IL FARMACO, 47 (1), 21-35; 1992

SYNTHESIS AND CHOLERETIC ACTIVITY OF 3-[2-(3-R', 4-R'', 5-R'''-BENZYL)-5-R-BENZIMIDAZOL-1-YL]-BUTANOIC ACIDS. (*)

GIUSEPPE GRELLA(**) - GIUSEPPE PAGLIETTI(**)

ISTITUTO DI CHIMICA FARMACEUTICA E TOSSICOLOGICA - UNIVERSITÀ DI SASSARI
VIA MURONI, 23 - 07100 SASSARI

FABIO SPARATORE

ISTITUTO DI SCIENZE FARMACEUTICHE - UNIVERSITÀ DI GENOVA
VIALE BENEDETTO XV, 3 - 16132 GENOVA

MARGHERITA SATTA - PEPPINA MANCA - ALESSANDRA PEANA

CATTEDRA DI FARMACOLOGIA E FARMACOGNOSIA - UNIVERSITÀ DI SASSARI
VIA MURONI, 23 - 07100 SASSARI

SUMMARY — *On the ground of the evidentiated choleretic activity of 3-[2-benzylbenzimidazol-1-yl]butanoic acid, 28 new acids were prepared in order to evaluate the influence of suitable substitutions in either C5 of heteroring or C3', C4', C5' of benzyl group in position 2 on the choleretic activity. Pharmacological results after i.v. administration of 0.5 mmol/Kg in rats confirmed a general high choleretic activity that in eleven cases showed during the first 4 hours an increase of bile volume higher than 80%, that is superior to that produced by dehydrocholic acid. Only in a few cases the bile volume increase was less than 37% of basal value.*

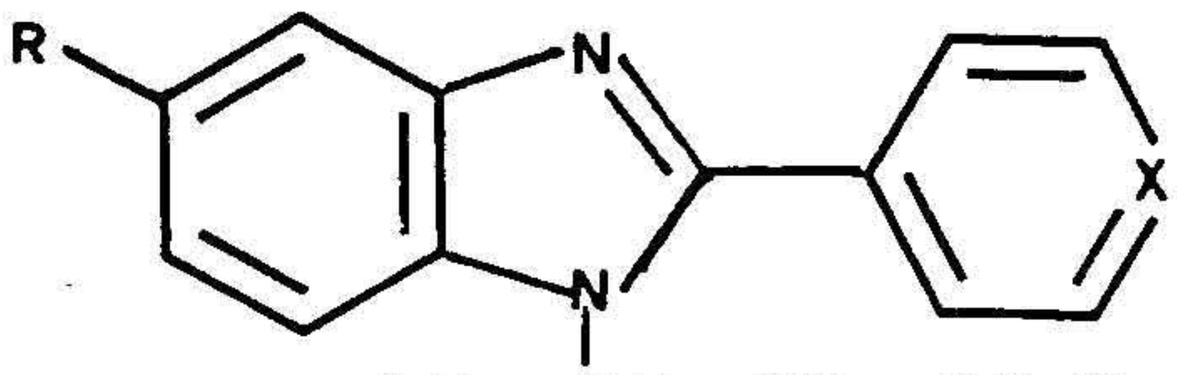
RIASSUNTO — *Sulla base dell'evidenziata attività coleretica dell'acido 3-[2-benzilbenzimidazol-1-il]-butanoico sono stati preparati 28 nuovi acidi recanti sostituenti nella posizione 5 del nucleo benzimidazolico e nelle posizioni 3', 4', 5' del benzile in 2 al fine di valutarne l'effetto sull'attività coleretica. L'indagine farmacologica effettuata su questi e altri acidi già descritti ha confermato, nel ratto alla dose di 0.5 mmol/Kg, una generale attività coleretica che in undici casi comporta una variazione percentuale media del flusso biliare nelle quattro ore superiore all'80% e quindi superiore a quella dell'acido deidrocolico. Solo in pochi casi l'aumento del volume della bile resta al di sotto del 37%.*

(*) Part of this work was presented as poster communication at 6th National Meeting of the Division of Medicinal Chemistry of the Società Chimica Italiana, Alghero, 14-18 October 1986.

(**) To whom correspondence should be addressed.

Introduction

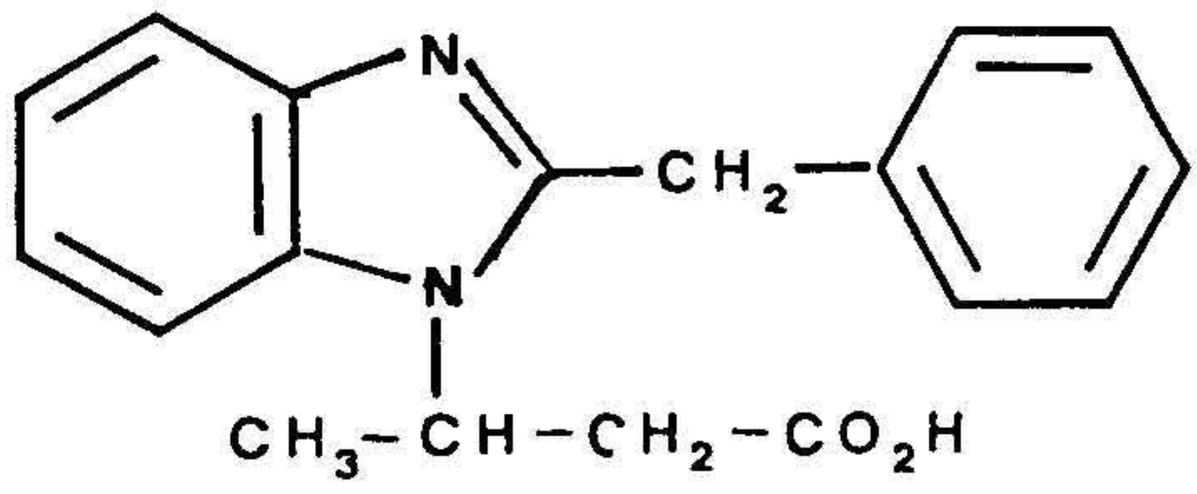
In a previous paper (1) we reported that 3-[2-aryl-5-R-benzimidazol-1-yl]-butanoic acids of structure **A** possess a generalised choleretic activity in rat by i.v. administration of 0.5 mmol/Kg of acid.



