Versatility of the Pyridazine System: Chemistry and Biology

G. Cignarella* and D. Barlocco

Ist. Chimico Farmaceutico e Tossicologico, Viale Abruzzi 42, 20131 Milano (Italy)

In accordance with the title of my lecture, I am going to briefly illustrate our researches on pyridazine derivatives active in different biological areas.

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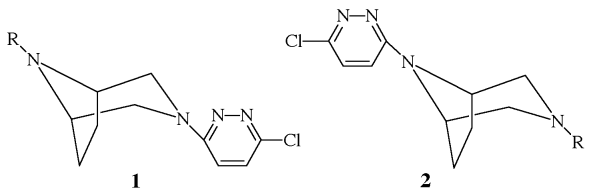
Central Analgesics.

The first topic is represented by analgesic pyridazines having the following isomeric structures **1** and **2**.

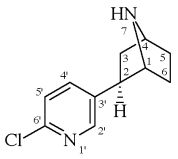


1 **2**

R = H, alkyl, acyl

Figure 1

They are constituted by a 3,8-diazabicyclo[3.2.1]octane (DBO) system, having two non equivalent nitrogens in 3 and 8, substituted by a chloropyridazine. These compounds were devised having epibatidine as the model.

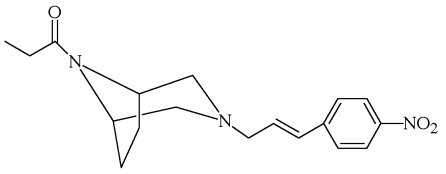


Epibatidine

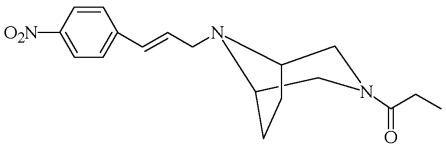
Figure 2

This alkaloid was isolated by Daly and coworkers [1] from the skin of an ecuadorian poison frog. It has the structure of 7-azabicyclo[2.2.1]heptane substituted at the 2-position by a 2-chloro pyridine with *exo*-configuration. It was shown to be about 200 fold more active than morphine in the hot-plate test (mouse), whith a non-opioid and non-cholinergic mechanism of action, but with the involvement of the nicotinic system.

Previous extensive studies from my group on the 3,8-diazabicyclo[3.2.1]octane system [2-4] led to the observation that, when substituted at the nitrogen atoms by a propionyl and by an arylpropenyl chain, it displayed a central analgesic activity comparable or even better than morphine. In particular, **DBO-11** and **DBO-17** were found 10 and 30 fold, respectively, more potent than morphine in the hot-plate test (mouse), with the advantage of definitively lower side effects like tolerance and physical dependence [5].



DBO 11 $K_i(\mu) = 25 \pm 3.5$ n*M*

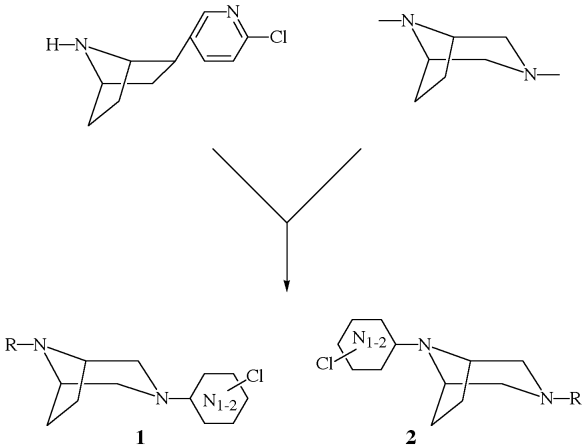


DBO 17 $K_i(\mu) = 5.1 \pm 0.33$ n*M*

Figure 3

Interestingly, despite their structural dissimilarity with morphine, their mechanism of action involved interaction with μ opioid receptors, similar to the reference drug, but with better μ/δ, and κ selectivity.

