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Synthesis and pharmacological properties of a series of antidopaminergic piperidyl benzamides*

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The synthesis and pharmacological screening for anti-apomorphine, stomach emptying and local anaesthetic activities of some new piperidylbenzamides is described. One of these, *N*-(1′-benzyl-4′-piperidyl)-2-methoxy-4-amino-5-chlorobenzamide (clebopride) is more potent than metoclopramide in tests related to blockade of cerebral dopamine receptors.

Metoclopramide (*N*-diethylaminoethyl-2-methoxy-4-amino-5-chlorobenzamide) is used clinically to prevent post-operative vomiting, to speed gastric emptying before anaesthesia or to facilitate radiological evaluation, and to correct a variety of disturbances of gastrointestinal function (Robinson, 1973a).

In animal tests, in addition to possessing properties directly related to such clinical uses (Reusse, 1973), metoclopramide has also been shown to exhibit a variety of pharmacological actions (induction of catalepsy and antagonism of behavioural changes induced by apomorphine or amphetamine in intact or nigrostriatal lesioned rodents, increase in dopamine turnover as measured by increased concentrations of HVA and DOPAC in dopamine containing areas of the rat brain, etc.) usually associated with blockade of dopamine receptors in both striatal and mesolimbic pathways (Boissier, Simon & others, 1964; Janssen, Niemegeers & others, 1967; Costall & Naylor, 1973; Ahtee & Buncombe, 1974; Dolphin, Jenner & others, 1975; Perringer, Jenner & Marsden, 1975). Although these properties are considered to be characteristic of the classical neuroleptic drugs (Janssen, Niemegeers & Schellekens, 1965; O'Keefe, Sharman & Vogt, 1970) the antipsychotic potential of metoclopramide in the clinic appears to be negligible (Borenstein & Bles, 1965; Nakra, Bond & Lader, 1975) despite the fact that its use has been associated with extrapyramidal side effects such as acute dystonic reactions (Casteels-van Daele, Jaeken & others, 1970; Robinson, 1973b).

In an attempt to increase the antidopaminergic activity of metoclopramide a series of substituted benzamides, in which the amide function was an *N*-substituted piperidine nucleus characteristically present in the potent butyrophenone compounds such as haloperidol, were prepared and subjected to pharmacological evaluation.

The experimental chemistry and pharmacology are given at the end of the paper.

DISCUSSION

Of the three tests used in the initial pharmacological screen:—antagonism of apomorphine induced gnawing behaviour in the rat, stomach emptying in the rat and local anaesthesia in the mouse, only in the anti-apomorphine test can the results be clearly related to blockade of cerebral dopamine receptors. Stomach emptying was included because it represents a unique action of metoclopramide-like compounds and could conceivably be related to antidopaminergic activity if a dopaminergic inhibitory mechanism is proved to be present in the myenteric plexus (Schulz & Goldstein, 1973). The inclusion of a test for local anaesthetic activity has its rationale in the chemical relation of metoclopramide to procainamide and in the possibility of the stomach emptying effect being related to blockade of transmission in inhibitory nerves.

In the first series of compounds tested (Table 1) the introduction of a variety of *N*-substituted piperidyl side chains into the basic 2-methoxy-4-amino-5-chloro benzamide nucleus of metoclopramide resulted in a wide range of activities in the

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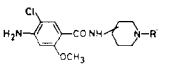
Table 1. N-(*1'-Benzyl-3'-piperidyl and* N-(*1'-benzyl-4'-piperidyl*)-*2-methoxy-4-amino-5-chloro benzamides. All compounds had C, H, N, Cl analyses within the usual limits.*



