ELSEVIER

Il Farmaco 54 (1999) 248–254

IL FARMACO

Quinolizidinyl derivatives of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylic acid and 1-homolupinanoyl benzimidazolones as ligands for 5-HT_3 and 5-HT_4 receptors☆

Anna Sparatore [a,*], Alfredo Cagnotto [b], Fabio Sparatore [c]

[a] *Istituto di Chimica Farmaceutica e Tossicologica, Università di Milano, Viale Abruzzi, 42, 20131 Milan, Italy*
[b] *Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62-20157 Milan, Italy*
[c] *Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV, 3-16132 Genoa, Italy*

Received 23 November 1998; accepted 20 February 1999

Abstract

Five 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylic acid derivatives of lupinine, *epi*-lupinine and lupinylamine, together with two 1-homolupinanoyl benzimidazolones were prepared and investigated for their ability to displace specific radioligands from 5-HT_3 and 5-HT_4 receptors. The synthesized compounds were only moderately active, with IC_{50} in the micromolar range. The compound with the highest affinity for 5-HT_4 receptor was tested for the enhancement of intestinal transit rate but was inactive at the oral dose of 100 mg/kg.

Keywords: Quinolizidine derivatives; Benzimidazolone derivatives; 5-HT_3 and 5-HT_4 receptors

1. Introduction

For many years some of us have investigated the effect of the exchange of dialkylaminoalkyl chains (or other basic cyclic head) of different drugs with the rigid and cumbersome lupinyl (1-quinolizidinylmethyl) residue, on the grounds that such a substitution, while preventing the interactions with some kinds of receptors, could allow highly selective interactions with others. Sometimes the *epi*-lupinyl residue was also considered in these studies.

Recently a set of *N*-lupinyl-2-methoxybenzamides as analogues of the most used 'orthopramides' (metoclopramide, sulpiride, tiapride, cisapride, etc.) was prepared [1].

The metoclopramide analogue **1** showed poor or very moderate affinity for D_2 and 5-HT_3 receptors ($K_i > 10$ μM and = 2.65 μM, respectively), but relaxed the previously contracted oesophageal *tunica muscularis mucosae* of rat (a 5-HT_4-mediated effect) with $EC_{50} = 1.7$ μM, whereas serotonin had $EC_{50} = 0.17$ μM [2]. The *N*-(*epi*-lupinyl)-4-amino-5-chloro-2-methoxybenzamide (the epimer of **1**) exhibited a slightly higher affinity for 5-HT_3 receptor, with $K_i = 0.62$ μM.

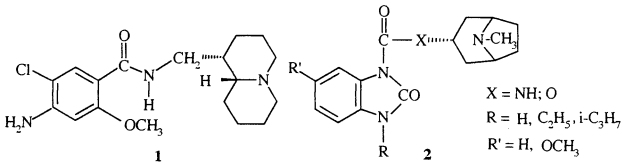
Compound **1** was found to be able to increase the progression of a charcoal bolus in mice in the same measure as domperidone and levo-sulpiride and much more than metoclopramide itself. This action could be related to the stimulation of the 5-HT_4 receptor, but could also depend on more direct interaction with muscarinic receptors.

On the other hand, it is known that other conformationally restricted basic chains (such as α-tropanyl) joined through an amidic (or esteric) bond to disparate aromatic or heteroaromatic moieties, give rise to compounds with high affinity for 5-HT_3 and 5-HT_4 receptors [3]. Particularly, selected derivatives of benzimidazolone (**2**) can interact with 5-HT_3 and 5-HT_4 receptors with different profiles of agonist or antagonist activity [4–6].

☆ Presented at the 'First Italian–Swiss Meeting on Medicinal Chemistry', September 23–26, 1997 Turin, Italy.