Based on the presence both in epibatidine and in analgesic DBO of a nitrogen substituted bicyclic system, we decided to build up hybrid compounds, aiming to verify if DBO could be a proper substrate to develop compounds provided with analgesic properties similar to those of epibatidine. If so, their mechanism of action would also be investigated.



1 **2**

Figure 4

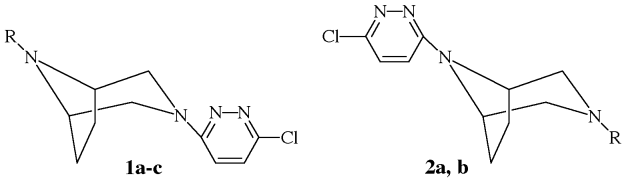
The choice of the heterocyclic substituent was extended from chloropyridine to chlorinated six-membered heterocycles having two nitrogens in their ring, *e.g.*, pyrazine, pyrimidine and pyridazines. In particular, amongst 15 derivatives tested, the 6-chloro-3-pyridazinyl-DBO (**1a**) was found to be the most interesting [6]. In the table the minimal analgesic dose (MAD) and the maximal non-toxic dose (MNTD) and efficacy of pyridazine derivatives **1** and **2** are compared, having as reference both epibatidine and morphine.

that **1a** presents one conformation similar to that of epibatidine, thus suggesting that this could be the bioactive conformer [6].

Chemistry.

The key intermediate in the synthesis of compounds **1** was the known 3-benzyl DBO (**3**) [7], which was first protected at the *N*-8 with di-*tert*-butyldicarbonate to give **4**, then transformed into **5** by catalytic debenzylation. Treatment of the latter with an equimolar amount of the

Table 1



1a-c **2a, b**

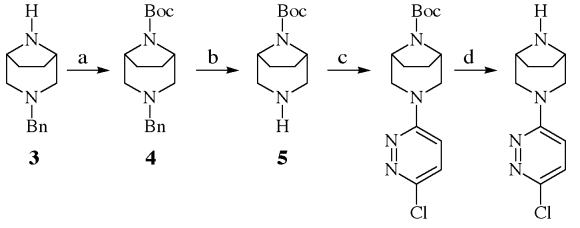
R = H (a), COEt (b), Et (c)

Compound	Minimal analgesic dose (mg/kg, sc) (MAD)	Maximal non toxic dose (mg/kg, sc) (MNTD)	% analgesic efficacy compared to Morphine	Epibatidine
1a	1	30	136	167
1b	inactive	>60	---	---
1c	10	20	61	74
2a	15	30	107	130
2b	inactive	>50	---	---
Morphine	2	20	100	122
Epibatidine	0.001	0.007	82	100

As indicated in the table, compound **1a** retained significant analgesic activity still at 1 mg/kg s.c., when evaluated in the hot-plate test (mouse). Its analgesic action remained almost unchanged after 45 minutes. Though definitely less potent than epibatidine, which is active in the same test at 1 μg/kg, **1a** favourably compared with morphine, active at 2 mg/kg. In addition, **1a** exhibited the highest ratio (30) between the maximal non-toxic dose and the minimal analgesic dose, when compared to morphine (10) and epibatidine (7). It should be noted that reversion of the chloropyridazine substituent from N_3 to N_8 as in **2a**, or the insertion on N_8 of an alkyl (**1c**) or a propionyl group (**1b**), markedly reduced or - as for **1b** - abolished the activity.