Compd.[a]	R'	Method	Crude yield %	m.p.[b] °C	Anti-apomorph.[c]	Stomach empyting[d]	Local anaesth.[e]	Toxicity[f]
1	H	A	89	209–11	—	—	—	1
2	CH_3	C	74	245–7	—	—	+	2
3	C_2H_5	C	87	253–5 dec	25	—	+	2
4	$CH_2C_6H_5$	D, F, G	94, 75, 85	217–9	1·56	+++	++	1
5	$CH_2CH_2C_6H_5$	B	90	229–31	—	NT	NT	1
6	$CH(CH_3)C_6H_5$	B	70	248–50	6·25	+	++	2
7	$CH_2CH{=}CHC_6H_5$	B	58	231–3	25	++	++	2
8	CH_2-β-Naphthyl	B	56	220–1	25	++	++	2
9	$CH(C_6H_5)_2$	B	40	235–7	100	++	—	1
10	2-Thienyl	B	84	231–3	25	+++	++	1
11	$CH_2C_6H_5$	B	55	163–5	—	—	++	2

[a] All compounds are 4-piperidyl derivatives except No. 11 which is a 3-piperidyl derivative; [b]hydrochlorides except compounds 1, 5 and 11 (fumarates) and compound 4 (hydrochloride monohydrate); [c]minimum effective oral dose (see text); [d]emptying responses + = 25–50%, ++ = 50–100% and +++ = >100% more than vehicle control; [e]active at + = 1% or ++ = 0·1% (see text); [f]approx. oral LD50 in mice 1 = >1000 mg kg^{-1}, 2 = >300 <1000 mg kg^{-1}. NT = not tested, — = no activity at maximal screening dose.

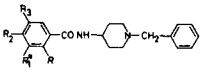
anti-apomorphine test without the appearance of any clear relations between structure and activity.

The position of the substituent in the piperidine ring appears to be important since compound 4 (*N*-benzyl-4-piperidyl) was active at 1·56 mg kg^{-1} while compound 11 (*N*-benzyl-3-piperidyl) was inactive at 100 mg kg^{-1}.

Increasing the size of the aromatic group (compound 8), changing the phenyl function to heterocyclic (compound 10) or the introduction of more carbon atoms (straight or branched chain) between the piperidine nitrogen and the phenyl group (compounds 5, 6, 7 and 9) resulted in a reduction, sometimes substantial, in anti-apomorphine activity. The absence of a substituent on the piperidine ring nitrogen (compound 1) or the introduction of a methyl group (compound 2) resulted in compounds inactive at 100 mg kg^{-1}. Some activity was regained when the *N*-substituent was ethyl (compound 3).

In the second series of compounds (Table 2),

Table 2. *1-Benzyl-4-substituted benzamido, piperidines. All compounds had C, H, N (and where appropriate halogen and S) analyses within the usual limits.*



Compd.	R	R_2	R_3	Method	Crude yield %	m.p. °C[b]	Anti-apomorph.[d]	Stomach emptying[e]	Local anaesth.[f]	Toxicity[g]
12	H	H	H	E	92	235–7[c]	50	NT	++	2
13	H	H	Cl	E	94	206–8	50	+	++	3
14	H	$NHCOCH_3$	H	B	51	287–9	100	+	—	1
15	H	$NHCOCH_2Cl$	H	F	37	270–1 dec	100	—	+	1
16	H	NH_2	H	G	86	250–2	100	+	+	2
17	OCH_3	H	H	E	95	213–4	100	—	+++	3
18	OCH_3	H	Cl	E	86	236–8	100	++	++	2
19	OCH_3	H	SO_2NH_2	F	63	176–8	—	—	—	1
20	OCH_3	H	SO_2CH_3	F	74	206–8 dec	—	—	—	1
21	OCH_3	$NHCOCH_3$	H	B	60	215–7	12·5	++	++	3
22	OCH_3	$NHCOCH_3$	Cl	E	98	210–2 dec	3·12	++	++	3
23	OCH_3	$NHCOCH_2Cl$	H	B	77	245–6	25	+++	+	1
24	OCH_3	$NHCOCH_2Cl$	Cl	E	71	179–80	6·25	+++	++	2
25	OCH_3	$NHCOCF_3$	H	B	83	238–9 dec	25	+	+	1
26	OCH_3	$NHCOCF_3$	Cl	E	98	227–9	1·56	—	++	2
27	OCH_3	$NHSO_2CH_3$	H	H	95	235–7	—	+	—	1
28	OCH_3	$NHCONHCH_3$	H	I	82	239–41 dec	—	+	—	2
29	OCH_3	$NHCOCH_2N(CH_3)_2$	Cl	J	94	241–3	12·5	++	++	2
30	OCH_3	NH_2	H	G, D	94, 93	215–7 dec	12·5	+	+	3
31	OCH_3	NH_2	Br	B	61	219–21	3·12	+++	++	1
32	OCH_3	OCH_3	OCH_3	E	31	226–7	—	—	+	1
33	H	OCH_3	OCH_3	E	88	290–2	—	—	+	2