A

X=CH; C-CH$_3$; C-Cl; C-OCH$_3$; C-OC$_2$H$_5$; N;
R=H; Cl; CF$_3$; CO-CH$_3$; CH(OH)CH$_3$; NO$_2$

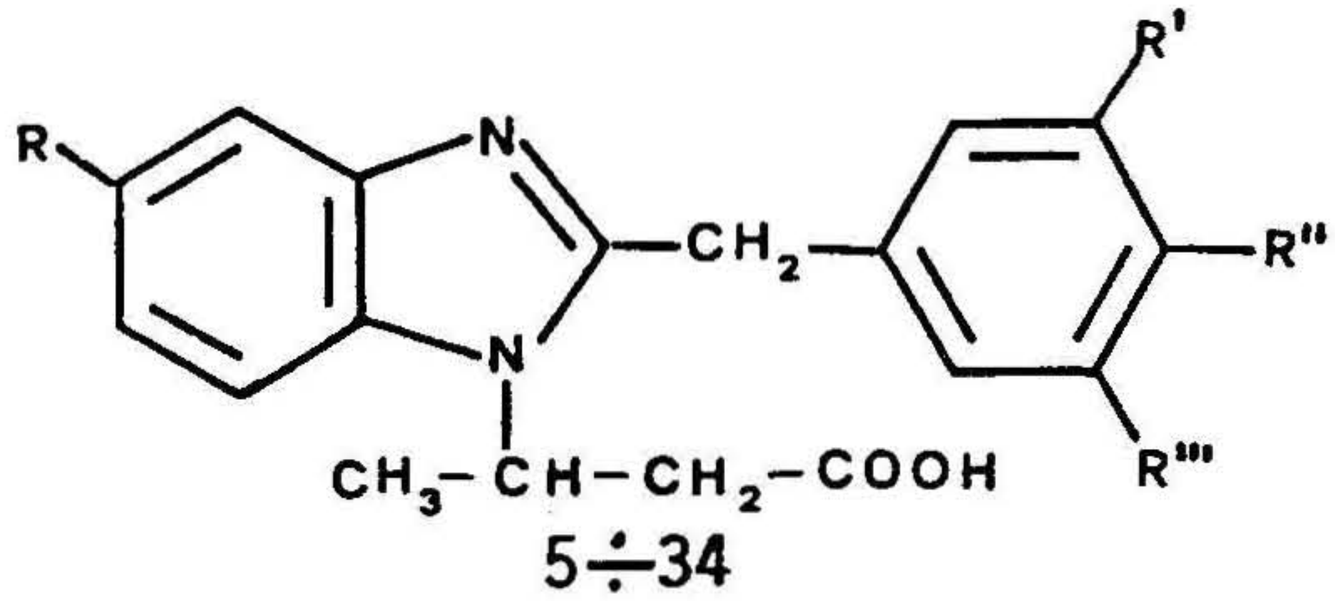
In several acids this activity was superior to that of 3-[benzotriazol-1-yl]butanoic acid, which prompted our research in this field (2, 3), and to that of dehydrocholic acid (**DCA**) taken as reference. In that context we also remarked that, in accordance with preliminary observations of Preziosi (4), compound **B** was a quite potent choleretic among a series of 3-(benzimidazol-1-yl)butanoic acids previously described (4).



B

Actually the acid **B** showed a persistently high choleretic activity during the first three hours after i.v. administration. On the basis of these encouraging results we have now designed a new series of acids (**5-34**) with the same structure of **B** where we considered the effect of further substitutions in either position 5 of the heterocycle or in position 3', 4', 5' of benzyl group attached in position 2 and for which we have evaluated the choleretic activity in comparison with **DCA** and **B**.

In order to support further the importance of an aromatic moiety on position 2 of the benzimidazole ring for the choleretic activity we have also tested the compounds **35-36**, previously described (4), as well as some pre-



5÷34

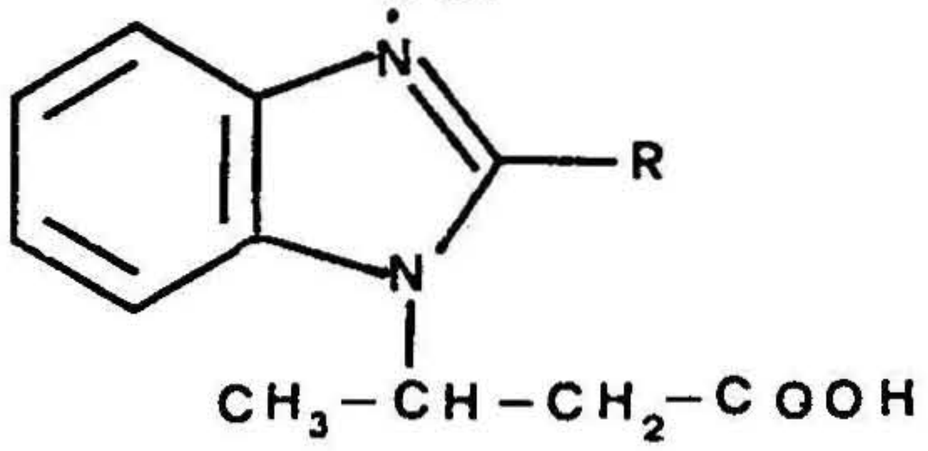
R=H, Cl, CF_3, CH_3, NO_2, CO-CH_3

R'= H, OCH_3

R''=H, CH_3, Cl, OCH_3, OC_2H_5, NO_2, NH_2, $NHCOCH_3$

R'''= H, OCH_3

cursors (**2c, 3a, 3c**) of Scheme 1 and the simple 3-aminobutanoic acid (**ABA**), all of which resulted endowed with very low activity or completely inactive.