Binding experiments indicated for **1a** high affinity and selectivity towards nicotinic receptors, in particular for the $\alpha_4\beta_2$ nAChR subtype (Ki = 4.1 n*M*). On the contrary, no binding at the opioid receptors was observed. Finally, the results of the modeling indicated

Scheme 1

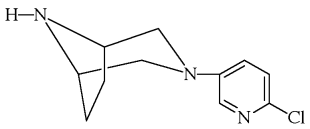


3 **4** **5** **6** **1a HCl**

a) *di-tert*-butyldicarbonate/CH_2Cl_2
b) Pd-C/H_2/EtOH
c) ArCl/Toluene/TEA/Δ
d) Et_2O/HCl

appropriate heterocycle in refluxing toluene and in the presence of equimolar triethylamine gave the Boc derivative **6** that was finally deprotected with a solution of HCl in Et_2O to give the desired **1**.

The reverted isomer **2a** was obtained from the known 3-propionyl DBO [8] by reaction with 2,6-dichloropyridazine as for **1a**, followed by *N*-deacylation in refluxing 2 *N* NaOH. Eventually, to further investigate this class, we synthesized and tested the chloropyridine DBO, having the same heterocyclic substituent of epibatine.



K_i = 0.95 n*M* (for **1a** K_i = 4.1 n*M*)
MNTD/MAD = 30/3 = 10

Figure 5

The results were somewhat disappointing. In fact, though the affinity towards $\alpha_4\beta_2$ nAchR nicotinic receptor was better than that of **1a**, the compound displayed a weaker *in vivo* activity, with lower MNTD/MAD ratio. In addition, its synthesis was more difficult than for **1a**. In fact, the needed 2-nitro-4-bromo-piperidine had to be prepared by H_2O_2 oxidation of the commercially available 2-amino-4-bromopiridine followed by condensation with Boc-DBO, reduction of the nitro group to the corresponding amino, diazotization of the latter and transformation into the chloro analog with concomitant removal of the protecting group.

Aldose Reductase Inhibitors.

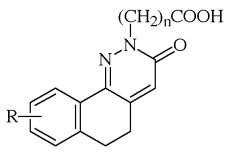
We started working on tricyclic pyridazinones active as aldose reductase (ALR2) inhibitors about 5 years ago. ALR2 is the first enzyme of the so-called polyol pathway, which in the presence of NADPH converts glucose to sorbitol, in turn processed to fructose by sorbitol dehydrogenase.

Under diabetic conditions, the increased glucose flux with consequent high intracellular accumulation of sorbitol is likely to be involved in late-onset diabetic complications such as neuropathy, nephropaty, retinopathy, and cataracts [9].

Inhibitors of ALR2 thus seem to have the potential to prevent or treat such diabetic complications. However, several problems are associated with ALR2-inhibitors therapy, *e.g.*, poor selctivity and decline of effectiveness over prolonged period of treatment.

At the beginning of our researches we were stimulated by a paper of French Authors [10] dealing with pyridazinones having acidic side chains at positions 2 and 4 of the heterocycle, which were reported to be potent inhibitors of ALR2. On these bases, we identified the benzocinnolinones having acidic side chains at the N-2 as potential new substrates.

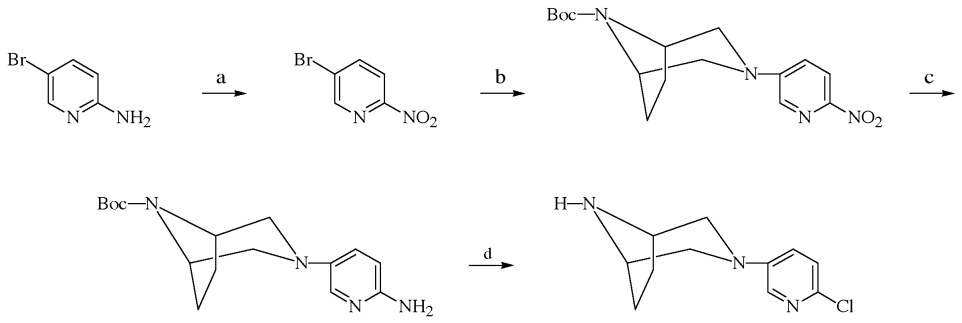
Table 2



Compound	R	n	Aldose red [a]	Aldehyde red [a]
	H	1	12.6 (7.14-22.1)	156 (141-173)
		2	133 (117-152)	32.7% (152μ*M*)
		3	11.4 (7.60-17.2)	92.6 (65.8-130)
sorbinil			3.04 (2.91-3.52)	1.74 (1.69-1.79)
tolrestat			0.096 (0.079-0.117)	1.21 (0.87-1.70)