R_1^4 is always hydrogen except in compound 33 where it is methoxy; [b]hydrochlorides except compound 22 (fumarate), 24 (hydrochloride monohydrate) and compounds 29 and 30 (dihydrochlorides); [c]Lit. m.p. 237° dec. (Archibald, Fairbrother & Jackson, 1974); [d]minimum effective oral dose (see text); [e]emptying responses + = 25–50%, ++ = 50–100% and +++ = >100% more than vehicle control; [f]active at + = 1%, ++ = 0·1% and +++ = 0·01% (see text); [g]approx. oral LD50 in mice 1 = >1000 mg kg^{-1}, 2 = >300<1000 mg kg^{-1} and 3=>100<300 mg kg^{-1}. NT = not tested, — = no activity at maximal screening dose.

where the side chain was consistently *N*-benzyl-4-piperidyl, the optimal substituents on the benzamide moiety were clearly shown to be 2-methoxy-4-amino-5-chloro (compound 4). Nevertheless, substitution of the chlorine in position 5 by bromine (compound 31) or the amino in position 4 by acetamide (compound 22), chloroacetamide (compound 24) or trifluoroacetamide (compound 26) resulted in anti-apomorphine activities equal to or greater than that of metoclopramide.

As a general rule, the other activities included in the screen followed the same general pattern in that high potency against apomorphine was usually associated with increased stomach emptying and local anaesthesia whereas weakly active compounds in the anti-apomorphine test seldom showed much activity in the other two tests. The most notable exceptions to the rule were compound 17 (high local anaesthetic activity, weak anti-apomorphine activity and negligible stomach emptying activity), compound 26 (high anti-apomorphine and local anaesthetic activities but negligible stomach emptying activity) and compound 9 (poor anti-apomorphine activity, good stomach emptying activity but weak local anaesthetic activity). The results are subject to the limitations of the screening process employed in that, in addition to the erratic nature of the responses obtained in the stomach emptying test, there are differences in the scoring systems for the different tests (i.e. anti-apomorphine scored in multiples of 2 using actual doses, stomach emptying scored as the response to a single dose and local anaesthesia scored in multiples of 10 using actual doses).

Table 3. *Antagonism of apomorphine-induced gnawing behaviour in the rat by orally administered metoclopramide and clebopride.*

Compound	Dose mg kg^{-1}	No. animals protected / No. animals in group	% inhib.	ED50 (95% conf. lim.)
Carboxymethyl-cellulose	—	0/20	0	
Metoclopramide hydrochloride	2	0/20	0	
	4	3/20	15	8·69
	8	9/20	45	
	16	17/20	85	(6·21–12·18)
	32	18/20	90	
Clebopride hydrochloride	0·5	2/20	10	
	1	3/20	15	2·40
	2	6/20	30	
	4	14/20	70	(1·74–3·33)
	8	19/20	95	

Apomorphine hydrochloride (2 mg kg^{-1}) administered subcutaneously 1 h after oral administration of the test compounds and 15 min before evaluation of gnawing behaviour.