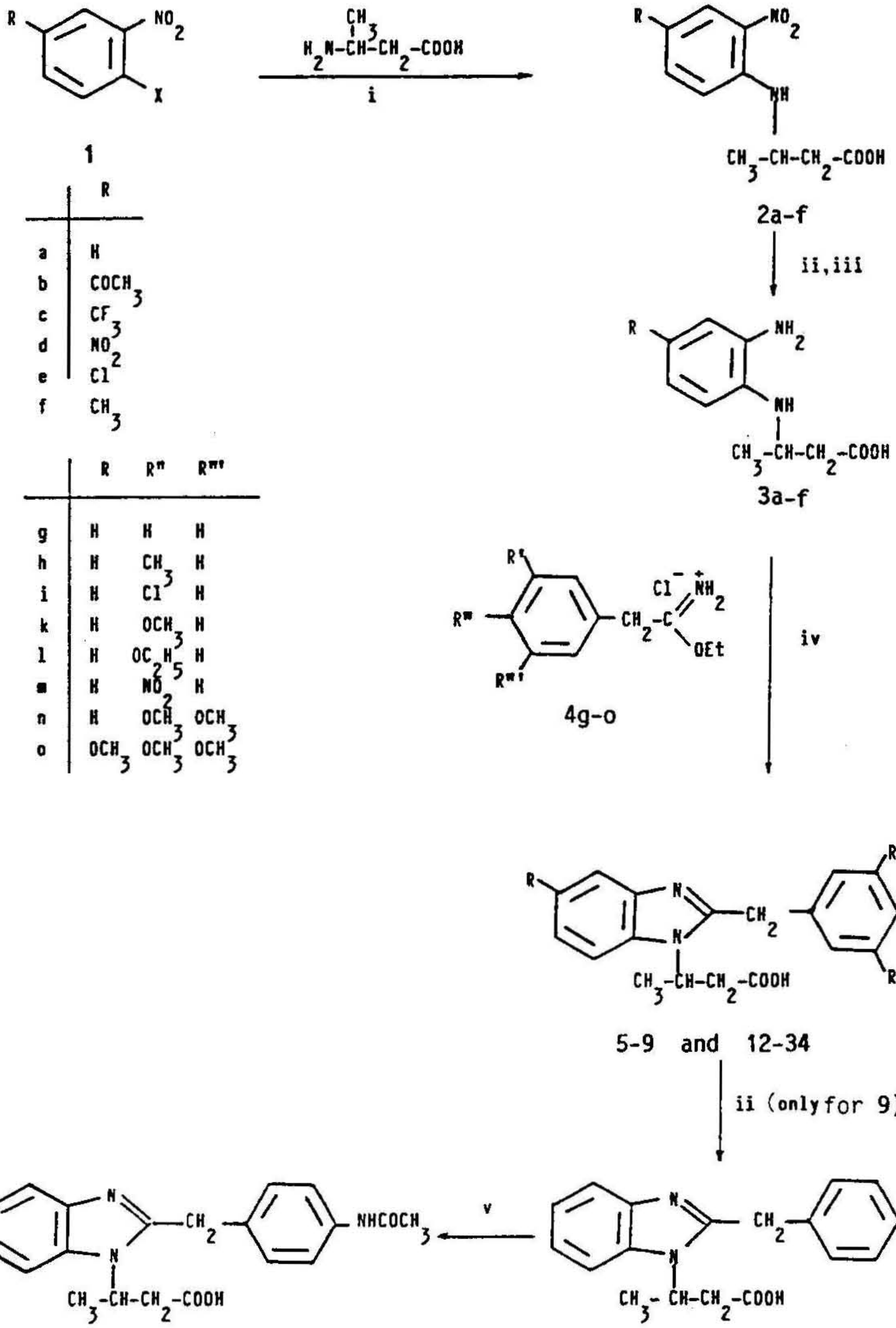


35 R= H

36 R= CH_3

Chemistry

The preparation of the set of acids listed in Table I was achieved through the known King and Acheson's procedure (5) previously used in similar cases (6) and reproduced in Scheme 1. The condensation of o-phenylendiamines with the corresponding iminoether hydrochlorides was carried out in dry methanol and the expected acids **5-34** were obtained in fair yields. Most of the required o-phenylendiamines **3** were previously described (1,4,7). However, two additional derivatives (**3a,f**) were purposely prepared using the sequence of reactions of Scheme 1. The suitable o-fluoronitrobenzenes were successfully reacted in isobutan-1-ol to obtain the intermediates (**2a,f**), while the corresponding chloroderivative failed to give the desired compounds. All iminoether hydrochlorides, prepared according to previously reported procedures (6), were isolated as crystalline solids and their mp's



SCHEME 1

i, isobutanol; ii, H_2/Pd; iii, NH_3/H_2S in ethanol; iv, dry methanol; v, Acetic anhydride.

are given for the first time. The starting nitriles were all but one available on the market. p-Ethoxy-phenylacetonitrile was prepared as indicated in ref. (6). Compound **10** was obtained by catalytic hydrogenation of **9** and on acetylation with acetic anhydride gave the acid **11**. Physical properties and yields of all the prepared acids are reported in Table I. The structures of all the

TABLE I

Physical properties and yields of 3-[2-(3,4,5-trisubstituted)benzyl-5-R-benzimidazol-1-yl]butanoic acids.

Comp.	R	R'	R''	R'''	M.P. °C	Yield %	Analysis for (C, H, N)	λ_{max}nm (log ε)·95% EtOH
B	H	H	H	H	175-178	79	(a)	283(3.83), 276(3.80), 253(3.86)
5	H	H	CH_3	H	195-96	73	$C_{19}H_{20}N_2O_2 + 0.5H_2O$	283(3.78), 276(3.72), 253(3.66)
6	H	H	Cl	H	170-72	96	(b)	—
7	H	H	OCH_3	H	190-92	74	(c)	283(4.03), 276(3.99), 253(3.97)
8	H	H	OC_2H_5	H	200-02	69	$C_{20}H_{22}N_2O_3 + 0.40H_2O$	—
9	H	H	NO_2	H	225-30	83	$C_{18}H_{17}N_3O_4 + 0.10H_2O$	—
10	H	H	NH_2	H	125-30	78 (d)	$C_{18}H_{19}N_3O_2 + 1.30H_2O$	—
11	H	H	$NHCOCH_3$	H	145	64 (e)	$C_{20}H_{21}N_3O_3 + 1.20H_2O$	—
12	H	OCH_3	OCH_3	H	145	73	$C_{20}H_{22}N_2O_4$	284(4.04), 277(4.00), 255sh(3.93), 234(4.06)
13	H	OCH_3	OCH_3	OCH_3	208-10	83	$C_{21}H_{24}N_2O_5$	—
14	$COCH_3$	H	H	H	204-07	73	$C_{20}H_{20}N_2O_3$	—
15	$COCH_3$	H	CH_3	H	98-103	57	$C_{21}H_{22}N_2O_3 + 0.50H_2O$	—
16	$COCH_3$	H	Cl	H	200	50	$C_{20}H_{19}N_2O_3Cl + 0.10H_2O$	—
17	$COCH_3$	OCH_3	OCH_3	H	222-27	46	$C_{22}H_{24}N_2O_5 + 0.25H_2O$	—
18	CF_3	H	H	H	168-70	74	$C_{19}H_{17}N_2O_2F_3$	—
19	CF_3	H	CH_3	H	185-90	72	$C_{20}H_{19}N_2O_2F_3$	283(3.92), 277(3.87), 255(3.85)
20	NO_2	H	H	H	175-80	57	$C_{18}H_{17}N_3O_4$	—
21	NO_2	H	CH_3	H	195-200	71	$C_{19}H_{19}N_3O_4$	—
22	NO_2	H	OC_2H_5	H	180-85	80	$C_{20}H_{21}N_3O_5$	—
23	Cl	H	H	H	207-09	41	$C_{18}H_{17}N_2O_2Cl + 0.10H_2O$	—
24	Cl	H	CH_3	H	157-58	60	$C_{19}H_{19}N_2O_2Cl + 0.75\ H_2O$	—
25	Cl	H	Cl	H	175	84	$C_{18}H_{16}N_2O_2Cl_2$	—
26	Cl	H	OC_2H_5	H	168-70	69	$C_{20}H_{21}N_2O_3Cl + 0.30H_2O$	—
27	Cl	H	NO_2	H	210-12	55	$C_{18}H_{16}N_3O_4Cl$	—
28	Cl	OCH_3	OCH_3	H	193-95	66	$C_{20}H_{21}N_2O_4Cl$	—
29	CH_3	H	H	H	178-80	66	$C_{19}H_{20}N_2O_2$	—
30	CH_3	H	CH_3	H	170-72	78	$C_{20}H_{22}N_2O_2 + 0.30H_2O$	—
31	CH_3	H	OCH_3	H	160-62	85	$C_{20}H_{22}N_2O_3 + 0.35H_2O$	—
32	CH_3	H	OC_2H_5	H	200-05	65	$C_{21}H_{24}N_2O_3 + 0.30H_2O$	—
33	CH_3	H	NO_2	H	198-200	71	$C_{19}H_{19}N_3O_4$	—
34	CH_3	OCH_3	OCH_3	H	163-65	79	$C_{21}H_{24}N_2O_4$	291(3.90), 282(3.95), 256sh(3.87), 234sh(4.01)

