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Pharmacological Studies of 1-(*p*-Chlorophenyl)propanol and 2-(1-Hydroxy-3-butenyl)phenol: Two New Non-narcotic Analgesics Designed by Molecular Connectivity

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Abstract

Molecular topology has been applied to the design of new analgesic drugs. Linear discriminant analysis and connectivity functions were used to design two potentially suitable drugs which were synthesized and tested for analgesic properties by the acetic acid-induced abdominal constriction test in mice and the tail-flick test in rats.

In mice, the compound 1-(*p*-chlorophenyl)propanol showed higher analgesic activity, both intraperitoneally and orally, than acetylsalicylic acid. 2-(1-Hydroxy-3-butenyl)phenol exhibited a lesser protective effect (70% of that shown by acetylsalicylic acid). In rats, acetylsalicylic acid gave the greatest protection against pain when administered intraperitoneally, while 1-(*p*-chlorophenyl)propanol was the most active orally. The 2-(1-hydroxy-3-butenyl)phenol, both intraperitoneally and orally, showed the least protective effect.

These results demonstrated the peripheral analgesic properties of the selected compounds, thus confirming the validity of the molecular design method.

Connectivity indices have shown their usefulness in the prediction of diverse physical, chemical and biological properties of various types of compounds (Kier & Hall 1986; García et al 1991; Soler et al 1992; Gálvez et al 1994, 1995; Julián-Ortiz et al 1996) and also in the design of new antiviral (Muñoz et al 1994) and hypoglycaemic agents (Antón-Fos et al 1994).

The first step in applying such method to a group of non-narcotic analgesics is the search for connectivity functions capable of discriminating between whether or not a particular compound has an analgesic character. We used discriminant linear analysis and multilinear regression for this. The second step is to construct chemical structures, possibly starting from a base structure taken as a reference, and then matching their properties against limits posed by discriminant analysis, selecting only those compounds which meet the determining criteria. The compounds which are designed are finally submitted to standard pharmacological tests for analgesics to corroborate their theoretical behaviour.

Materials and Methods

Synthesis of analgesics

Infrared absorption spectra were obtained on a Perkin-Elmer Model 843 spectrophotometer (Norwalk, CT, USA) with samples as neat liquids. Nuclear magnetic resonance (NMR) spectra were recorded on Hitachi Perkin-Elmer Model R-24 B or Brucker WP 80 SY (Fällanden, Switzerland) spectrometers. ^{1}H data are reported as follows: chemical shift (ppm) [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, interpretation]. ^{13}C data are reported as follows: chemical shift (ppm) (interpretation). Reactions were carried out under a blanket of argon. The organic synthesis was achieved as follows:

1-(p-chlorophenyl)propanol (1). To a stirred solution of 4 g (28·5 mM) of *p*-chlorobenzaldehyde (Aldrich, Steinheim, Germany) freshly sublimated in 10 mL of ether (SDS, Peypin, France) previously dried, was added a solution of 85 mM of ethylmagnesium bromide (DePuy & Klein 1973) in 50 mL of dry ether with cooling. The reaction was left stirring overnight at room temperature, then neutralized with 40 mL of 25% aqueous ammonium chloride (Scharlau, Barcelona). The ethereal layer was extracted, dried with sodium sulphate (Panreac, Barcelona) and concentrated to yield **1** as a colourless oil. IR 1090 cm^{-1}; ^{1}H-NMR ($CDCl_3$): 0·9 (t, 3 H, CH_3–), 1·8 (m, 2 H, C–CH_2–C), 2·0 (broad s, 1 H, C–OH), 4·6 (t, 1 H, AR–CH–O), 7·3 (AR–H); ^{13}C–NMR ($CDCl_3$): 9·8 (–CH_3), 31·8 (–CH_2–), 75·0 (CH–OH), 127·3 (AR C–2), 128·3 (AR C–3), 132·9 (AR C–1), 143·0 (AR C–Cl).