Table 4. *Antagonism of amphetamine-induced stereotypic behaviour in the mouse by orally administered metoclopramide and clebopride.*

Compound	Dose mg kg^{-1}	Stereotypy score*	% inhib.	ED50 (95% conf. limits)
Carboxymethyl-cellulose		2·85	0	
Metoclopramide hydrochloride	2·5	2·5	24·56	
	5	1·85	35·08	
	10	2·00	29·82	11·40
	20	0·95	66·66	6·08–21·37)
	40	0·70	75·43	
	80	0·00	100	
Carboxymethyl-cellulose		2·75	0	
Clebopride hydrochloride	1·25	1·95	29·09	
	2·5	1·90	30·91	
	5	0·95	65·46	5·17
	10	1·25	54·56	(2·36–11·33)
	20	1·10	60·00	
	40	0·30	89·09	

* Costall, Naylor & Pettit (1974).
(+)-Amphetamine sulphate (20 mg kg^{-1}) administered intraperitoneally 30 min after oral administration of the test compounds and 30 min before evaluation of stereotypic behaviour.

Of the compounds prepared, compound 4 was chosen for more detailed testing because it was the most active in the anti-apomorphine test, was one of the most active in reducing stomach emptying time in rats and was one of the least toxic in acute toxicity in mice. It was therefore compared with metoclopramide in tests considered to be related to the blockade of cerebral dopamine receptors, i.e. antagonism of apomorphine-induced gnawing behaviour in the rat (Table 3), antagonism of amphetamine-induced stereotypic behaviour in the

Table 5. *Induction of catalepsy in the rat by orally administered metoclopramide and clebopride.*

Compound	Dose mg kg^{-1}	No. animals with catalepsy / No. animals in group	% cata-lepsy	ED50 95% conf. limits)
Metoclopramide hydrochloride	6·25	0/15	1·67	
	12·5	2/15	13·33	
	25	2/14	14·29	93·00
	50	5/15	33·33	(50·73–170·51)
	100	6/15	40·00	
	200	12/15	80·00	
Clebopride hydrochloride	6·25	2/15	13·33	
	12·5	2/15	13·33	
	25	1/15	6·67	47·58
	50	6/15	40·00	(28·36–79·81)
	100	12/15	80·00	
	200	15/15	100·00	

Catalepsy was evaluated 1 h after oral administration of test compounds.

mouse (Table 4) and induction of catalepsy in the rat (Table 5); in all these tests compound 4 was the more active.

This compound (rec. INN, Clebopride) was therefore selected for intensive pharmacological and toxicological testing and is now undergoing clinical evaluation in appropriate diseases of both gastrointestinal and central origin.

CHEMICAL METHODS

The compounds in Tables 1 and 2 were prepared by several methods which are illustrated in the following examples. 1-Substituted-4-aminopiperidines and carboxylic acids were prepared by known methods (Harper & Chignell, 1964; Thominet, 1966). 1-benzyl-3-aminopiperidine was prepared from its *N*-acetyl derivative (Moragues, Prieto & others, 1976).

Melting points were recorded on a Mel-Temp capillary apparatus and are uncorrected. The structure of all compounds was confirmed by infrared and nmr spectrometry (Perkin-Elmer 257 and Hitachi-Perkin-Elmer R-24 respectively). Elemental analyses (C,H,N) were determined (Perkin-Elmer 240 Elemental Analyzer) and results were within $\pm 0{\cdot}4\%$ of the theoretical value. Halogens and sulphur were determined by the oxygen flask method, and H_2O determinations by the Karl-Fischer method.

Method A

N-(*4'-piperidyl*)-*2-methoxy-4-amino-5-chlorobenzamide fumarate* (*cpd No. 1*). A suspension of *N*-(1'-benzyl 4'-piperidyl)-2-methoxy-4-amino-5-chlorobenzamide hydrochloride monohydrate (cpd No. 4) (10 g, 0·0233 mol), 10% palladium/charcoal catalyst (1 g) and absolute EtOH (250 ml) was shaken under hydrogen (0·5 atm pressure) for 48 h. The mixture was filtered and the solvent removed from the filtrate. The residue was dissolved in H_2O and the solution made alkaline with NaOH (6 N) and extracted with $CHCl_3$. The organic extracts were washed with H_2O, dried (Na_2SO_4) and evaporated to dryness to give 5·9 g (89% yield) of free base. The fumarate salt, prepared by addition of the stoichiometric amount of fumaric acid in hot EtOH to the base, was recrystallized from EtOH m.p. 209–211°.