(a) = known (4), m.p. 148-50°C from EtOH; (b) = known (4), m.p. 142-44°C from EtOH; (c) = known (4), m.p. 182-83.5°C from EtOH; (d) = yield from **9**; (e) = yield from **10**.

compounds described are supported by elemental analyses (C,H,N) and spectroscopical data. IR spectra were not characteristic and showed an absorption between 1710-1695 cm^{-1} due to acid carbonyl. UV spectra in ethanol exhibited two sharp absorption maxima in the range 291-283 and 282-276 nm in accordance with the literature data for 2-alkylbenzimidazoles (8, 9). These maxima become broad when electronwithdrawing substituents are present in the position 5 of benzimidazole. A few examples are reported in Table I.

^{1}H-NMR spectra at 200 MHz in $CDCl_3$ or DMSO-d_6 are very similar, resonances due to aromatic protons being well defined as well as those of the aliphatic chain. Benzylic methylene protons can appear either as singlet or as an AB system because of the non equivalence arising from free rotation of the phenyl ring. A typical spectrum of this class of compounds is reported for the acid **34**:

δ_H200 MHz ($CDCl_3$): 7.46 (s, 1H, C-4 H), 7.34 (d, 1H, *J* 8.3, C-7 H), 7.03 (d, 1H, *J* 8.3, C-6 H), 6.75 (s, 1H, C-6' H), 6.70 (s, 2H, C-2', 5' H), 5.08 (m, 1H, N-*CH*), 4.41 (s, 2H, C-2 CH_2), 3.80 (s, 3H, C-3' OCH_3), 3.73 (s, 3H, C-4' OCH_3), 3.13 (dd, 1H, *J* 16.4 and 9.9, CH_2COOH), 2.78 (dd, 1H, *J* 16.4 and 4.7, CH_2COOH), 2.39 (s, 3H, C-5 CH_3), 1.25 (d, 3H, *J* 6.8, CH-CH_3).

Experimental

A) CHEMISTRY

Melting points were taken on a Kofler apparatus and are uncorrected. IR spectra are from nujol mulls and were recorded on Perkin-Elmer 781 spectrophotometer. UV spectra are in nm for ethanolic solutions and were recorded with Perkin-Elmer Lambda 5 instrument. ^{1}H-NMR spectra are in ppm (δ) and were recorded with a Varian XL-200 instrument. Elemental analyses were performed at Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, University of Padova, and the results were within $\pm 0.4\%$ of the theoretical values for C,H,N.

Intermediates

Most o-phenylendiamines (**3**) of Scheme 1 were known (1, 4, 7) and were prepared. Compounds **3a,f** not previously described were prepared as reported below.

*3-[(2-Nitro)anilino]butanoic acid (**2a**)*

A mixture of 2-nitrofluorobenzene (27.2 ml, 258 mmol) and 3-amino-butanoic acid (**ABA**) (29.9 g, 290 mmol), in isobutyl alcohol (480 ml) and in the presence of triethylamine (TEA) (35.3 ml, 253 mmol) was heated under reflux for 24 h. After evaporation of the solvent *in vacuo* the oily residue was taken up with 1M NaOH aqueous solution (300 ml) and then extracted with diethyl ether. On acidification of the alkaline solution with 6M HCl aqueous solution a pure yellow-orange crystalline compound **2a** (42.6 g, 74% yield) was obtained. Mp 98-100°C. Evaporation of the ethereal extract gave some starting 2-nitrofluorobenzene.

*3-[(2-Nitro-4-methyl)anilino]butanoic acid (**2f**)*

In a similar manner from equimolar amounts (81.4 mmol) of 2-fluoro-5-methylnitrobenzene (10.0 ml) and TEA (11.3 ml) and a slight excess of 3-aminobutanoic acid (9.44 g, 91.5 mmol), in isobutanol (150 ml), after cooling of the reaction mixture some starting acid (2.4 g) was recovered by filtration and the work-up of the mother liquors as above gave deep yellow-orange crystals of **2f** (8.6 g, 44% yield), mp 128-130°C.

*3-[(2-Amino)anilino]butanoic acid (**3a**)*

A solution of **2a** (3.0 g, 13.4 mmol) in ethanol (60 ml) was hydrogenated under moderate pressure (2 atm) in the presence of 10% palladium on charcoal (0.3 g); the hydrogen uptake was complete within 2 h.

After filtration of the catalyst the ethanolic solution was concentrated *in vacuo*; a first crop of compound **3a** (2.0 g; mp 135-38°C) was collected and on further concentration more **3a** (0.3 g) was obtained, with an overall yield of 84%.

*3-[(2-Amino-4-methyl)anilino]butanoic acid (**3f**)*

This acid was obtained in 70% yield in a similar manner as above. Mp 118-120°C.

*Iminoether hydrochlorides (**5g-o**). General procedure*

A solution of equimolar amounts (28.4 mmol) of the suitable benzyl-cyanide and absolute ethanol in chloroform (30 ml) was saturated with dry hydrogen chloride at 0-5°C and stopped. After two hour standing at room temperature the solvent was removed *in vacuo* without heating. The obtained solid was washed out twice with dry diethyl ether and dried in a dessicator. All iminoether hydrochlorides were obtained in good yield (>80%) as crystalline compounds with defined mp's (°C) as listed: **5g**: 98; **5h**: 124-126; **5i**: 128; **5k**: 102-104; **5l**: 115-118; **5m**: 195; **5n**: 118; **5o**: 128.

*Acids **5-9** and **12-34**. General procedure*

A mixture of equimolar amounts (3.60 mmol) of o-phenylendiamine derivative **3** and iminoether hydrochloride in methanol (10 ml) was stirred at room temperature for 1 h. Ring closure was complete after this time. The acids mostly precipitated and were collected: sometimes to induce precipitation an equal volume of water was added to the methanol solution. On concentration of the mother liquors or by further adding of water more acid was obtained. A thorough washing of the precipitates with water was sufficient to obtain pure compounds, which were analysed without further purification.