2-(1-hydroxy-3-butenyl)phenol (2). An ethereal solution of allylmagnesium bromide (0·2 M in 60 mL) (Grummitt et al 1963) was added dropwise to a stirred solution of 3 g (26·6 mM) of salicylaldehyde (Aldrich) in 10 mL of dry ethyl ether (SDS). The reaction mixture was refluxed in a steam bath for one hour, left overnight, poured slowly into a cold saturated aqueous ammonium chloride solution (80 mL) and extracted with three 30-mL portions of ether. The organic phase was dried (sodium sulphate) and evaporated to dryness obtaining **2** as a colourless oil. IR 1480, 1460, 1240, 760 cm^{-1}; ^{1}H–NMR ($CDCl_3$): 2·7 (t, 2 H, C–CH_2–C=), 3·9 (broad s, 1 H, alcohol), 4·7 (m, 3 H, C = CH_2 + AR–CH–O), 5·6 (m, 1 H, C–CH=C), 6·7 (m, 4 H, AR–H), 8·2 (s, 1 H, phenol); ^{13}C–NMR ($CDCl_3$): 41·5 (–CH_2–), 73·3 (CHOH), 116·4, 117·8, 119·6 (=CH_2, AR C–6, AR C–2), 126·9, 127·3, 128·4 (AR C–4, AR C–3, AR C–5), 133·9 (–CH=), 154·5 (AR C–OH).

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1-(p-chlorophenyl)-propanamine (3). To a stirred solution of 4·22 g (30 mM) of *p*-chlorobenzaldehyde (Aldrich) freshly sublimated in 10 mL of previously dried tetrahydrofurane (SDS) was added dropwise a solution of lithium bis-(trimethylsilyl)amide (prepared with 23·2 mL of a 1·55 M hexane solution of *n*-butyllithium and 8·2 mL (39 mM) of 1,1,1,3,3,3-hexamethyldisilazane (Aldrich) in 10 mL of tetrahydrofurane) with cooling in an ice-water bath (Hart et al 1983). To the resulting solution containing *N*-trimethylsilyl imine was added 90 mM of ethylmagnesium bromide in 50 mL of ether. The mixture was stirred at room temperature for 30 min, poured into 200 mL of saturated aqueous ammonium chloride, and extracted with three 30-mL portions of dichloromethane. The organic phase was then treated with 10 mL of 1 M sulphuric acid, the aqueous phase extracted, and neutralized with 3 M potassium hydroxide (Panreac) in the presence of ether. The ethereal phase was dried (sodium sulphate) and concentrated 'in vacuo' to give the amine as a yellow oil, which could not be crystallized. IR 1500 cm^{-1}; ^1H–NMR (D_2O): 0·7 (t, 3 H, CH_3–), 1·5 (m, 2 H, C–CH_2-C), 3·6 (t, 1 H, AR–CH–N), 4·7 (d, 3 H, C–NH_2), 7·1 (m, 4 H, AR–H); ^{13}C–NMR (D_2O): 10·2 (–CH_3), 31·7 (–CH_2–), 56·3 (CH–NH_2), 127·3 (AR C–2), 127·9 (AR C–3), 131·7 (AR C–Cl), 144·2 (AR C–1).

Animals used for testing of analgesia

Swiss female mice (25–30 g) and female Wistar rats (200–250 g) purchased from Panlab (Barcelona) were used. Animals were allowed free access to food and water. In the experimental groups with oral administration, animals were fasted for 8 h before the experiments.

Abdominal constriction test in mice

Products, standards and vehicle alone were administered intraperitoneally and orally (0.25 mL), 30 min and 1 h, respectively, before the intraperitoneal administration of 0.25 mL of a 3% acetic acid (Panreac) aqueous solution. Acetylsalicylic acid (Sigma, St. Louis, MO, USA), the reference peripheral analgesic compound, was used at its ED50 of 100 mg kg^{-1} (18 μmol kg^{-1}) intraperitoneally and 200 mg kg^{-1} (36 μmol kg^{-1}) orally. The control group received only the vehicle: absolute ethanol (Panreac)/Tween 80 (Panreac)/water, 2:2:35, v/v/v. Each group was composed of ten mice. Immediately after the injection of the algic compound, each animal was isolated in an individual box (24 × 24 × 20 cm) to be observed during 20 min (Witkin et al 1961; Nakamura & Shimizu 1981). The number of abdominal constrictions and stretchings were recorded and the percentage of protection (% A) was expressed using the following ratio:

$$\%A = \frac{C - P}{C} \cdot 100 \quad (4)$$

where C = control mean, P = treated mean number of abdominal constrictions and stretchings.