Method B

N-(*1'-benzyl-3'-piperidyl*)-*2-methoxy-4-amino-5-chlorobenzamide fumarate* (*cpd No. 11*). To a suspension of 2-methoxy-4-amino-5-chlorobenzoic acid (9·86 g, 0·049 mol) in dry THF (350 ml), a solution of triethylamine (TEA, 4·9 g, 0·049 mol) in dry THF (35 ml) was added. The mixture was gently heated to dissolve the reactants and then cooled to between −10° and −5°. A solution of ethyl chloroformate (5·3 g, 0·049 mol) in dry THF (35 ml) was slowly added, and the mixture was maintained at the same temperature for 0·5 h when a solution of 1-benzyl-3-aminopiperidine (9·3 g, 0·049 mol) in dry THF (50 ml) was added. The mixture was stirred for 1 h at between −10 and −5° and the temperature was allowed to rise to room temperature overnight. The precipitate was filtered, the filtrate was evaporated to dryness and the residue dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with NaOH (4%) (this step should be avoided in the case of compound 25), and with H_2O, dried (Na_2SO_4) and evaporated to dryness to give a paste. After washing with hot light petroleum (b.p. 30–60°) the solid was crystallized from Me_2CO-Et_2O to give 10·0 g of free base (55% yield). Treatment of the base with the stoichiometric amount of fumaric acid in hot EtOH and recrystallization from EtOH gave the pure fumarate m.p. 163–165°.

Method C

N-(*1'-ethyl-4'-piperidyl*)-*2-methoxy-4-amino-5-chlorobenzamide hydrochloride* (*cpd No. 3*). The same procedure as described in Method B was followed using 2-methoxy-4-amino-5-chlorobenzoic acid (7·0 g, 0·035 mol), TEA (3·5 g, 0·035 mol), ethyl chloroformate, 3·8 g, 0·035 mol) 1-ethyl-4-aminopiperidine (4·5 g, 0·035 mol) and dry THF (295 ml). After stirring overnight at room temperature, the precipitate was collected by filtration, washed with Et_2O and dried to give the title compound 10·6 g (87% yield) which was crystallized from MeOH m.p. 253–255° dec.

Method D

N-(*1'-benzyl-4'-piperidyl*)-*2-methoxy-4-amino-5-chlorobenzamide hydrochloride monohydrate* (*cpd No. 4*). A mixture of *N*-(1'-benzyl-4'-piperidyl)-2-methoxy-4-trifluoroacetamido-5-chlorobenzamide hydrochloride (cpd No. 26) (7·0 g, 0·0138 mol), EtOH (21·6 ml), H_2O (13 ml) and NaOH (8 N, 21·6 ml) was stirred at room temperature for 12 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The organic extracts were washed with H_2O, dried (Na_2SO_4) and evaporated to give 4·85 g (94% yield) of free base. The hydrochloride salt was isolated as a monohydrate, m.p. 217–219°.

Method E

N-(*1'-benzyl-4'-piperidyl*)-*3,4,5-trimethoxybenzamide hydrochloride* (*cpd No. 33*). To a cooled (0–5°), stirred solution of 1-benzyl-4-aminopiperidine (9·5 g, 0·05 ml) in dry MEK (75 ml) was added a solution of 3,4,5-trimethoxybenzoyl chloride (12·7 g, 0·55 mol) in dry MEK (75 ml) at such a rate that the temperature was maintained within the above limits. When the addition was complete, the reaction mixture was stirred at the same temperature for 1 h and then for 4 h at room temperature. The solid was filtered, washed with Et_2O and dried to give 18·5 g (88% yield) of cpd No. 33. Crystallization from ethanol gave pure hydrochloride, m.p. 290–292°.