*3-[2-(4-Amino)benzylbenzimidazol-1-yl]butanoic acid (**10**)*

Compound **9** (2.3 g, 6.76 mmol), dissolved in a mixture of a 5% sodium hydrogen carbonate aqueous solution (11.4 ml, 6.78 mmol) and ethanol (92 ml), was hydrogenated in the presence of 10% palladised charcoal (0.23 g) at room temperature and under moderated pressure (2 atm) within 2 h. After removal of the catalyst the solution was made neutral and evaporated *in vacuo*. The thick oily residue was triturated with water (30 ml) allowing the acid **10** to crystallize (1.64 g, 78% yield).

*3-[2-(4-Acetylamino)benzylbenzimidazol-1-yl]butanoic acid (**11**)*

A solution of compound **10** (1.50 g, 4.85 mmol) in acetic anhydride (1.4 ml)

was stirred at 65°C for 10 min. On cooling, water (12 ml) was added and the mixture re-heated at 65°C for 5 min. A precipitate was collected and thoroughly washed with water to give pure **11** (1.39 g, 82% yield).

B) PHARMACOLOGY

Materials and methods

Pharmacological testing was carried out for all the compounds listed in Table I and for the derivatives **35** and **36** previously described (4). In addition the intermediates **2a, 3a, 3c** and 3-aminobutanoic acid were tested. Dehydrocholic acid (**DCA**) was taken as reference. The procedure used for the determination of choleretic activity was previously described (3). 0.1 M aqueous solutions of both sodium salts of the acids and **DCA**were used. 0.5 mmol/Kg (5 ml/Kg) was given by i.v. route to male Wistar-Morin weighing 300-420 g rats fed with Nossan synthetic feed. In one case (**16**) on account of the low solubility only 0.25 mmol/Kg was administered in the same volume of solution. In the case of the acids **B, 5** and **12** a dose of 0.25 mmol/Kg was given to check any dose-dependent activity, while for the acids **13, 18** and **24** this dose was used because of an high mortality encountered at 0.5 mmol/Kg. However this dose was still too toxic in the case of the acids **19** and **22**; hence the dose was further reduced to 0.12 mmol/Kg in latter cases. The acids **25** and **26**, which gave 100% mortality at 0.5 mmol/Kg were not further investigated. Controls were given either 5 ml/Kg of physiological solution or 5 ml/Kg of 0.2 M sodium hydrogen carbonate aqueous solution.

During the experiments animals were housed at 29°C. Each compound was tested over a number of animals varying from 4 to 7 (Table II).

The dry residue of the excreted bile was determined for a limited number of acids which were considered as representative of high, medium and low activity (Table III). This was accomplished drying standard volumes of bile in the thermostated oven at 90°C to constant weight. On the dry residue, at the indicated times, it was determined by UV photometric titration the amount of the given acid excreted with the bile. Standard ethanol solutions (100 ml) containing a weighed amount of dry residue were used each time.

Comparison of extinction coefficient (log ϵ) recorded at the highest wavelength maximum against a calibration curve previously obtained at established concentrations at the same wavelength (Table I) of the examined compounds allowed us to determine the amount of acid associated with the bile excretion (Table IV). To be noted that the basal bile does not exhibit any absorption around 280-290 nm, thus allowing a reliable determination of benzimidazole derivatives contents.

Result and discussion

The volumes of the excreted bile and their percent variations at the indicated times against the basal values are reported in Table II. Fig. 1 shows the average percent variation for each compound within the considered time. A certain number of derivatives have shown to be too toxic at 0.5 mmol/Kg (**2c, 13, 18, 19, 22, 24, 25, 26**) and 100% mortality of the animals was reached within 1-3 h after administration.

TABLE II

Effects of the acids on bile output in rats in comparison with physiological solution, $NaHCO_3$ solution, DCA.