Tail-flick test in rats

Products, reference substance and vehicle were administered intraperitoneally and orally. Oral treatment was followed by a delay of 30 min before time zero. The tail was exposed to a heat and light source covering a photocell, and tested at 45, 75 and 120 min. When the rat experienced discomfort and moved its tail, it automatically stopped the timer (Glassman 1971). For each group (composed of five rats), the average reaction times (t) were calculated, and the percentages of variation were expressed using the following ratio:

$$\%A = \frac{t_p - t_c}{t_c} \cdot 100 \quad (5)$$

where t_P = treated mean time, t_c = control mean time

Analgesic properties of newly designed and synthesized compounds

The analgesic effects of **1** and **2** were demonstrated in the abdominal constriction and tail-flick tests. In the abdominal constriction test in mice, by the intraperitoneal route, com-

Table 1. Analgesic effects of compounds **1**, **2** and acetylsalicylic acid (ASA) against chemical-induced nociception.

Compound	Dose (mg kg^{-1})	No. of constrictions per 20 min	Inhibition (%)	ED50 (mg kg^{-1})
A. Administered intraperitoneally.				
Control		38·4 ± 4·8		
1	60	32·5 ± 4·8	15·4	
	80	23·4 ± 4·8*	39·1	84
	100	8·6 ± 1·4***	77·7	(70–98)
2	100	22·7 ± 4·1*	41·0	
	125	18·7 ± 3·3**	51·4	116
	150	8·0 ± 2·0***.	79·2	(89–143)
ASA	100	19·3 ± 1·2***	49·7	100
B. Administered orally.				
Control		40·8 ± 4·6		
1	140	33·3 ± 3·6*	18·5	
	180	21·5 ± 3·5***	47·3	193
	210	17·4 ± 4·2***	57·3	(156–230)
2	250	24·5 ± 3·0***	40·1	
	290	21·5 ± 1·7***	47·2	299
	330	17·5 ± 2·8***	57·0	(198–400)
ASA	200	24·5 ± 2·8***	40·1	> 200

Number of constrictions per 20 min expressed as means ± s.e.m. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ED50 95% confidence limits given in parentheses.

Table 2. Analgesic effects of compounds **1**, **2** and acetylsalicylic acid (ASA) against heat-induced nociception.

Compound	Dose ($mg\,kg^{-1}$)	Maximum response latency (s)			Variation (%)			ED50# ($mg\,kg^{-1}$)
		45 min	75 min	120 min	45 min	75 min	120 min	
A. Administered itraperitoneally.								
Control		2·8 ± 0·2	2·6 ± 0·1	2·4 ± 0·2				
1	80	2·8 ± 0·3	2·8 ± 0·2	2·4 ± 0·3	0·0	7·7	0·0	
	100	3·5 ± 0·3	3·8 ± 0·3**	3·4 ± 0·4*	25·0	46·2	41·7	114
	120	4·3 ± 0·4**	3·9 ± 0·3***	3·6 ± 0·2***	53·4	50·0	50·0	(95–133)
2	125	3·8 ± 0·2*	3·1 ± 0·4	3·4 ± 0·2*	35·7	19·2	41·7	
	150	4·2 ± 0·3***	4·7 ± 0·3***	3·8 ± 0·3***	50·0	80·8	58·3	141
	175	5·2 ± 0·5***	4·9 ± 0·5***	3·8 ± 0·2***	85·7	88·5	58·3	(130–152)
ASA	100	4·5 ± 0·3***	4·7 ± 0·3***	4·0 ± 0·3***	60·7	80·8	66·7	< 100
B. Administered orally.								
Control		2·4 ± 0·1	2·5 ± 0·1	2·2 ± 0·1				
1	160	3·7 ± 0·2***	4·0 ± 0·2***	3·3 ± 0·2***	54·2	60·0	50·0	
	200	3·9 ± 0·2***	4·4 ± 0·2***	3·5 ± 0·2***	62·5	86·0	59·1	133
	250	4·2 ± 0·1***	4·7 ± 0·2***	3·7 ± 0·2***	75·0	88·0	68·2	(108–158)
2	250	3·2 ± 0·3*	3·5 ± 0·3**	3·0 ± 0·1***	33·3	41·6	36·4	
	300	3·6 ± 0·2***	4·2 ± 0·2***	3·3 ± 0·2***	50·0	68·0	50·0	270
	350	4·0 ± 0·2***	4·3 ± 0·4***	3·7 ± 0·2***	66·7	72·0	68·2	(223–317)
ASA	200	4·2 ± 0·2***	4·3 ± 0·2***	3·8 ± 0·3***	75·0	72·0	72·7	< 200

