Synthesis and Pharmacological Properties of Phenyl-Substituted Biurets

J. D. McColl, F. L. Chubb, C. F. Lee, A. Hajdu, and J. Komlossy

Research Laboratories, Frank W. Horner Limited, Montreal, Canada

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A series of phenyl-substituted biurets was synthesized and tested for their effect on gastric secretion and the inhibition of ulcer formation in the pyloric ligated rat. Phenethyl and phenpropylbiuret proved to be the most potent. Phenethylbiuret did not possess any anticholinergic or antispasmodic activity. It was shown to inhibit both polysynaptic and monosynaptic pathways, to inhibit conditioned avoidance response in the rat, and to potentiate hexobarbital and ether-induced loss of righting reflex. Compared to other central nervous system agents, phenethylbiuret was relatively weak as a central depressant.

Although the synthesis of some phenyl-substituted biurets has been described,[1,2] few pharmacological properties other than weak anticonvulsive activity have been reported. The observation of oral hypoglycemic properties of phenformin[3] led Boggiano, *et al.*,[2] and ourselves to synthesize and test phenethylbiuret for such activity. While devoid of hypoglycemic action, other investigations[4] on the pharmacological properties of hypoglycemic agents suggested the study of this compound, and some congeners, on gastric secretion and ulcer formation in the pyloric ligated rat. In addition, the anticonvulsant profile of these compounds was investigated.

Experimental

Chemistry.—The 1-substituted biurets (Table I) were prepared by the reaction of nitrobiuret with the appropriate primary or secondary amine according to the procedure of Davis and Blanchard,[5] modified by Dunnigan and Close.[1] The primary amines, when not available commercially, were prepared by low pressure hydrogenation of the corresponding nitriles over Raney nickel. 3-Phenylpropionitrile and 4-phenylbutyronitrile were hydrogenated according to the procedure of Skinner, *et al.*,[6] and *p*-tolylacetonitrile and *p*-methoxyphenylacetonitrile were hydrogenated according to the procedure of Dominguez, *et al.*[7]

N-Methylphenethylamine.—The procedure of Barger and Ewins[8] was modified as follows. Phenethyl chloride (14 g.) and 15.5 g. of methylamine in 60 ml. of methanol were heated at 100–110° in a pressure reaction apparatus for 4 hr. The solvent was distilled and the residue, after treatment with sodium hydroxide, was extracted with ether. The ether layer was dried over sodium sulfate. Distillation of the residue yielded 9.5 g. (70%) of product, b.p. 92–94° (15 mm.).

Pharmacological Activity.—Acute toxicity of these compounds was studied in CFW mice by the intraperitoneal route, and the LD_{50} was determined by the method of Litchfield and Wilcoxon.[9]

Action of the compounds on gastric secretion and incidence of ulcer formation was investigated using the 8 hr. pyloric ligated rat.[10] The details of the procedure employed have been described.[4] Test compounds were administered subcutaneously in two equally divided doses, one immediately following ligation and the second 4 hr. later. In one experiment, phenethylbiuret (II) was administered intraduodenally below the ligation at time of operation in a single dose.

The ability of the compounds to modify the seizure patterns produced by injection of nicotine, pentylenetetrazole, picrotoxin, and strychnine was tested in the CFW mouse. A given dose was administered i.p. and the CD_{50} (mg./kg.) for each convulsant agent was determined 30 min. later.

Phenethylbiuret was chosen for further examination of its pharmacological properties. The following tests were employed: antichromodacryorrhea,[11] intestinal motility,[12] potentiation of loss of righting reflex produced by hexobarbital and ether, conditioned avoidance response,[13] effect on monosynaptic reflex (patellar) and polysynaptic reflex (linguomandibular), and histamine-induced gastric secretion.[14]

Results

Injection of the compounds produced ataxia, depression followed by loss of righting reflex, paralysis, and at higher doses vasodilatation. Some congeners (*e.g.*, III, Table II) produced tonic extensor seizures at lethal dose levels. Death appeared to be due to respiratory collapse.