Compound	Dose mmol/kg	Animals N.	Volumes ml ± S.D.) of bile output at times					% variation of bile output at times				% mean variation
			Basal	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h	
Physiol. sol.	—	7	0.87 ± 0.03	0.80 ± 0.03	0.72 ± 0.04	0.69 ± 0.04	0.58 ± 0.04	− 8.0	− 17.2	− 20.7	− 33.3	− 19.8
$NaHCO_3$(O.2M)	—	4	0.85 ± 0.02	0.95 ± 0.05	0.79 ± 0.04	0.89 ± 0.11	0.47 ± 0.06	+ 11.8	− 7.1	+ 4.7	− 44.7	− 8.8
DCA	0.50	4	0.59 ± 0.03	1.69 ± 0.32	1.31 ± 0.20	0.67 ± 0.05	0.60 ± 0.10	+186.4	+122.0	+ 13.6	+ 1.7	+ 80.9
ABA	0.50	6	0.75 ± 0.05	0.64 ± 0.04	0.62 ± 0.03	0.72 ± 0.06	0.57 ± 0.05	− 14.7	− 17.3	− 4.0	− 24.0	− 15.0
B	0.50	7	0.78 ± 0.04	1.94 ± 0.12	1.95 ± 0.07	1.55 ± 0.07	1.45 ± 0.06	+148.7	+150.0	+ 98.7	+ 85.9	+120.8
B	0.25	5	0.59 ± 0.05	0.99 ± 0.07	1.22 ± 0.05	0.89 ± 0.05	0.82 ± 0.06	+ 67.8	+106.8	+ 50.8	+ 39.0	+ 66.1
3a	0.50	5	0.66 ± 0.03	0.68 ± 0.04	0.82 ± 0.02	0.73 ± 0.03	0.70 ± 0.03	+ 3.0	+ 24.2	+ 10.6	+ 6.1	+ 11.0
3c	0.50	7	0.67 ± 0.03	1.02 ± 0.10	0.78 ± 0.05	0.73 ± 0.04	0.61 ± 0.04	+ 52.2	+ 16.4	+ 9.0	− 9.0	+ 17.2
5	0.50	4	0.71 ± 0.07	1.35 ± 0.21	1.32 ± 0.08	1.32 ± 0.07	0.94 ± 0.05	+ 90.1	+ 85.9	+ 85.9	+ 32.4	+ 73.6
5	0.25	6	0.61 ± 0.02	1.16 ± 0.05	1.20 ± 0.04	0.84 ± 0.04	0.60 ± 0.03	+ 90.2	+ 96.7	+ 37.7	− 1.6	+ 55.8
6	0.50	6	0.49 ± 0.07	0.58 ± 0.05	0.60 ± 0.04	0.70 ± 0.08	0.80 ± 0.12	+ 18.4	+ 22.4	+ 42.9	+ 63.3	+ 36.8
7	0.50	7	0.65 ± 0.05	1.39 ± 0.13	1.28 ± 0.10	1.40 ± 0.11	0.99 ± 0.05	+113.8	+ 96.9	+115.4	+ 52.3	+ 94.6
8	0.50	6	0.53 ± 0.03	1.04 ± 0.11	1.26 ± 0.07	1.13 ± 0.09	0.85 ± 0.07	+ 96.2	+137.7	+113.2	+ 60.4	+101.9
9	0.50	5	0.62 ± 0.07	1.14 ± 0.12	1.47 ± 0.10	1.38 ± 0.11	1.19 ± 0.12	+ 83.9	+137.1	+122.6	+ 91.9	+108.9
10	0.50	5	0.59 ± 0.01	0.95 ± 0.10	0.85 ± 0.02	0.68 ± 0.04	0.52 ± 0.05	+ 61.0	+ 44.1	+ 15.3	− 11.9	+ 27.1
11	0.50	5	0.44 ± 0.10	0.89 ± 0.14	0.83 ± 0.09	0.84 ± 0.12	0.52 ± 0.06	+102.3	+ 88.6	+ 90.9	+ 18.2	+ 75.0
12	0.50	7	0.69 ± 0.06	1.83 ± 0.16	1.85 ± 0.14	1.50 ± 0.06	1.16 ± 0.10	+165.2	+168.1	+117.4	+ 68.1	+129.7
12	0.25	7	0.49 ± 0.03	1.14 ± 0.12	0.77 ± 0.07	0.69 ± 0.07	0.53 ± 0.07	+132.7	+ 57.1	+ 40.8	+ 8.2	+ 59.7
13 (a)	0.25	6 (b)	0.87 ± 0.09	0.98 ± 0.23	1.12 ± 0.15	1.03 ± 0.12	0.59 ± 0.06	+ 12.6	+ 28.7	+ 18.4	− 32.2	+ 6.9
14	0.50	5	0.91 ± 0.13	1.32 ± 0.17	1.48 ± 0.09	1.19 ± 0.11	1.00 ± 0.05	+ 45.1	+ 62.6	+ 30.8	+ 9.9	+ 37.1
15	0.50	6	0.98 ± 0.08	1.60 ± 0.08	1.65 ± 0.19	1.48 ± 0.05	1.26 ± 0.03	+ 63.3	+ 68.4	+ 51.0	+ 28.6	+ 52.8
16	0.25	4	0.77 ± 0.05	0.86 ± 0.09	0.73 ± 0.06	0.85 ± 0.03	0.63 ± 0.06	+ 11.7	− 5.2	+ 10.4	− 18.2	− 0.3
17	0.50	7	0.63 ± 0.03	1.23 ± 0.10	1.55 ± 0.19	0.97 ± 0.06	0.95 ± 0.03	+ 95.2	+146.0	+ 54.0	+ 50.8	+ 86.5
18 (c)	0.25	7	0.83 ± 0.10	0.93 ± 0.09	1.05 ± 0.09	0.90 ± 0.08	0.88 ± 0.07	+ 12.0	+ 26.5	+ 8.4	+ 6.0	+ 13.2
19[d, e]	0.12	7	0.58 ± 0.04	0.94 ± 0.03	0.93 ± 0.03	0.60 ± 0.02	0.41 ± 0.02	+ 62.1	+ 60.3	+ 3.4	− 29.3	+ 24.1
20	0.50	5	0.61 ± 0.11	1.26 ± 0.13	1.26 ± 0.13	1.17 ± 0.09	1.04 ± 0.03	+106.6	+106.6	+ 91.8	+ 70.5	+ 93.9

(a) 100% mortality at 0.5 mmol/kg within 3 h after administration; (b) during the experiment two animals died 2 h after administration; (c) 100% mortality at 0.5 mmol/kg within 2 h after administration; (d) 100% mortality at 0.5 mmol/kg within 1 h after administration; (e) 50% mortality at 0.25 mmol/kg within 4 h after administration.

(Continuation table II)

Compound	Dose mmol/kg	Animals N.	Volumes ml ± S.D.) of bile output at times					% variation of bile output at times				% mean variation
			Basal	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h	
21	0.50	6 (b)	0.93 ± 0.10	1.04 ± 0.09	1.54 ± 0.17	1.53 ± 0.27	1.18 ± 0.05	+ 11.8	+ 65.6	+ 64.5	+ 26.9	+ 42.2
22 (d)	0.12	7	0.66 ± 0.02	0.78 ± 0.03	0.94 ± 0.04	0.76 ± 0.04	0.51 ± 0.02	+ 18.2	+ 42.4	+ 15.2	− 22.4	+ 13.3
23	0.50	5	0.65 ± 0.06	1.24 ± 0.08	1.09 ± 0.15	1.13 ± 0.18	1.05 ± 0.05	+ 90.8	+ 67.7	+ 73.8	+ 61.5	+ 73.4
24 (a)	0.25	6	0.69 ± 0.04	0.80 ± 0.08	0.74 ± 0.06	0.54 ± 0.06	0.44 ± 0.04	+ 15.9	+ 7.2	− 21.7	− 36.2	− 8.7
27	0.50	4	0.59 ± 0.07	0.67 ± 0.04	0.76 ± 0.05	0.80 ± 0.04	0.79 ± 0.06	+ 13.6	+ 28.8	+ 35.6	+ 33.9	+ 28.0
28	0.50	6	0.66 ± 0.07	0.99 ± 0.06	0.93 ± 0.07	0.88 ± 0.09	0.84 ± 0.10	+ 50.0	+ 40.9	+ 33.3	+ 27.3	+ 37.9
29	0.50	7	0.70 ± 0.07	1.09 ± 0.10	1.30 ± 0.12	1.27 ± 0.10	1.07 ± 0.09	+ 55.7	+ 85.7	+ 81.4	+ 52.9	+ 68.9
30	0.50	5	0.52 ± 0.03	0.98 ± 0.05	1.14 ± 0.10	1.39 ± 0.15	0.88 ± 0.08	+ 88.5	+ 119.2	+ 167.3	+ 69.2	+ 111.0
31	0.50	5	0.56 ± 0.04	1.14 ± 0.23	1.19 ± 0.07	1.02 ± 0.14	0.87 ± 0.11	+ 103.6	+ 112.5	+ 82.1	+ 55.4	+ 88.4
32	0.50	6	0.88 ± 0.07	1.43 ± 0.12	1.78 ± 0.15	1.99 ± 0.14	1.02 ± 0.08	+ 62.5	+ 102.3	+ 126.1	+ 15.9	+ 76.7
33	0.50	6	0.66 ± 0.05	1.44 ± 0.09	1.79 ± 0.11	1.63 ± 0.15	1.19 ± 0.14	+ 118.2	+ 171.2	+ 147.0	+ 80.3	+ 129.2
34	0.50	6	0.56 ± 0.05	1.39 ± 0.15	1.15 ± 0.12	1.02 ± 0.14	0.68 ± 0.08	+ 148.2	+ 105.4	+ 82.1	+ 21.4	+ 89.3
35	0.50	7	0.66 ± 0.07	0.86 ± 0.05	0.70 ± 0.04	0.79 ± 0.05	0.61 ± 0.05	+ 30.3	+ 6.1	+ 19.7	− 7.6	+ 12.1
36	0.50	5	0.57 ± 0.05	0.75 ± 0.05	0.52 ± 0.05	0.50 ± 0.03	0.46 ± 0.02	+ 31.6	− 8.8	− 12.3	− 19.3	− 2.4