ASA, acetylsalicylic acid. Maximum response latency expressed as means ± s.e.m. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. #Calculated from variation percentages at 75 min, 95% confidence limits given in parentheses.

pounds **1** and **2** showed dose-dependent analgesic activity, having ED50 values of $84\,mg\,kg^{-1}$ ($14{\cdot}3\,\mu mol\,kg^{-1}$) and $116\,mg\,kg^{-1}$ ($19\,\mu mol\,kg^{-1}$), respectively (Table 1). Orally, they gave minor steep dose-response curves and the ED50 values were $193\,mg\,kg^{-1}$ ($32{\cdot}9\,\mu mol\,kg^{-1}$) and $299\,mg\,kg^{-1}$ ($49{\cdot}1\,\mu mol\,kg^{-1}$), respectively.

In the tail-flick test in rats, the ED50 values of **1** and **2** were $114\,mg\,kg^{-1}$ ($19{\cdot}5\,\mu mol\,kg^{-1}$) and $141\,mg\,kg^{-1}$ ($23{\cdot}2\,\mu mol\,kg^{-1}$), respectively, after intraperitoneal administration, and $133\,mg\,kg^{-1}$ ($22{\cdot}7\,\mu mol\,kg^{-1}$) and $270\,mg\,kg^{-1}$ ($44{\cdot}3\,\mu mol\,kg^{-1}$), respectively, in the oral administration group (Table 2).

It is noteworthy that **1** is a new kind of analgesic structure. Analgesic potencies (ED50), summarized in Tables 1 and 2, show that the analgesic activity is dose-dependent and that compound **1** is more active than **2**. In chemical-induced nociception (Table 1) **1** is more active than acetylsalicylic acid, and acetylsalicylic acid more than **2**. The reference drug, acetylsalicylic acid, showed the most protective effect against heat (Table 2). It was not possible to assign any clear analgesic activity to compound **3** because it caused hyperactive behaviour in mice, thus making the abdominal constriction test impossible. We will study this effect in further investigations.

Acute lethal toxicity

The intraperitoneal lethal toxicities of **1** and **2** were compared with that of the vehicle in mice. Mortality was observed for 48 h. The intraperitoneal LD50 values of **1** and **2** in mice were $719\,mg\,kg^{-1}$ ($122{\cdot}7\,\mu mol\,kg^{-1}$) and $778\,mg\,kg^{-1}$) ($127{\cdot}8\,\mu mol\,kg^{-1}$), respectively; that of acetylsalicylic acid was $795\,mg\,kg^{-1}$ ($143{\cdot}2\,\mu mol\,kg^{-1}$) (Nakamura et al 1983). The therapeutic indices of **1** and **2**, calculated from the intraperitoneal LD50 and ED50 values in mice were 1·1 and 0·8 times that of acetylsalicylic acid for the acetic acid-induced abdominal constriction test. These results demonstrate the wide safety margin of compounds **1** and **2**.

Table 3. Predicted and experimental cyclooxygenase inhibition values for a set of established analgesics.