* Corresponding author. Fax: +39-02-295-14197.
E-mail address: anna.sparatore@unimi.it (A. Sparatore)

0014-827X/99/$ - see front matter
PII: S0014-827X(99)00025-7



1 2

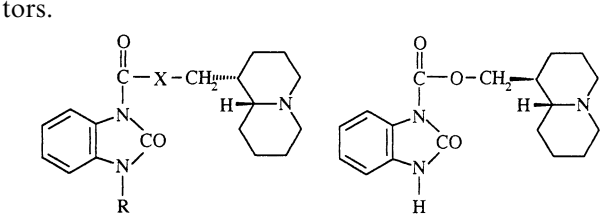
Benzimidazolone derivatives **2** were potent antagonists towards the 5-HT_3 receptor, with high affinity (pK_i = 8.42–8.70), comparable to, or superior than, that of ondansetron (pK_i = 8.42). On the other hand, their behaviour towards 5-HT_4 receptors was rather peculiar: when X = NH and R = H (DAU 6215), no appreciable interaction was seen, while with the introduction of ethyl (BIMU-1) or isopropyl (BIMU-8) on the benzimidazolone nitrogen, partial agonism or full agonism were exhibited. In the case of ester derivatives, the introduction of a methoxy group (**2**, X = O; R = H; R′ = OCH_3; DAU-6285) reduced affinity versus the 5-HT_3 receptor and shifted the activity on the 5-HT_4 receptor from agonism to antagonism. An analogous shift of activity was seen when the α-tropanyl residue was exchanged with a (1-butylpiperidin-4-yl)methyl moiety [7].

It should be noted that the (1-butylpiperidin-4-yl)methyl analogue of metoclopramide was an antagonist towards the 5-HT_4 receptor [8] and that still more potent and selective antagonists were the amides recently described by Schaus et al. [6].

Thus, the shift from agonist to antagonist activity should not be related merely to the presence of an ester function, but also to the prescence of peculiar basic chains.

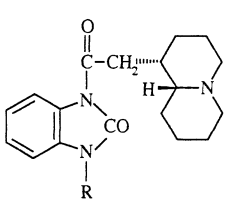
On these grounds we deemed it worthy to investigate the affinity for 5-HT_3 and 5-HT_4 receptors of a small set of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylic acid derivatives of lupinine, *epi*-lupinine and lupinylamine **1**–**7**, together with two 1-homolupinanoyl benzimidazolones **8** and **9**.

The latter compounds are also related to the recently studied homolupinanoyl anilides [9], some of which were endowed with a very high affinity for muscarinic receptors.



	X = O	X = NH
R = H,	**3**	**5**
C_2H_5	**4**	**6**

7



R = H, **8**
C_2H_5 **9**

2. Chemistry

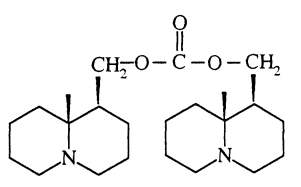
Benzimidazolone was treated with trichloromethylchloroformate in refluxing tetrahydrofuran and the obtained chlorocarbonyl derivative was allowed to react at room temperature with lupinylamine in tetrahydrofurane to give the amide **5**, or heated with lupinine or *epi*-lupinine in *o*-dichlorobenzene at 110°C to give the esters **3** and **7**.

During the reaction of benzimidazolone carbonylchloride with *epi*-lupinine, some di-*epi*-lupinyl carbonate **10** was formed, possibly by an alcoholysis of the N–CO bond of ester **7**.

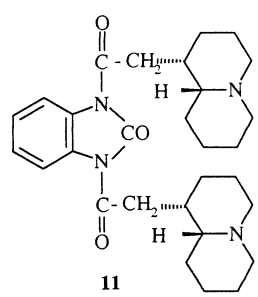
Finally, the ethyl chain was introduced onto the benzimidazolone nitrogen of compounds **3** and **5**, by means of ethyl iodide and sodium hydride in dimethylformamide.

The homolupinanoyl benzimidazolones **8** and **9** were obtained in low yield by reacting benzimidazolone or its *N*-ethyl derivative with homolupinanoyl chloride hydrochloride in anhydrous pyridine solution.

In spite of the use of an excess of benzimidazolone with respect to the acylchloride, 34% of 1,3-dihomolupinanoylbenzimidazolone **11** was formed with only 14% of the desired monosubstituted benzimidazolone **8**.



10



11

The structures of the prepared compounds were derived straight from the synthetic procedures and were supported by the elemental analyses.

IR and ^1H NMR spectra were conforming to the structures and did not exhibit any unusual features (see Section 3).

3. Experimental

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected.

The elemental analyses were performed at the Microanalytical Laboratory of the 'Dipartimento di Scienze Farmaceutiche' of Genoa University and the analytical results for the indicated elements were within ±0.3% of the calculated values.