[a] $\mathbf{IC_{50}}$ (μ*M*)

Scheme 2

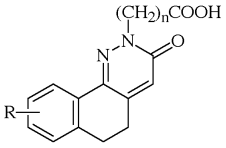


a) H_2SO_4/H_2O_2
b) DBO-Boc/TEA/toluene/Δ
c) H_2/Pd/C 10 %/EtOH
d) $NaNO_2$/HCl

The preliminary results [11] indicated that acetic- and butyric acid derivatives were significantly effective as ALR2 inhibitors and, contrary to the well known ALR2 inhibitor sorbinil, showed good selectivity with respect to other oxoreductase like aldehyde reductase.

Based on these models, we evaluated the effect of substituents like the methoxy group on the various positions of the phenyl moiety. For the acetic series, we found that positions 7 and 8 gave the best compounds, while in the higher homologs position 9 gave the most active terms.

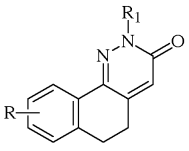
Table 3



R	n	$IC_{50}(\mu M)$	R	n	$IC_{50}(\mu M)$
7-OCH_3	1	6.65	7-OCH_3	3	19.62
8-OCH_3	1	10.31	8-OCH_3	3	25.81
9-OCH_3	1	43.62	9-OCH_3	3	4.79
10-OCH_3	1	175.0	10-OCH_3	3	73.8
Sorbinil		3.04	Sorbinil		3.04

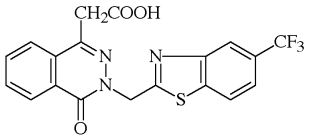
We also explored the insertion of an unsaturation in the butyric chain. Also in this case the effect is dependent on the position of the methoxy group. The activity was improved in the 7-OCH_3 butenoic derivative, whereas in the 9-OCH_3 a slight decrease of potency was observed.

Table 4



R	R_1	$IC_{50}(\mu M)$
7-OCH_3	$CH_2CH_2CH_2$-COOH	19.62 (15.58-24.70)
7-OCH_3	CH_2CH=CH-COOH	4.25 (3.65-4.95)
9-OCH_3	$CH_2CH_2CH_2$-COOH	4.79 (3.63-6.31)
9-OCH_3	CH_2CH=CH-COOH	8.99 (7.98-10.13)
Sorbinil		3.04 (2.91-3.52)

However, the most interesting result in this field derived from a research developed in collaboration with the Dept. of Pharmaceutical Sciences of the University of Modena, selecting zopolrestat as the reference drug.



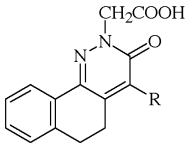
Zopolrestat

Figure 6

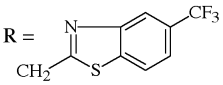
Some results in this direction have already been presented in Sopron [12] and I will only reproduce them briefly for completness.

By docking the tricyclic pyridazinone **Ia**, taken as the model, into the crystal structure of ALR2, it appeared that the carboxylate occupies the same anionic site as the carboxylate moiety of zopolrestat, while the carbonyl group interacts with Cys288. However, our compound did not participate in binding the benzothiazolyl site as zopolrestat does. It was therefore speculated that if **Ia** was functionalyzed with a benzothiazolyl moiety properly oriented, the interaction with the enzyme should improve. The modeling of the complex with **Ia** also indicated that the side chain had to be introduced at position 4 of the pyridazinone moiety [13].