Compound	IC_{obs}[a]	IC_{calc}[b]
Acetylsalicylic acid	700	929
Flufenamic acid	6	3
Meclofenamic acid	15	4
Mefenamic acid	4	14
Salicylic acid	1000	679
Alclofenac	18	51
Aminopyrine	10 000	4841
Antipyrine	5	6
Azapropazone	169	76
Diclofenac	100	22
Diflunisal	0·34	16
Etodolac	4	18
Phenacetin	11	12
Fenbufen	6	5
Fenclofenac	32	10
Phenylbutazone	5	17
Fenoprofen	662	284
Flurbiprofen	120	343
Ibuprofen	28	9
Indomethacin	12	28

[a]Cyclooxygenase inhibition values (IC) taken from Flower & Vane (1974). [b]Values calculated from equation 1.

Results and Discussion

In a previous work (Gálvez et al 1994), we demonstrated that it is possible to obtain a high degree of molecular characterization of analgesic activity by an adequate choice of connectivity indices. We used connectivity indices up to 4th order (Kier et al 1975; Kier & Hall 1986) and obtained two suitable functions. The first function was obtained through the optimization of multiple linear regressions between logarithm of half-inhibition concentration of cyclooxygenase (IC50) and path (p),

Table 4. Discriminant functions, derived from equations 1–3, applied to a group of 46 compounds known to possess analgesic activity and 56 molecules without known analgesic activity.

Compound	X[a]	log IC[b]	Class[c]	Compound	X[a]	log IC[b]	Class
Analgesics				*Anti-inflammatory agents*			
2,4-Dimethyl acetophenone	0·43	0·82	+	Bucloxic acid	0·05	−0·50	−
2-Amino-4-picoline	0·41	2·48	+	Bucolome	−0·47	2·04	−
2-(1-Propenyl)phenol	0·24	1·98	+	Butibufen	−0·60	1·43	−
Acemetacin	0·34	1·32	+	Clidanac	0·30	−0·30	−
Alminoprofen Minalfene	0·11	1·39	+	Difenpiramide	−0·08	0·20	−
Benorylate	0·46	2·47	+	Glucametacin	−1·34	3·02	−
Benoxaprofen	−0·04	1·05	−	Ibuproxam	−0·19	1·37	−
Aminochlorthenoxazin	0·33	2·92	+	Oxaceprol	−0·78	4·23	−
4-Hydroxy isophthalic acid	0·32	3·00	+	Oxametacine	−0·42	1·10	−
Ammonium salicylate	0·14	2·91	+	Proglumetacin	−0·80	−0·73	−
Diplosal acetate	0·38	2·74	+	*Bronchodilators*			
Antipyrine	0·37	2·78	+	3-Methylxanthine	0·39	4·00	−
Antrafenine	0·20	−1·41	−	Acephylline	−0·30	4·57	−
Apazone	0·31	3·95	−	Indoramin	0·44	0·08	+
Benzydamine	−0·50	1·43	−	Clenbuterol	−24·30	−1·65	−
Bufexamac	−0·79	1·42	−	Dametralast	0·33	4·14	−
Bumadizon	0·46	1·46	+	Theophylline	0·05	4·17	−
Cinchophen	0·15	0·99	+	Dioxyephedrine	−1·40	2·57	−
Cinmetacin	−0·53	1·10	−	Flutropium	−17·24	1·81	−
Acetanilide	0·41	2·26	+	Theobromine	0·39	4·13	−
Chlortenoxicam	0·47	2·74	+	6-Thiocaffeine	0·44	3·33	+
Epirizole	−1·08	3·71	−	*Antivirals*			
Etersalate	−0·33	2·46	−	Bromovirin	−0·62	4·74	−
Ethenzamide	−3·25	2·64	−	Cytarabine	−2·23	6·43	−
Etodolac	−27·76	1·69	−	Idoxuridine	0·16	3·13	+
Fenbufen	0·47	0·37	+	Desciclovir	−2·67	3·95	−
Fenclofenac	0·42	1·52	+	2′-Deoxy-5-iodo uridine	0·35	2·51	+
Phenylbutazone	0·47	1·88	+	Arildone	−1·84	−0·26	−
Phenol	0·46	2·26	+	Ribavirin	−2·09	6·63	−
Fentiazac	0·17	−0·91	−	9-(4-Hydroxybutyl) guanine	−0·20	3·40	−
Glafenine	0·45	1·44	+	Methyl galate	−0·37	3·51	−
Ibufenac	0·30	1·52	+	*Hypoglycaemics*			
Indoprofen	0·21	1·17	+	Acetohexamide	0·20	0·67	+
Ketorolac	0·45	2·07	+	Buformin	0·04	2·65	+
Metofoline	−2·31	0·22	−	Benfluorex	−0·28	0·77	−
Morazone	0·46	2·54	+	Glibencyclamide	0·40	−1·45	−
Oxypizone	0·28	2·08	+	Miglitol	0·47	5·99	−
Paracetamol	0·42	2·45	+	Imipramine	−0·17	0·53	−
Pirprofen	0·42	1·17	+	Tolbutamide	−0·09	0·59	−
Ramifenazone	0·18	3·38	+	Carbutamide	0·09	0·96	+
Salsalate	0·47	2·59	+	Gliquidone	−8·50	−1·13	−
Salverine	−6·85	0·93	−	Chlorpropamide	−0·19	0·79	−
Tiaprofenic acid	0·47	−0·44	−	Dichloroacetic	−0·62	2·60	−
Viminol	−0·30	0·24	−	Glisoxepid	0·44	0·68	+
Zomepirac	0·47	1·61	+	Emiglite	−0·13	5·31	−
Flufenamic acid	0·47	0·53	+				
Antifungals							
Buclosamide	−0·97	1·66	−				
Candicidin	−31·44	4·95	−				
Chlormidazol	0·47	0·93	+				
Diamthazol	−5·64	0·25	−				
Enilconazole	−1·38	1·3	−				
Filipin	−7·58	4·03	−				
Fungichromin	−17·04	5·05	−				
Halethazole	−5·92	−1·24	−				
Miconazole	−0·89	0·06	−				
Natamycin	−19·19	6·40	−				
Nifuratel	0·25	3·45	+				
Oligomycins	−7·57	2·12	−				
Siccanin	−90·64	1·78	−				
Sulconazole	−0·35	−2·49	−				