IR spectra were recorded on a Perkin–Elmer 1310 spectrophotometer; ^1H NMR spectra were taken on a Bruker AC 200 spectrometer using either $CDCl_3$ or DMSO-d_6 as solvent.

As a rule spectra in DMSO-d_6 were less resolved than those in $CDCl_3$, but in the cases of compounds **3**, **5**, **7** and **8** they exhibit at δ 11.20–11.50 a broad singlet due to the benzimidazolone N–H (collapsing after exchange with D_2O), which was lacking in the $CDCl_3$ spectra.

3.1. Lupinyl 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylate (3)

A suspension of 2.75 g (14 mmol) of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carbonyl chloride [10] and 2.14 g (10.4 mmol) of lupinine hydrochloride in 25 ml of *o*-dichlorobenzene was heated at 110°C for 4 h, with stirring and under a stream of nitrogen. The reaction progression was followed through TLC on silica gel using a mixture of dichloromethane, methanol and conc. ammonia (20:3:0.4) as eluent.

The reaction mixture was cooled on ice and filtered; the solid was washed several times with dry ether and then with ethyl acetate. The crude hydrochloride was crystallized from ethanol yielding 0.52 g (13.7% yield) of pure salt. The ethanol solution was evaporated and the residue was dissolved in water. After washing with ether, the solution was basified with ammonia up to pH 9 and extracted with ether. The ether was evaporated and the residue was rinsed with light petroleum ether to remove some traces of lupinine: 1.96 g = 57.2% yield.

Total yield of pure base plus pure hydrochloride = 71% (Table 1). IR (KBr): 3240, 2910, 2840, 2780, 2740, 1742, 1700, 1600 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.88–7.78 (d, 1H, aromatic), 7.22–6.97 (m, 3H, aromatic), 4.86–4.52 (m, 2H, $-O-CH_2-$), 3.00–2.85 (d, 2H, 2CH quinolizidine), 2.25–1.10 (m, 14H, $7CH_2$ quinolizidine).

Table 1
Quinolizidinyl derivatives of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylic acid (**3**–**7**) and *N*-homolupinanoylbenzimidazolones (**8**–**9**)

Compound	Formula [a]	M.p. (°C)	Solvent [b]	Yield (%)
3	$C_{18}H_{23}N_3O_3$	163–164	C	71
3 · HCl	$C_{18}H_{24}ClN_3O_3$	222–223	B	
4	$C_{20}H_{27}N_3O_3$	Oil		65
4 · HCl	$C_{20}H_{28}ClN_3O_3$	195–196	D	
5	$C_{18}H_{24}N_4O_2$	187–189	E	64
5 · HCl	$C_{18}H_{25}ClN_4O_2$	180–182	B	
6	$C_{20}H_{28}N_4O_2$	82–83	F	65
6 · HI	$C_{20}H_{29}IN_4O_2$	223–224	A	
6 · HCl	$C_{20}H_{29}ClN_4O_2$	220–222	B	
7	$C_{18}H_{23}N_3O_3$	136–140	E	34
7 · HCl	$C_{18}H_{24}ClN_3O_3$	172–180	C	
8	$C_{18}H_{23}N_3O_2$	205–206	E	14
8 · HCl	$C_{18}H_{24}ClN_3O_2$	262–264	B	
9	$C_{20}H_{27}N_3O_2$	90–91	F + G	16
9 · HCl	$C_{20}H_{28}ClN_3O_2$	232.5–233.5	B	

[a] Analytical results for C, H, N were within ± 0.3% of the calculated values.

[b] A = ethanol 95°; B = absolute ethanol; C = acetonitrile; D = acetone; E = dry ether; F = isopropyl ether; G = petroleum ether.

3.2. Epi-lupinyl 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylate (7)

This ester was prepared with the same procedure used for **3**, starting from 360 mg (1.75 mmol) of *epi*-lupinine hydrochloride [11] and 410 mg (2 mmol) of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carbonyl chloride. The crude hydrochloride was dissolved in water, the solution was washed with ethyl acetate and basified with ammonia and extracted several times with ether. After removing the solvent a very viscous oil was obtained that solidified when taken up with petroleum ether and then with isopropyl ether. Finally the solid was crystallized from dry ether (Table 1).