Method F

N-(*1'benzyl-4'-piperidyl*)-*4-α-chloroacetamidobenzamide hydrochloride* (*cpd No. 15*). The reaction was carried out as described in Method B using the following reagents: 4-α-chloroacetamidobenzoic acid (17·5 g, 0·082 mol), TEA (8·3 g, 0·082 mol), ethyl chloroformate (8·9 g, 0·082 mol), 1-benzyl-4-aminopiperidine (15·6 g, 0·082 mol) and dry THF (460 ml). After standing overnight at room temperature, the mixture was concentrated *in vacuo*, poured into H_2O and extracted with $CHCl_3$. The solvent layers were washed successively with NaOH (4%) and H_2O, dried (Na_2SO_4), decolorized and evaporated to dryness to give a solid, which was triturated with Et_2O and filtered to give 11·7 g (37% yield) of free base. The hydrochloride salt crystallized from MeOH, m.p. 270–271° dec.

Method G

N-(*1'-benzyl-4'-piperidyl*)-*2-methoxy-4-aminobenzamide dihydrochloride* (*cpd No. 30*). A mixture of *N*-(1'-benzyl-4'-piperidyl)-2-methoxy-4-acetamidobenzamide (cpd No. 21). (12·5 g, 0·032 ml), conc. HCl (9·4 ml) and H_2O (30 ml) was heated under reflux with stirring for 1·5 h. The solution was cooled, diluted with H_2O, made alkaline with NaOH (6 N) and extracted with $CHCl_3$. The organic extracts were washed with H_2O, dried (Na_2SO_4), decolorized and evaporated to give 10·2 g (94% yield) of the free base as an oil. This was dissolved in Me_2CO and converted into its dihydrochloride by treatment with a dry saturated ethanolic solution of HCl, m.p. 215–217° dec.

Method H

N-(*1'-benzyl-4'-piperidyl*)-*2-methoxy-4-methylsulfonamido-benzamide hydrochloride* (*cpd No. 27*). To a solution of *N*-(1'-benzyl-4'-piperidyl)-2-methoxy-4-aminobenzamide (cpd No. 30 free base) (4·2 g, 0·0124 mol) in dry C_6H_6 (50 ml), a solution of methansulphonyl chloride (1·53 g, 0·134 mol) in dry C_6H_6 (10 ml) was slowly added with stirring at room temperature. After standing for 12 h at room temperature the precipitate was filtered, washed successively with C_6H_6 and Et_2O and dried to give 5·3 g (95% yield) of the title compound, m.p. 235–237° (EtOH).

Method I

N-(*1'-benzyl-4'-piperidyl*)-*2-methoxy-4-*(N'-*methyl-ureido*) *benzamide hydrochloride* (*cpd No. 28*). To a solution of *N*-(1'-benzyl-4'-piperidyl)-2-methoxy-4-aminobenzamide (cpd No. 30 free base) (5·8 g, 0·017 mol) in Me_2CO–Et_2O (1:1, 20 ml), a solution of methylisocyanate (2·56 ml) in dry Et_2O (10 ml) was added at room temperature with stirring. After standing at room temperature for 12 h, the white precipitate was filtered, washed with Et_2O and dried to give 5·5 g of free base (82% yield). The hydrochloride salt was crystallized from MeOH, m.p. 239–241° dec.

Method J

N-(*1'-benzyl-4'-piperidyl*)-*2-methoxy-4-*[α-(*1''-piperidyl*)-*acetamido*]-*5-chlorobenzamide dihydrochloride* (*cpd No. 29*). A mixture of *N*-(1'-benzyl-4'-piperidyl)-2-methoxy-4-α-chloroacetamido-5-chlorobenzamide (cpd No. 24 free base) (11 g, 0·0244 mol) and piperidine (5·07 ml, 0·0512 mol) in dry C_6H_6 (50 ml) was refluxed for 12 h, cooled and the precipitate filtered and washed with Et_2O. The solvent was removed to give a paste which was triturated with a mixture of Et_2O-light petroleum (b.p. 30–60°) and filtered to give 11·4 g (94% yield) of the free base. The solid was dissolved in Me_2CO and after addition of a dry saturated ethanolic HCl solution, the dihydrochloride was isolated and crystallized from EtOH, m.p. 241–243°.