Actually, the structure of the minimized complex of the enzyme with **Ib** indicated that the benzothiazolyl fragment is correctly placed in a site suitable for interaction. The compound was then synthesized and tested. As expected, the result was very interesting, the new derivative being two orders of magnitude more potent than the model **Ia**.



Ia R = H $IC_{50} = 12 \pm 3\ \mu M$

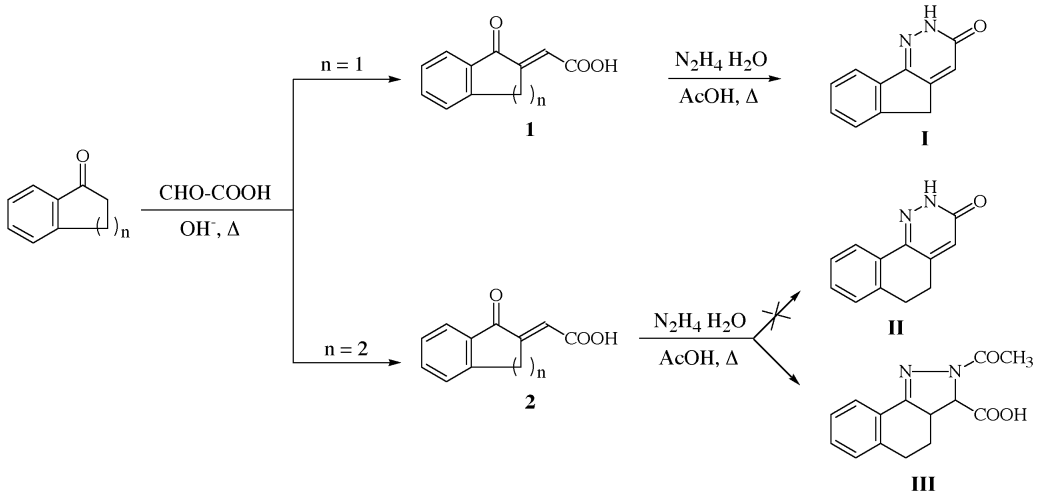


Ib R = $IC_{50} = 0.15 \pm 0.03\ \mu M$

Figure 7

Finally, there is a chemical aspect on the synthesis of the benzocinnolinone system I wish to illustrate. The first attempts at obtaining this moiety was based on the synthetic pathway followed for the lower homolog indenopyridazinone. It involved heating of indanone with glyoxylic acid in alkaline medium to give the unsaturated acid **1** which was condensed with hydrazine hydrate in acetic acid to the desired **I**. However, repetition of this procedure to obtain **II** failed, the only product isolated being the dihydro benzindazolo **III**.