(a) 100% mortality at 0.5 mmol/kg within 3 h after administration; (b) during the experiment two animals died 2 h after administration; (c) 100% mortality at 0.5 mmol/kg within 2 h after administration; (d) 100% mortality at 0.5 mmol/kg within 1 h after administration; (e) 50% mortality at 0.25 mmol/kg within 4 h after administration.

TABLE III

*Effect of the acids **B, 5, 7, 12, 19** and **34** on bile dry residue.*

Comp.	Dose mmol/kg	Animals N	Dry residue of bile (absolute mean values in mg) at indicated times (percent variations against basal value)					% mean variation over 4 h	% mean corrected variat. (*)
			Basal	1 h	2 h	3 h	4 h		
Phys. sol.	—	7	24.4	21.6 (− 11.5)	18.7 (− 23.4)	16.3 (−33.2)	13.8 (−43.4)	−27.9	—
$NaHCO_3$	—	4	27.5	27.7 (+ 0.7)	21.8 (− 20.7)	25.1 (− 8.7)	13.7 (−50.2)	−19.7	—
DCA	0.50	4	19.5	59.8 (+206.7)	41.7 (+113.8)	17.7 (− 9.2)	17.0 (−12.8)	+74.6	—
B	0.50	7	26.7	43.1 (+ 61.4)	42.1 (+ 57.7)	34.4 (+28.8)	31.3 (+17.2)	+41.3	+17.2
5	0.50	4	26.0	39.7 (+ 52.7)	38.3 (+ 47.3)	35.6 (+36.9)	24.8 (− 4.6)	+33.1	− 4.5
7	0.50	7	22.9	41.6 (+ 81.7)	37.2 (+ 62.4)	41.3 (+80.3)	26.1 (+14.0)	+59.6	+24.1
12	0.50	7	24.8	57.6 (+132.3)	56.8 (+129.0)	46.4 (+87.1)	34.8 (+40.3)	+97.2	+32.6
19	0.12	7	18.0	25.9 (+ 43.9)	23.4 (+ 30.0)	15.0 (−16.7)	10.5 (−41.7)	+ 3.9	−10.4
34	0.50	6	22.2	43.0 (+ 93.7)	34.0 (+ 53.2)	29.4 (+32.4)	19.7 (−11.3)	+42.0	− 9.1

(*) deduced of the amount of acids detected according to Table IV.

TABLE IV

Evaluation of the excreted acids in the dry residue of bile.

Compd	Dose mmol/kg	Average weigth/rat (g)	Quantity of acid (in mg) detected by U.V. titration at times(*)				Total excreted acid (mg)	% excreted acid during 4h
		Total ccmpd given (mg)	1 h	2 h	3 h	4 h		
B	0.50	334 / 49.2	10.1	6.2	5.3	4.1	25.7	52.2
5	0.50	310 / 47.8	12.2	11.6	10.0	5.3	39.1	81.8
7	0.50	379 / 61.5	9.7	9.0	9.0	4.8	32.5	52.8
12	0.50	402 / 71.2	20.1	22.9	14.1	7.0	64.1	90.0
19	0.12	323 / 15.2	4.5	3.2	1.7	0.9	10.3	67.8
34	0.50	400 / 73.7	16.1	12.4	10.4	6.5	45.4	61.6

(*)These quantities were calculated assuming that the injected acids were excreted unchanged and using for each of them the extinction coefficient indicated in Table I.

Some of these compounds were toxic even at lower dosage (Table II) and the most toxic seems to be **19**. For the remaining acids it is supposed that the lethal dose is quite far from that (0.5 mmol/Kg) used for choleretic activity determination, since the animals did not show any sign of suffering during the experiment and afterwards.

From the results of Table II and Fig. 1 it is evident that eleven acids [in decreasing order of activity: **12, 33, B, 30, 9, 8, 7, 20, 34, 31, 17**] were significantly more active than **DCA** (+81%) and two (**12,33**) were superior to the model compound **B** (+121%). Five acids (**32, 11, 5, 23, 29**) were almost as active as **DCA**, with a bile output increase ranging from +77 to +69%, while for other five (**15, 21, 28, 14, 6**) the volume increase goes progressively down to +37% of the basal value; the remaining acids showed poor activity or were completely inactive.

Reducing the dose from 0.5 to 0.25 mmol/Kg, the activity of the acid **B** and **12** dropped to an half, while that of **5** was reduced of only 18%. It has to be noted that generally the activity of benzimidazolylbutanoic acids is rather long lasting, while that of dehydrocholic acid is pratically estinguished after 2 h from administration. Limiting the comparison of the mean activities of **DCA** and of benzimidazolylbutanoic acids recorded during the first two h after administration it must be noted that only the acid **12**

