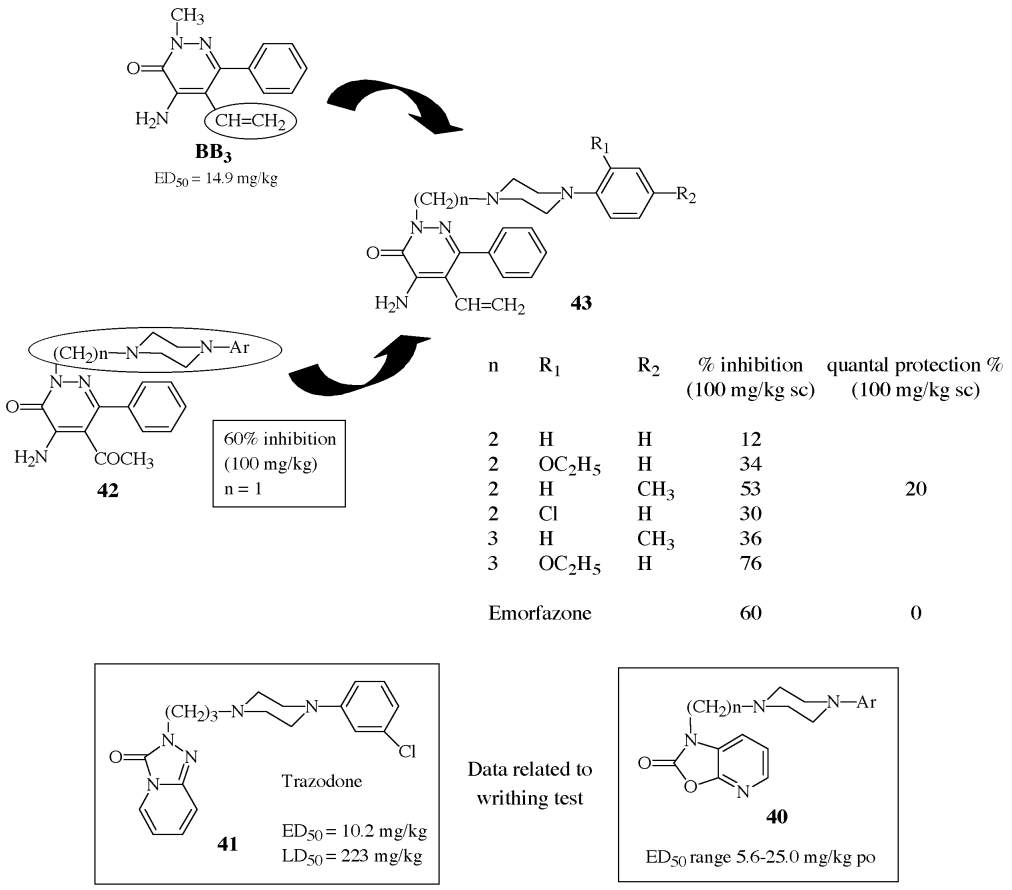


Figure 14. 4-Amino-2-[(4-arylpiperazin-1-yl)-alkyl]-5-vinylpyridazinones: antinociceptive activity.