IR (KBr): 3200, 2910, 2840, 2740, 1775sh, 1740, 1725sh, 1705sh, 1620 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.90–7.70 (dd, 1H, aromatic), 7.25–7.00 (m, 3H, aromatic), 4.60–4.30 (m, 2H, $-O-CH_2$), 2.95–2.75 (m, 2H, 2CH quinolizidine), 2.20–1.15 (m, 14H, $7CH_2$ quinolizidine).

The petroleum ether washings were chromatographed on alumina eluting with dichloromethane and a growing concentration of methanol. Thus, an oil was obtained, that through IR and ^1H NMR spectra was identified as di-*epi*-lupinyl carbonate.

IR (film): 2910, 2840, 2790, 2740, 1735 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.20–3.95 (m, 4H, 2 $-COO-CH_2-$), 2.95–2.72 (m, 4H, 4CH quinolizidine), 2.2–1.08 (m, 28H, $14CH_2$ quinolizidine).

3.3. N-Lupinyl-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxamide (5)

To a solution of freshly distilled lupinylamine [12] (2.2 g = 13.1 mmol) in 31 ml of dry tetrahydrofuran, at room temperature and under a stream of nitrogen, 3 g (15.26 mmol) of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carbonyl chloride were added, divided in 3–4 portions. The mixture was stirred for 3 h monitoring the reaction through TLC on silica using dichloromethane, methanol and conc. ammonia (20:3:0.3) as eluent.

After removing the solvent, the residue was dissolved in 1 N hydrochloric acid and the acid solution was extracted with ethyl acetate. The ice-cooled acid solution was basified with ammonia and extracted with ether. The ether solution was dried over sodium sulfate and evaporated to dryness leaving 2.75 g of crystals (64% yield) that were recrystallized from ether (Table 1).

^1H NMR ($CDCl_3$): δ 9.00–8.88 (t, 1H, CO–*NH*–CH_2), 8.30–8.10 (m, 1H, aromatic), 7.23–6.93 (m, 3H, aromatic), 3.87–3.62 and 3.62–3.35 (2m, 2H, CO–NH–*CH_2*–), 3.10–2.75 (m, 2H, 2CH quinolizidine), 2.45–1.15 (m, 14H, $7CH_2$ quinolizidine).

3.4. N-Ethyl derivatives **4** and **6**

To a stirred solution of 3 mmol of ester **3** or amide **5** in 12–15 ml of anhydrous dimethylformamide, 97 mg (3.2 mmol) of an 80% dispersion in mineral oil of sodium hydride were added portionwise. The mixture was stirred for 80 min under a stream of nitrogen and then 475 mg (3.04 mmol) of ethyl iodide were added, with further stirring for 2 h. The reaction progression was monitored as usual through TLC on silica.

The solvent was removed under reduced pressure as completely as possible and the residue was taken up in water plus 3 ml of 1 N hydrochloric acid.

(a) In the case of ester **4**, the acidic solution was extracted with ethyl acetate, basified with sodium carbonate and thoroughly extracted with ether. After removing the ether, the oily residue was chromatographed on silica (18 g) using dichloromethane with increasing content (from 2 to 10%) of methanol as eluent; finally the column was eluted with dichloromethane containing 12% methanol and 0.5% conc. ammonia. The *N*-ethyl derivative (700 mg = 65% yield) was dissolved in cold absolute ethanol and treated with the equivalent of ethanolic 1 N hydrochloric acid. The solution was evaporated to dryness avoiding any heating. The residue was treated with acetone to induce crystallization; the obtained crystals were further crystallized from the same solvent.

^{1}H NMR ($CDCl_3$) of **4** hydrochloride: δ 8.05–7.80 (t, 1H, aromatic), 7.30–6.95 (m, 3H, aromatic), 5.00–4.75 and 4.55–4.15 (2 m, 1H each, –O–CH_2–), 4.05–1.50 (m, 18H, N–CH_2–CH_3 + 2CH and 7CH_2 quinolizidine), 1.45–1.20 (t, 3H, N–CH_2–CH_3).

(b) In the case of amide **6**, its hydroiodide separated from the acidic solution and was filtered and washed with water.