Scheme 3

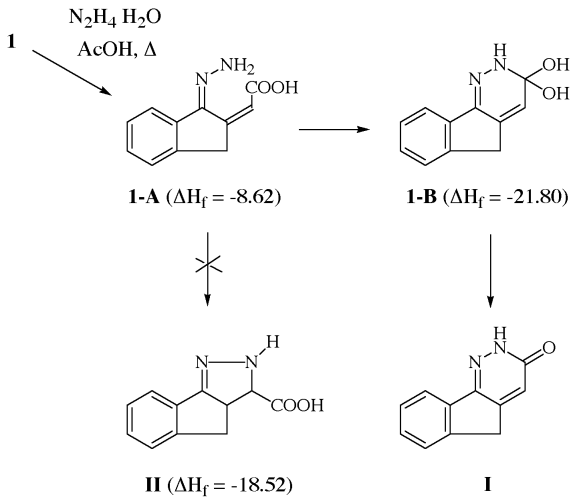


Possible reasons of failure to obtain **II** could be due to a) different configuration of the double bond b) geometrical constraints dictated by different size of the ring c) electronic reasons. Theoretical calculations [14] of the isomers *E* and *Z* of **1** and **2** indicated that on the basis of the heat of formation the *E* configuration is preferred for **1** (ΔH_f = -85.15 Kcal/mol versus ΔH_f = -83.63 Kcal/mol), while for **2** the *E* and *Z* geometrical isomers exhibited almost the same heat of formation (ΔH_f = -90.20 Kcal/mol for *E* and –90.12 Kcal/mol for *Z*). However, by submitting solutions of **1** and **2** to photoirradiation, monitoring by UV-visible and ^{1}H-NMR spectroscopy indicated for both compounds a photoisomerization to a stable *Z*-configuration. As a consequence other factors than configuration should influence the course of the condensation of **1** and **2** with hydrazine. Theoretical calculations of the heat of formation of the possible intermediates suggested that in the case of the indanylidene acetic acid **1** the evolution of the initially formed hydrazone (**1-A**) towards the pyridazinone hydrate (**1-B**) is thermodinamically favoured by the heat of formation lower than that of the carboxylic acid **II**.

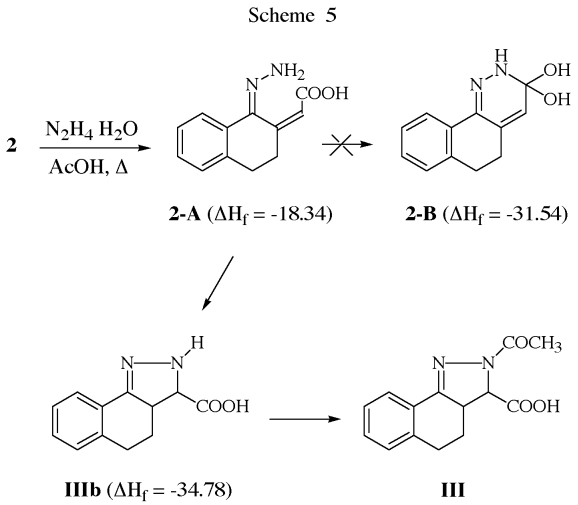
On the contrary, for the higher homolog **2** the addition of the N-amino group to the double bond to give **IIIb** is favoured with respect to the condensation to **2-B**.

In conclusion, the choice of the reaction path is determined by thermodinamic control. In any case, the synthesis of the desired benzocinnolinones was easily accomplished according to a procedure I presented at the 6th Symposium in Florida [15]. Briefly, by carrying out the condensation with glyoxylic acid at room temperature, the hydroxy acid was isolated, which easily cyclized with hydrazine to give the desired benzocinnolinone, passing through the 4-hydroxy derivative.

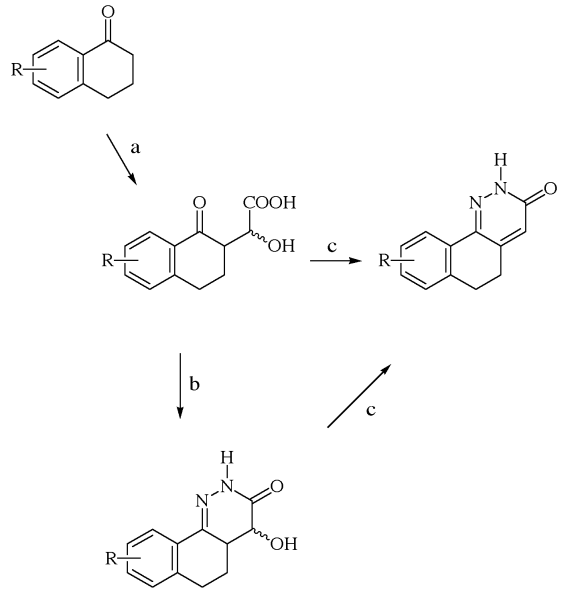
Scheme 4



Scheme 5



Scheme 6



a) CHO-COOH, NaOH, H_2O, r.t.; b) NH_2NH_2 H_2O, EtOH, Δ, 1 hour; c) NH_2NH_2 H_2O excess, Δ, 0.5 hour.

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