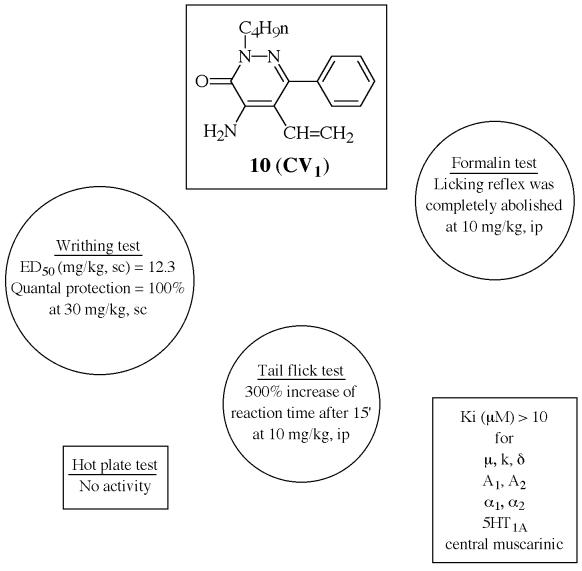
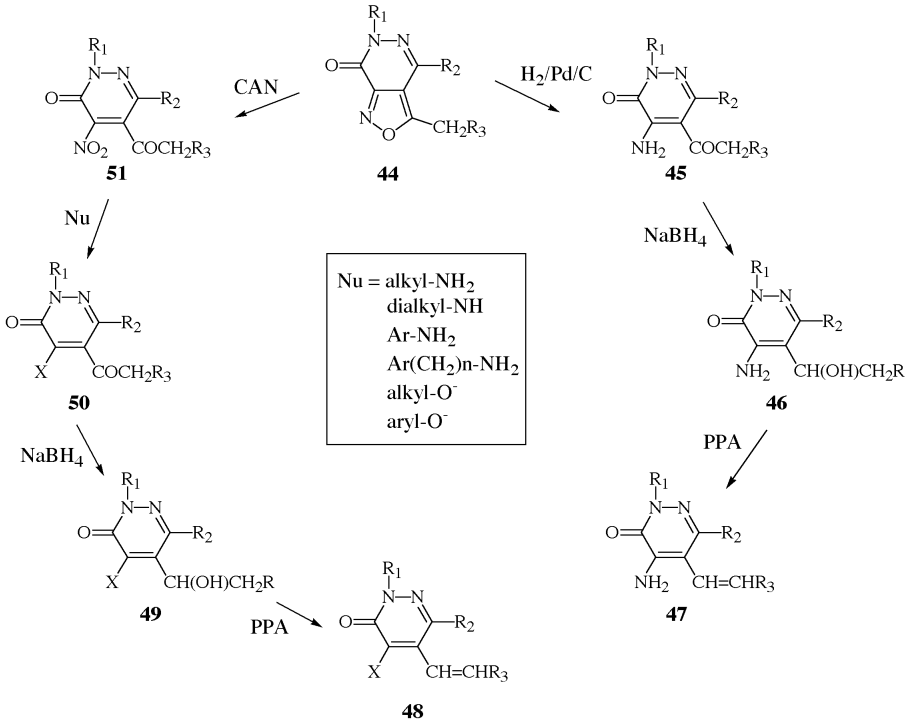


Figure 15. Pharmacological profile of 4-amino-2-n-butyl-6-phenyl-5-vinylpyridazinone (CV1).

Treatment of compound **52** with Lawesson's reagent (Scheme 2) afforded the key intermediate from which the final compound **24** was obtained using the usual three steps procedure based on reductive ring cleavage, reduction with sodium borohydride, followed by dehydratation with PPA. Alkylation of **53** with iodomethane gave rise to the 7-methylthio-isoxazolo-pyridazinone **54** which was converted in the 4-amino-5-vinylpyridazine **25** through the above described three steps procedure. When compound **54** was treated with sodium methoxyde the corresponding 7-methoxy derivative **55** was obtained. From this precursor the final **26** was prepared by the usual three steps procedure.