The acidic solution was extracted with ethyl acetate, basified with 2 N sodium hydroxide solution and extracted with ether. The ether was removed and the solid residue was rinsed with a mixture (1:1) of dry ether–petroleum ether.

A little of the crude hydroiodide was crystallized from ethanol, while the main part of the salt was suspended in water, treated with an excess of 2 N sodium hydroxide, stirred for a few minutes and then extracted with ether. The ether solution was evaporated and the oily residue crystallized when treated with a small amount of ether.

^{1}H NMR ($CDCl_3$): δ 8.95–8.78 (m, 1H, –CO–NH–CH_2), 8.30–8.15 (dd, 1H, aromatic), 7.25–6.85 (m, 3H, aromatic), 4.05–3.85 (q, 2H, CH_2–CH_3), 3.84–3.42 (m, 2H, NH–CH_2), 2.95–2.75 (d, 2H, 2CH quinolizidine), 2.10–1.10 (m, 17H, 7CH_2 quinolizidine + CH_2–CH_3).

3.5. 1-Homolupinanoyl-2,3-dihydro-2-oxo-1H-benzimidazole (**8**)

The homolupinanoyl chloride hydrochloride [13,14] (1.40 g = 5.56 mmol) and 2,3-dihydro-2-oxo-1H-benzimidazole (0.90 g = 6.72 mmol) were suspended in 20 ml of anhydrous pyridine and heated to reflux, with stirring and under a stream of nitrogen, for about 2 h.

After standing for one night in the refrigerator, the reaction mixture was filtered and the precipitate was washed with dry ether and then with ethyl acetate.

(a) The product was dissolved in water and after treatment with sodium carbonate solution the base was extracted with ether.

After removing the solvent the crystalline residue was washed with petroleum ether leaving 470 mg (= 33.8% yield referred to the acyl chloride) of 1,3-di-homolupinanoyl-2,3-dihydro-2-oxo-1H-benzimidazole (**11**) with m.p. 180–182°C.

Analysis (C,H,N) for $C_{29}H_{40}N_4O_3 + 0.5H_2O$. IR (KBr): 3400, 2900, 2850, 2770, 2740, 1730, 1700 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.30–8.10 (m, 2H, aromatic), 7.35–7.18 (m, 2H, aromatic), 3.70–3.45 and 3.27–3.07 (2 m, 4H, 2 CO–CH_2), 2.95–2.72 (d, 4H, 4CH quinolizidine), 2.50–1.10 (m, 28H, 14CH_2 quinolizidine).

(b) The pyridine solution was evaporated to dryness under reduced pressure and the residue was taken up with diluted hydrochloric acid. The acid solution was extracted with ethyl acetate, basified with sodium carbonate and thoroughly extracted with ether. The ether solution was dried over sodium sulfate and evaporated; the residue was rinsed with petroleum ether and then with a little ether to leave 240 mg (14% yield) of **8** (Table 1).

^{1}H NMR ($CDCl_3$): δ 8.25–8.07 (d, 1H, aromatic), 7.25–6.90 (m, 3H, aromatic), 3.62–3.42 and 3.30–3.10 (2 m, 1H each, CO–CH_2), 3.00–2.75 (m, 2H, 2CH quinolizidine), 2.55–1.17 (m, 14H, 7CH_2 quinolizidine).

3.6. 1-Ethyl-3-homolupinanoyl-2,3-dihydro-2-oxo-1H-benzimidazole (**9**)

The homolupinanoyl chloride hydrochloride (1.40 g = 5.56 mmol) was reacted with 1-ethyl-benzimidazolone (960 mg = 5.8 mmol) in 18 ml pyridine as described above. The heating was extended to 4 h monitoring the reaction progression through TLC as usual.

Pyridine was evaporated under reduced pressure and the residue was taken up with 1 N hydrochloric acid and the solution was extracted several times with ether to remove the unreacted ethylbenzimidazolone.

The solution was basified with sodium carbonate and extracted with ether. After removing the solvent the residue was rinsed with petroleum ether and then with a little ether to have 300 mg of **9** (Table 1).

^{1}H NMR ($CDCl_3$): δ 8.30–8.10 (d, 1H, aromatic), 7.30–6.90 (m, 3H, aromatic), 4.05–3.80 (q, 2H, N–CH_2), 3.75–3.50 and 3.30–3.08 (2 dd, 2H, CO–CH_2), 2.95–2.72 (d, 2H, 2CH quinolizidine), 2.47–1.08 (m, 17H, 7CH_2 quinolizidine + CH_2–CH_3).