[a]Calculated from equations 2 and 3. [b]Calculated from equation 1. [c]Classification as an analgesic according to the criteria $X > 0$ and $3{\cdot}5 > \log IC > 0$.

cluster (c), path-cluster (pc), valence (v) and non-valence connectivity indices. It was:

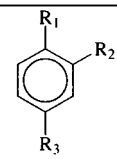
$$\log IC = -2{\cdot}64\ {}^{3}\chi_p^{v} + 1{\cdot}8\ {}^{4}\chi_p - 10{\cdot}28\ {}^{4}\chi_c + 1{\cdot}24\ {}^{4}\chi_{pc} + 3{\cdot}05 \quad (1)$$

$n = 20$, $r = 0{\cdot}846$, s.e. $= 0{\cdot}63$, $F = 9{\cdot}4$, $P < 0{\cdot}0005$,

where r = correlation coefficient and F = F-Snedecor function.

Table 3 shows the predicted results for this property using equation 1 for a number of established analgesics. The accuracy of prediction of IC, considering the structural heterogeneity of the group of drugs used and the wide range over which the cyclooxygenase inhibition value varies ($0{\cdot}34 < IC < 10\ 000$), is quite acceptable.

Table 5. Base structure used in the design stage and chemical structures of the compounds selected as theoretical new analgesics.



Compound	R_1	R_2	R_3	X[a]	Log IC[b]	Class[c]
1	Cl	H	$CHOH-CH_2-CH_3$	0·47	1·16	+
2	OH	$CHOH-CH_2-CH=CH_2$	CH_3	0·43	2·30	+
3	Cl	H	$CHNH_2-CH_2-CH_3$	0·45	0·90	+
4	OH	COOH	$COCH_3$	0·37	2·58	+
5	COOH	H	$C(CH_3)=CH_2$	0·47	1·74	+
6	OH	$CH=CH-CH=CH_2$	H	0·26	1·79	+
7	OH	Cl	$CH=CH_2$	0·45	1·57	+
8	Cl	CH_3	$COCH_3$	0·47	0·81	+
9	NH_2	$NHCH_3$	$COCH_3$	0·22	2·13	+

[a]Calculated from equations 2 and 3.
[b]Calculated from equation 1.
[c]Classification from equations 1 and 2.