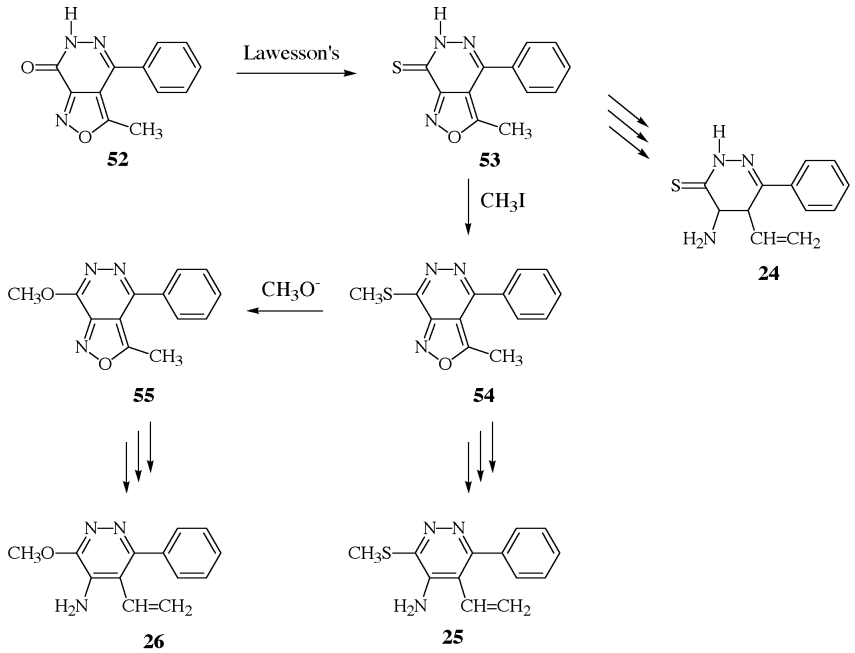
The 4-amino-5,6-diphenylpyridazinone **58** (Scheme 3) was synthesized starting from **56** which was alkylated to give compounds **57**, in which the amino group was introduced by heating at 190 °C in sealed tube with hydrazine, following the procedure described by Coates *et al.*, [15].

Scheme 1



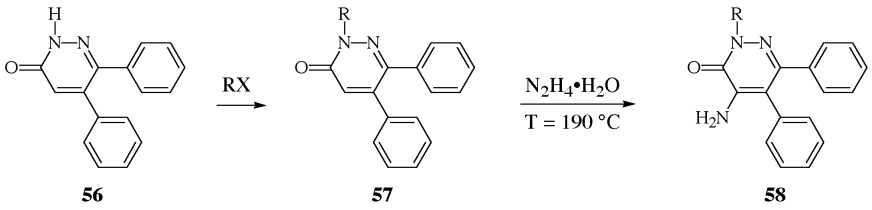
Synthesis of 4-Amino-5-vinylpyridazinones and Related Compounds.

Scheme 2



Synthesis of 3-Thiopyridazinones and Related Compounds.

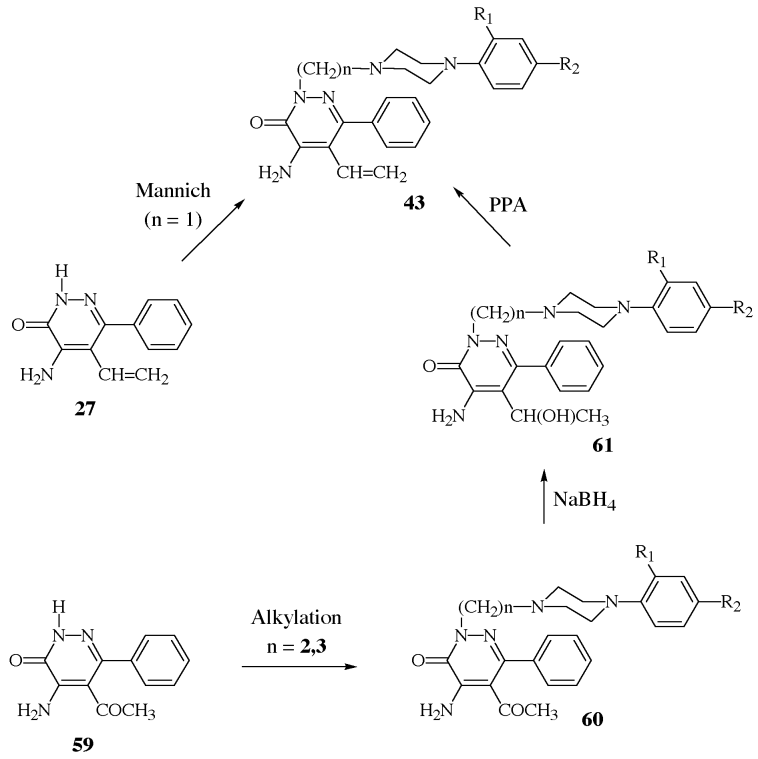
Scheme 3



56 **57** **58**

Synthesis of 4-Amino-5,6-diphenylpyridazinones.