3.7. Hydrochlorides

For all compounds whose hydrochlorides were not obtained directly from the synthetic procedure, the free base was dissolved in absolute ethanol, the stoichiometric volume of 1 N ethanolic hydrochloric acid was added and the solution evaporated to dryness avoiding any heating.

The residue was taken up in absolute ethanol and the solution was again evaporated to dryness in vacuo. For crystallization solvent see Table 1.

4. Pharmacology

Compounds **3**–**9** were subjected to radioligand binding assays towards serotonin 5-HT_3 and 5-HT_4 receptors.

Moreover, the gastro-enteroprokinetic activity of compound **6** was explored by MDS Panlabs Inc., Bothell, WA, USA.

4.1. Binding assay to serotonin 5-HT_3 receptor

Male CRL:CD(SD)BR-COBS rats (125–150 g, Charles River, Calco, Italy) were killed by decapitation; their cortices were rapidly removed and stored at −80°C until the day of assay. The frozen tissue was homogenized in about 50 vol. of ice-cold Tris–HCl, 25 mM, pH 7.4 using an Ultra Turrax TR 1810 homogenizer (2 × 20s) and centrifuged at 50 000 × *g* for 10 min (Beckmann model J-21B refrigerated centrifuge). The pellet was resuspended in the same volume of fresh buffer, incubated at 37°C for 10 min and centrifuged again for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The pellet obtained was finally resuspended in Tris–HCl, 25 mM, pH 7.4, containing 10 μM pargyline.

[^{3}H]-Zacopride binding was assayed [15] in a final incubation volume of 1 ml consisting of 0.5 ml of membrane suspension (20 mg tissue/sample), 0.5 ml of [^{3}H]-zacopride (s.a. 84 Ci/mmol, final concentration 0.4 nM) and 20 μl of displacing agents solution or solvent. Incubation (30 min at 25°C) was stopped by rapid filtration in vacuo (Brandell MR 48 R) through GF/B filters which were then washed with 12 vol. of cold buffer and counted in Wallach 1204 betaplate BS liquid scintillation counter with a counting efficiency of 45%.

Dose–inhibition curves were analyzed by the Allfit program [16] to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding.

The non specific binding was measured in the presence of 1 μM ondansetron (GR38032).

4.2. Binding to serotonin 5-HT_4 receptor

Male Cri:(HA)BR guinea pigs (180–200 g, Charles River) were killed by decapitation; their striata were rapidly removed and stored at −80°C until the day of assay. The frozen tissue was homogenized in about 33 vol. of ice-cold Hepes HCl (50 mM, pH 7.4) with the same procedure described above. The pellet obtained was finally resuspended in Hepes HCl, 50 mM, pH 7.4, containing 10 μM pargyline.

[^{3}H]-GR113808 binding was assayed in a final incubation volume of 1 ml consisting of 0.5 ml of membrane suspension (20 mg tissue/sample), 0.5 ml of [^{3}H]-GR113808 (s.a. 84 Ci/mmol, final concentration 0.4 nM) and 20 μl of displacing agents or solvent [17]. Incubation, counting and dose–inhibition curve analyses were performed as described for the 5-HT_3 binding assay.

The non specific binding was measured in the presence of 10 μM serotonin.

4.3. Gastro-entero prokinetic activity [18]

Groups of ten male ICR mice, weighing 22 ± 2 g and fasting for 16 h were used. A dose of 100 mg/kg of test compound suspended in 2% 'Tween 80' was administered p.os; the control group received the vehicle alone. Some 60 min after the administration of the drug, the animals received a 5% suspension of charcoal in a 10% arabic gum solution (0.3 ml/animal) and 15 min later were sacrificed. The intestines were removed and the intestine total length (GL) and the charcoal distance from pylorus (CP) were measured.

The intestinal transit (IT) was calculated as IT = (CP/GL) × 100. The mean ± standard error (SEM) for each treatment was calculated and the Student's *t*-test was used to compare the treated groups with those receiving the vehicle. Differences were considered significant when $P < 0.05$.