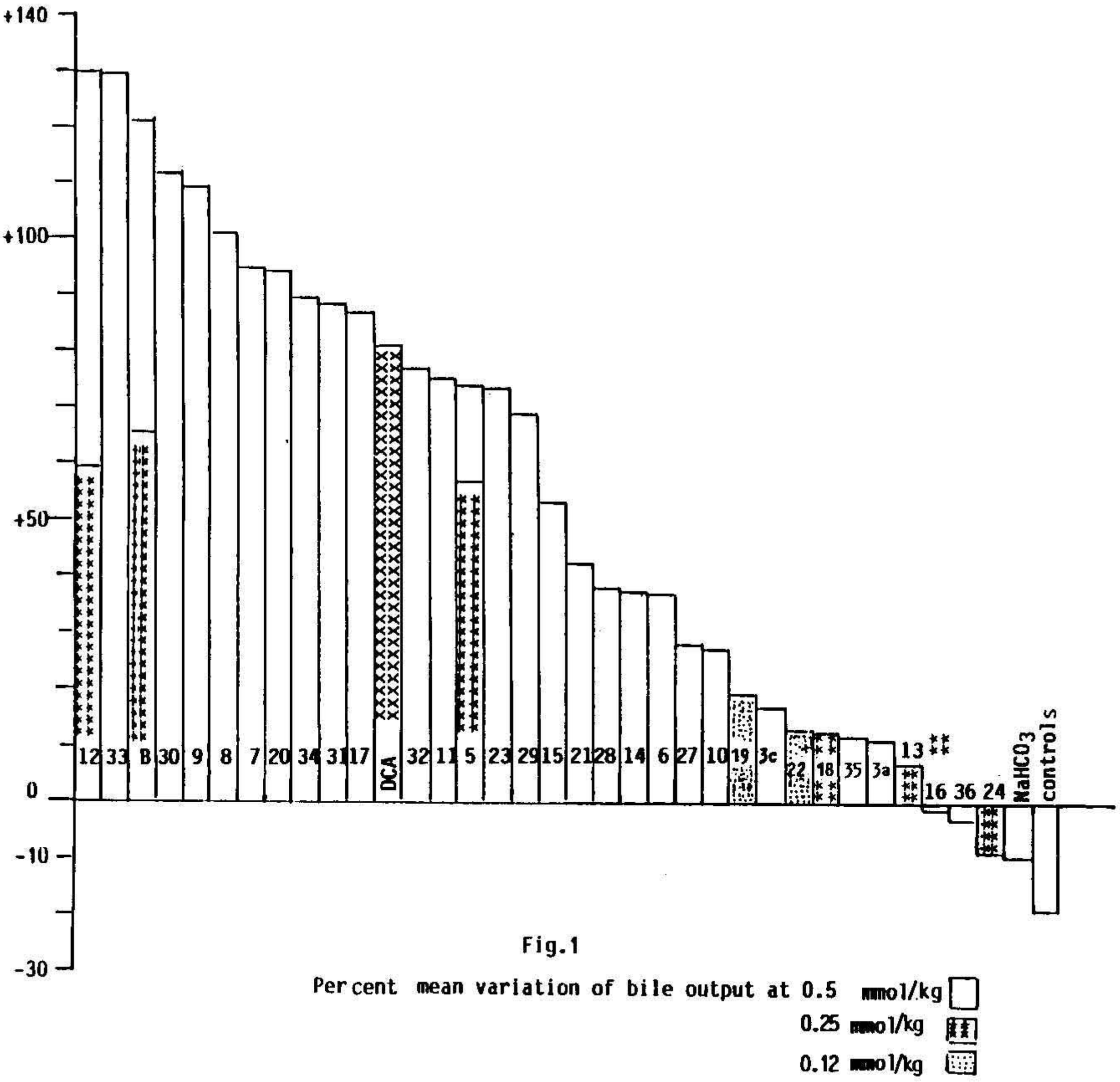


Fig.1

(+167%) was more active than **DCA** (+154%) (Table II), while the remaining ten more active acids previously mentioned still exhibited high activities with a bile output mean increase after 2h ranging from +149 to +104% of the basal value.

The increase of bile volume is associated with an increase of the bile dry residue (Table III), the latter being always lower thus indicating that the bile is generally more diluted than the basal one. It is noteworthy our finding that the tested acids are eliminated with the bile, but no attempts were made so far to establish if the acids were excreted unchanged or in any modified or conjugated form. Assuming that the excreted benzimidazole derivatives had the same extinction coefficient of the injected acids we observed that the amounts of acids excreted during the four hours of the experiment varyed from 52% (**B**) to 90% (**12**) of the injected quantity. This observation well accounts for an increase of the bile dry residue imputable to the excreted acids or their possible conjugation derivatives. As consequence we must deduce that a real increase in dry residue is very limited or only

apparent. Among the six acids examined (Table III and IV), only **B,7** and **12** were able to produce a significant increase of the bile dry residue and deserve a further investigation in order to control any possible variation of both bile cholesterol and cholic acid contents. As regard as the structure-activity relationships it is noteworthy that, with the only exceptions of acids **12** and **33**, all tested compounds are less active than the model acid **B**. The detrimental effects of the substituents on position 5 of the benzimidazole ring or on position 4 of the benzyl residue are generally additive. Very peculiar effects were due to methoxy groups. In fact, going from the 4'-methoxy- to the 3', 4'-dimethoxy derivative the activity is significantly increased, whereas introduction of a third methoxyl cancelled the activity and strongly enhanced the toxicity. Similarly a special mention deserve the 5-methylbenzimidazole derivatives **29-34**; in contrast with the general trend the introduction of a substituent on position 4' gives rise to compounds more active than the 4'-unsubstituted **29**, and in the case of compounds **30** and **33** the activities are also superior to those of the corresponding compounds **5** and **9**, which do not bear substituent on position 5.

Finally we must remark that 3-aminobutanoic acid and its aryl derivatives **3a** and **3c**, as well as the two benzimidazole derivatives **35** and **36** were scarcely active or pratically inactive. Thus we can assert that both the integrity of the benzimidazole nucleus and the presence of a cumbersome substituent in its position 2 are important for the expression of the choleretic activity. Moreover, the 2-benzylsubstituted benzimidazolyl-butanoic acids are, on the whole, more active than the 2-phenyl or 2-pyridil derivatives previously described (1).

In conclusion, the finding of new strong and long lasting choleretic agents (mainly of hydrocholeretic type) warrant the study of additional benzimidazolylbutanoic acids to find the optimal substitution pattern. Moreover, in order to simulate more closely the circumstances under which such agents might be used clinically, the most active compounds will be administered into the animal duodenum via a catheter and the bile collected hourly will be returned to the animal through the same way.

Acknowledgement

This work was supported by a grant of University of Sassari (MURST Research Funds 60%).

REFERENCES

1) GRELLA G., PAGLIETTI G., SPARATORE F., MANCA P., SATTA M., *Il Farmaco Ed. Sc.*, **42**, 475; 1987.
2) SPARATORE F., PAGANI F., *Il Farmaco, Ed. Sc.*, **19**, 55; 1964.
3) PREZIOSI P., MANCA P., PAGLIETTI G., *Il Farmaco Ed. Sc.* **20**, 238; 1965.

4) PAGLIETTI G., SPARATORE F., *Il Farmaco, Ed. Sc.* **27**, 471; 1972
5) KING F.E., ACHESON R.M., *J. Chem. Soc.*, 1396; 1949.
6) PAGLIETTI G., SPARATORE F., *Il Farmaco, Ed. Sc.*, **27**, 333; 1972.
7) PAGLIETTI G., SPARATORE F., *Il Farmaco, Ed. Sc.*, **27**, 380; 1972.
8) LEANDRI G., MANGINI A., MONTANARI F., PASSERINI R., *Gazz. Chim. It.*, **85**, 769; 1955.
9) PRESTON P.N.: "Benzimidazoles and Congeneric Tricyclic Compounds" Part I, The Chemistry of Heterocyclic Compounds, vol. **40**; pag. 64;
A. WEISSBERGER and E.C., TAYLOR, *Eds, John Wiley & Sons*, New York; 1980.

Received July 24, 1991; accepted August 26, 1991.