PHARMACOLOGICAL METHODS

Methods

Acute toxicity. Male Swiss mice, 20 ± 2 g, were used, 3 animals per dose level. Approximate LD50 values were determined from deaths occurring within 72 h of oral administration of the test compounds.

Apomorphine-induced gnawing behaviour in the rat. Albino Wistar rats of either sex (150 ± 30 g) were used and the test was essentially that described by Janssen, Niemegeers & Jageneau (1960). Animals were injected subcutaneously with apomorphine hydrochloride (2 mg kg^{-1}), dissolved in distilled water containing a few crystals of sodium bisulphite as antioxidant, 1 h after the oral administration of the test compounds suspended in 2·5% w/v carboxymethylcellulose. Fifteen min later the absence or presence of gnawing behaviour was noted.

(a) *Screening test.* All compounds were tested in groups of 2 rats at progressively lower (halved) doses (starting at 100 mg kg^{-1}) until protective activity was clearly lost.

(b) *Potency.* The potency of the test compounds was compared with that of metoclopramide at 5 dose levels using randomized groups of 20 rats.

Stomach emptying in the rat. Albino Wistar rats of either sex (100 ± 30 g) were used following starvation overnight and the method followed was similar to that described by Jacoby & Brodie (1967). Test compounds (suspended in 2·5% w/v carboxymethylcellulose) were administered orally 30 min before the placement of 40 enteric coated glass pellets (1·75 ± 0·15 mm diameter) into each stomach by means of a polythene tube attached to a syringe. Fifteen min later the animals were killed by a blow on the head and the stomach rapidly removed between two ligatures at the cardia and the pylorus. The number of pellets remaining in the stomach were counted and subtracted from 40 to obtain the number that had passed into the duodenum.

All compounds were tested in groups of 10 rats in parallel with metoclopramide and vehicle controls at a single dose of 3 mg kg^{-1} orally. Results were calculated

as a percentage increase in emptying over the vehicle control and any experiment in which the metoclopramide control failed to give at least a 25% increase over the vehicle was repeated.

Local anaesthetic activity in the mouse. Swiss albino mice (22 ± 2 g) of either sex were used in a modified version of the test described by Truant (1958) for rats using conduction block in the sciatic nerve.

Test compounds (1% w/v in normal saline 0·1 ml) were injected intramuscularly into the thigh close to the sciatic nerve in randomized groups of 10 mice and a similar injection of normal saline was made in the other hind limb. Five min later each animal was evaluated for presence of anaesthesia (dragging of the injected hind foot). Compounds showing effect in 3 or more of the 10 mice were retested at 0·1% w/v and yet again at 0·01% w/v if 3 or more animals still showed signs of nerve block.

Amphetamine-induced stereotyped behaviour in the mouse. Albino Swiss mice (22 ± 3 g) of either sex were used randomly distributed into groups of 10. Test compounds (suspended in 2·5% carboxymethylcellulose) were administered orally 30 min before the injection of amphetamine sulphate (10 mg kg^{-1}, i.p.) and after a further 30 min each animals was observed for the presence of stereotypic behaviour which was scored according to the system described by Costall and others (1974).

Induction of catatonia in the rat. Wistar rats of either sex (120 ± 30 g) were used randomly distributed into groups of 15. Test compounds (suspended in 2·5% carboxymethylcellulose) were administered orally 60 min before placing the front paws of the animals on a Perspex bar 5 cm above floor level. Catatonia was considered to be present in those animals which remained motionless in this abnormal position for 2 min.

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