5. Results and discussion

The results of radioligand binding assays on serotonin 5-HT_3 and 5-HT_4 receptors are shown in Table 2.

Compounds **3**–**9** were able to displace [^{3}H]-zacopride from the 5-HT_3 receptor, but only at micromolar concentrations with IC_{50} in the range 2.32–16.50 μM.

Table 2
Inhibition of binding of [^{3}H]-zacopride and [^{3}H]-GR113808 on 5-HT_3 and 5-HT_4 receptors

Compound	IC_{50} (μM)	
	5-HT_3	5-HT_4
3	10.20	>10.00
4	3.64	[a]
5	4.51	[b]
6	8.10	1.18
7	16.50	5.50
8	6.99	2.56
9	2.32	7.32
Ondansetron	0.00816	
Serotonin		0.137

[a] % Inhibition of specific binding at 10^{-5} and 10^{-7} M is 81.7 and 9.8, respectively.
[b] % Inhibition of specific binding at 10^{-5} and 10^{-7} M is 71 and 2.9, respectively.

These values of IC_{50} were quite far from those of ondansetron (0.00816 μM) and of benzimidazolone derivatives described by Turconi et al. [4,5,9], but comparable with that of the lupinyl analogue of metoclopramide **1** [2].

Although the differences of activity among the tested compounds were rather limited, the lupinyl and *epi*-lupinyl esters with R = H (**3** and **7**) were the least active.

Similarly the affinity for 5-HT_4 receptors, measured through the displacement of [^{3}H]-GR113808, was rather modest. Once more the esters **3** and **7** were poorly active. However, the amide **6** with $IC_{50} = 1.18$ μM was only one order of magnitude less active than serotonin ($IC_{50} = 0.137$ μM).

Generally the introduction of an ethyl group on benzimidazolone nitrogen somewhat improved the affinity for both 5-HT_3 and 5-HT_4 receptors, but in two cases an inverted influence was observed.

Compound **6** exhibiting the highest affinity for 5-HT_4 receptor was subjected to a charcoal bolus intestinal progression test.

Contrary to what should be expected in the case of 5-HT_4 agonism, compound **6** was unable to enhance the intestinal transit rate at the oral dose of 100 mg/kg. This negative result could be related either to 5-HT_4 antagonist activity or to the overlapping of serotoninergic and antimuscarinic activities. Actually antimuscarinic action was often observed among the quinolizidine derivatives. Anyhow, having no information about the pharmacokinetic and metabolic features of this compound no conclusive interpretation for the mentioned lack of activity can be drawn and further investigations are needed to define the pharmacological profile of all the presently described compounds.

6. Conclusions

The benzimidazole derivatives **3**–**9** were able to displace the specific radioligands from 5-HT_3 and 5-HT_4 receptors; however, their activity was just moderate, with IC_{50} in the micromolar range. Thus, the lupinyl (quinolizidin-1-yl methyl) residue appears as quite less suitable than α-tropanyl and (1-butylpiperidin-4-yl)-methyl moieties for binding on these receptors.

Compound **6**, exhibiting the highest affinity for 5-HT_4 receptor, failed to enhance the intestinal transit rate of a charcoal bolus in mice, suggesting a possible antagonism towards that receptor, even though other interpretations of the negative results cannot be excluded.

Since 5-HT_4 antagonists could be of interest for the treatment of irritable bowel syndrome, atrial arrhythmias and various CNS diseases, further investigations are warranted to define the pharmacological profile of the synthesized compounds.

Acknowledgements

Financial support from CNR and MURST is gratefully acknowledged. The authors thank Dr Tiziana Mennini (Istituto di Ricerche Farmacologiche 'Mario Negri', Milan, Italy) for her collaboration.