Scheme 4



27 **43** **61** **59** **60**

Synthesis of 4-Amino-2-[(4-arylpiperazin-1-yl)-alkyl]-5-vinylpyridazinones.

In Scheme 4 are depicted synthetic procedures followed to obtain compounds **43**. Products with n=1 were obtained by condensing the precursor **27** with formaldehyde and the appropriate arylpiperazine in the conditions of the Mannich reaction. The superior homologues **43** (n=2,3) were prepared by condensing the 5-acyl-4-aminopyridazinone **59** with appropriate 1-aryl-4-bromoalkylpiperazine, followed by reduction with sodium borohydride and dehydratation with PPA.

Conclusions.

We have identified a series of 4-aminopyridazinones endowed with relevant antinociceptive activity in animal models. A sub-series of these compounds is characterized by the presence of an unusual functional group, the vinyl group, whose replacement led to a dramatic loss of activity.

The high level of potency and the absence of behavioural effects make these agents, whose

activity doesn't depend on opiod affinity and prostaglandins system, very promising leads for further development.

Aknowledgements.

Support of this work by the MURST (40%) is gratefully acknowledged.

REFERENCES AND NOTES

[1] H. Gregory and L. F. Wiggings, *J. Chem. Soc*., 2546 (1949).

[2] W. N. Haworth and L. F. Wiggings, British Patent 631, 755 (1949); *Chem. Abstr*., **44**, 4046g (1950).

[3] W. N. Haworth and L. F. Wiggings, British Patent 656, 228 (1951); *Chem. Abstr*., **46**, 7593i (1952).

[4] P. Schmidt and J. Druey, *Helv. Chim. Acta*, **40**, 1749, (1957).

[5] G. C. Wermuth, *Revue Aggressologie*, **4**, VI, 383 (1965).

[6] H. Laborit and G. C. Wermuth, *Aggressologie*, **6**, 415, (1965).

[7] M. Takaya, M. Sato, A. Tereshima and J. Maki, *J. Med. Chem*., **22**, 53 (1968).

[8] M. C. Viaud, P. Jamoneau, C. Flouzat, J.-G. Bizot-Espiard, B. Pfeiffer, P. Renard, D.-H. Caignard, G. Adam and G. Guillaumet, *J. Med. Chem*., **38**, 1278 (1995).

[9] M. Williams, E. A. Kowaluk and J. P. Arneric, *J. Med. Chem*., **42**, 1481 (1999).

[10] V. Dal Piaz, G. Ciciani, G. Turco, M. P. Giovannoni, M. Miceli, R. Pirisino and M. Perretti, *J. Pharm. Sci*., **80**, 341 (1991).

[11] V. Dal Piaz, M. P. Giovannoni, G. Ciciani, D. Barlocco, G. Giardina, G. Petrone and G. D. Clarke, al *Eur. J. Med. Chem*., **31**, 65 (1996).

[12] S. Pieretti, V. Dal Piaz, R. Matucci, M. P. Giovannoni and A. Galli, *Life Sciences*, **65**, 1381 (1999).

[13] G. Nannini, G. Biasoli, E. Perrone, A. Forgioni, A. Buttinoni and M. Ferrari, *Eur. J. Med. Chem*., **14**, 53 (1979).

[14] P. Valeri, G. Pimpinella, L. A. Morrone and L. Romanelli, *Gen. Pharmacol*., **22**, 127 (1991).

[15] W. J. Coates and A. Mc Killop, *Heterocycles*, **29**, 1077 (1989).