With the purpose of finding a complementary discriminant function, able to identify the active or inactive analgesic character, an extensive group of compounds was selected (40 with contrasting analgesic activity and 42 with unknown analgesic activity) to which discriminant analysis was applied. The chosen function, obtained from Gálvez et al (1994), was:

$$Y = -1{\cdot}325\ {}^{0}\chi + 4{\cdot}67\ {}^{1}\chi + 1{\cdot}96\ {}^{1}\chi^{v} - 6{\cdot}56\ {}^{2}\chi^{v} - 4{\cdot}25\ {}^{3}\chi_{p} - 4{\cdot}11\ {}^{3}\chi_{c} + 2{\cdot}68\ {}^{3}\chi_{p}{}^{v} + 13{\cdot}31\ {}^{3}\chi_{c}{}^{v} + 1{\cdot}28\ {}^{4}\chi_{p} + 11{\cdot}75\ {}^{4}\chi_{c} + 1{\cdot}22\ {}^{4}\chi_{pc} - 0{\cdot}04 \quad (2)$$

$n = 82$, $F = 9{\cdot}3$, U-statistics (Wilks' λ) $= 0{\cdot}798$.

The method used for descriptors selection was a stepwise linear discriminant analysis (SLDA) from F-Snedecor parameter. The classification criteria used was the minimum value of Mahalanobis. The quality of the discriminant function was evaluated through the Wilks' U-statistical parameter.

If we define

$$X = 0{\cdot}47 - Y^2 \quad (3)$$

the value that X takes for a certain compound will indicate its analgesic character, therefore, if $X > 0$, the compound will have a theoretical analgesic activity and if $X < 0$, the compound will be inactive. Thus, X represents a normalized value of Y. Cross-validation test was applied to X with a group of 29 analgesics and 142 theoretical inactives, not used in the discriminant function (Gálvez et al 1994). The results showed that in the active group the prediction success was 83%, and in the group of inactives 77%.

In this manner, and working with both equations (eqn 1 and eqn 2), a molecule will be selected as active if $X > 0$, and if $3{\cdot}5 > \log IC > 0$.

Table 4 displays the classification results obtained after applying the discriminant functions to a number of bioactive compounds, including those known to possess analgesic activity. In the analgesic group, we get an average measure of correct prediction of the order of 67%, while in the non-analgesic group this increases to approximately 82%.

These results show that the discriminant conditions which are imposed are intended to minimize the percentage of error in design, that is, to give the lowest possible number of false positives, although this may force us to discard a greater number of active compounds. The false negatives do not seem to share any common noticeable feature and its number varies randomly within the sructural group of analgesics that were considered. Thus, aryl propionics show 2 false negatives out of 8, *N*-aryl anthranilics 1 out of 3, pyrazolones and derivatives 3 out of 7, salycilates 3 out of 6, aryl acetics 4 out of 8 and there are no cases of false negatives among the 5 phenols.

Design of analgesic compounds

Once we obtained the ideal conditions of discrimination with which to assign the property of analgesic activity to a particular compound, the next step was to identify new active compounds. To do this, we used a software package of molecular design developed in our research unit. This program built chemical structures and used their properties in the discrimination functions. The designed molecule was selected if it passed the imposed barriers set by the discriminant functions (in our case $X > 0$; $3.5 > \log IC > 0$ indicated analgesic properties).

Using, as a base structure, a benzene with possible substituents at positions 1, 2 and 4, we found several chemicals that theoretically fitted the model. Table 5 shows the values for the discriminant functions, X and log IC, for each of the designed compounds (**1–9**). As it can be seen, they are relatively simple and poorly explored structures, such as phenols, benzoic acids and acetophenones. Finally, of the 9 compounds, it was decided to synthesize only 1-(*p*-chlorophenyl)propanol (**1**), 2-(1-hydroxy-3-butenyl)phenol (**2**) and 1-(*p*-chlorophenyl)propanamine (**3**), due solely to their ease of experimental synthesis.

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