References

[1] G. Iusco, V. Boido, F. Sparatore, Synthesis and preliminary pharmacological investigation of *N*-lupinyl-2-methoxy-benzamides, Farmaco 51 (1996) 159–174.
[2] G. Iusco, V. Boido, F. Sparatore, G. Colombo, P.L. Saba, Z. Rossetti, A. Vaccari, New benzamide-derived 5-HT_3 receptor antagonists which prevent the effects of ethanol on extracellular dopamine, and fail to reduce voluntary alcohol intake in rats, Farmaco 52 (1997) 141–146.
[3] L.M. Gaster, F.D. King, Serotonin 5-HT_3 and 5-HT4 receptors antagonists, Med. Res. Rev. 17 (1997) 163–214.
[4] M. Turconi, P. Schiantarelli, F. Borsini, C. Rizzi, H. Ladinsky, A. Donetti, Azabicycloalkylbenzimidazoles: interaction with serotonergic 5-HT_3 and 5-HT_4 receptors and potential therapeutic implications, Drugs Future 16 (1991) 1011–1026.
[5] A. Dumuis, H. Gozlan, M. Sebben, H. Ausanay, C.A. Rizzi, M. Turconi, E. Monferini, E. Giraldo, P. Schiantarelli, M. Ladinsky, J. Bockaert, Naunyn-Schmiedebergs Arch. Pharmacol. 345 (1992) 264–269.
[6] J.M. Schaus, D.C. Thompson, W.E. Bloomquist, A.S. Susemichel, D.O. Calligaro, M.L. Cohen, Synthesis and structure–activity relationships of potent and orally active 5-HT_4 receptor antagonists: indazole and benzimidazolone derivatives, J. Med. Chem. 41 (1998) 1943–1955.
[7] F.D. King, L.M. Gaster, P.A. Wyman, Nitrogen containing heterocyclic compounds useful as pharmaceuticals, PCT Int. Appl. WO 93-18027; Chem. Abstr. 120 (1994) 107013.
[8] L.M. Gaster, A.J. Jennings, G.F. Joiner, F.D. King, K.R. Mulholland, S.K. Rahman, S. Starr, P.A. Wyman, K.A. Wardle, E.S. Ellis, G.J. Sanger, (1-Butyl-4-piperidinyl)methyl 8-amino-7-

chloro-1,4-benzodioxane-5-carboxylate hydrochloride: a highly potent and selective 5-HT_4 receptor antagonist derived from metoclopramide, J. Med. Chem. 36 (1993) 4121–4123.

[9] A. Sparatore, F. Sparatore, Preparation and pharmacological activities of homolupinanoyl anilides, Farmaco 50 (1995) 153–166.

[10] M. Turconi, M. Nicola, M. Gil Quintero, L. Maiocchi, R. Micheletti, E. Giraldo, A. Donetti, Synthesis of a new class of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylic acid derivatives as highly potent 5-HT_3 receptor antagonists, J. Med. Chem. 33 (1990) 2101–2108.

[11] V. Boido, A. Sparatore, Chinolizidinil derivati di sistemi triciclici, Farmaco Ed. Sci. 67 (1982) 63–73.

[12] F. Sparatore, V. Boido, P. Preziosi, E. Miele, G. De Natale, Sintesi e proprietà farmacodinamiche di alcuni derivati lupinanici, Farmaco Ed. Sci. 24 (1969) 587–621.

[13] A. Sparatore, M. Veronese, F. Sparatore, Derivati chinolizidinici ad attività antimicrobica, Farmaco Ed. Sci. 42 (1987) 160–174.

[14] A. Sparatore, V. Boido, F. Sparatore, Preparation of quinolizidinyl derivatives of phenothiazine and thioxanthene of pharmacological interest, Farmaco 44 (1989) 1193–1203.

[15] F.J. Bolanos, L.E. Scechter, M.C. Miquel, M.B. Emerit, J.F. Rumigny, M. Hamon, H. Gozlan, Common pharmacological and physico-chemical properties of 5-HT3 binding sites in the rat cerebral cortex and NG 108-15 clonal cells, Biochem. Pharmacol. 40 (1990) 1541–1550.

[16] K.W. De Lean, P.J. Munson, D. Rodbard, Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves, Am. J. Physiol. 235 (1978) E97–E102.

[17] G. Campiani, A. Cappelli, V. Nacci, M. Anzini, S. Vomero, M. Hamon, A. Cagnotto, C. Fracasso, C. Uboldi, S. Caccia, S. Consolo, T. Mennini, Novel and highly potent 5-HT_3 receptor agonist based on a pyrroloquinoxaline structure, J. Med. Chem. 40 (1997) 3670–3678.

[18] M. Omosu, I. Komine, R.H.A. Becker, B.A. Schölkens, General pharmacology of ramipril, Arzneim. Forsch. 38 (1988) 1309–1317.