The LD_{50} values are summarized in Table II. In general, increasing the length of the alkyl substituents resulted in a decrease in toxicity. Benzylbiuret was the exception and was least toxic. Methyl and methoxyl substituents in the *para* position of the benzene ring also resulted in a decrease in toxicity.

Table II summarizes the effect of graded doses of the compounds on gastric secretion and on the incidence of ulcer formation in pyloric ligated rat. Included are some standard compounds as reference agents.

Depending on the dose, all derivatives decreased gastric secretion to some degree. Benzylbiuret (I) was least potent and phenethylbiuret (II) was most potent, within the dose ranges investigated. Certain compounds (VII, III) were more effective at lower dose levels than at higher. This apparent stimulation of gastric function at higher doses may be evidence of systemic toxicity. As far as decrease in gastric volume was concerned, the ethyl or propyl congeners were the most effective.

Free and total hydrochloric acid concentration was diminished by those compounds containing the shorter alkyl chains. Those with the longer chains, or those

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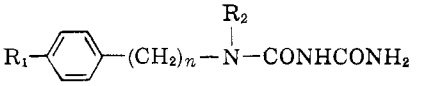
TABLE I

SUBSTITUTED BIURETS



Number	Substitutions R_1	Substitutions n	Substitutions R_2	Yield, %	M.p., °C.	Analyses Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
I	H	1	H	80	175–176						
II	H	2	H	58	140–141[a]	58.0	6.3	20.3	58.20	6.68	20.89
III	H	3	H	55	141–142	59.71	6.83	18.99	59.15	7.02	19.16
IV	H	4	H	66	135–136	61.26	7.28	17.86	61.12	6.96	18.18
V	H	2	CH_3	70	147–148	59.71	6.83	18.99	59.83	6.91	18.56
VI	CH_3	2	H	67	174–175	59.71	6.83	18.99	60.11	6.83	19.18
VII	CH_3O	2	H	62	159–160	55.63	6.33	17.71	55.55	6.56	17.71

[a] After completion of this work Boggiano, *et al.*,[2] reported m.p. 140–141°.

TABLE II

EFFECT ON GASTRIC SECRETION AND ULCER FORMATION IN THE RAT

Number	Mouse LD_{50}, i.p., mg./kg. (95% confidence limits)	Dose, mg./kg., and route	Gastric vol., ml./100 g. B.W. ± S.E.M.	pH	Acid concentration, ml. 0.01 *N* NaOH ± S.E.M. Free	Acid concentration, ml. 0.01 *N* NaOH ± S.E.M. Total	Macroscopic ulcer rating	Ulcer index
Control	..	..	4.2 ± 0.2	1.5	50 ± 1.8	105 ± 2.1	9.0 ± 0.6	37.2 ± 2.8
I	1300	200 s.c.	4.3 ± 0.7	2.0	22 ± 17	75 ± 18	8.0	44.0
	(1140–1480)	300 s.c.	2.6 ± 0.6	1.6	44 ± 21	118 ± 7	0	3.5
		400 s.c.	3.9 ± 1.5	2.9	7 ± 14	40 ± 22	1.0	10.0
II	570	25 s.c.	3.8 ± 0.3	1.3	54 ± 9	100 ± 10	8.0	30.0
	(515–635)	50 s.c.	3.3 ± 0.5	1.3	53 ± 9	111 ± 10	6.0	22.5
		62.5 s.c.	3.5 ± 0.6	4.2	40 ± 7	108 ± 9	9.5	18.5
		125 s.c.	2.2 ± 0.8	..	27 ± 11	80 ± 12	3.5	14.0
		250 s.c.	0.8 ± 0.1	5.5	0	45 ± 9	1.5	10.0
		400 s.c.	1.2 ± 0.3	3.4	0	26 ± 9	0	5.5
		500 s.c.	0.9 ± 0.1	4.9	2 ± 0.6	70 ± 10	0	3.0
		188 i.d.	1.0 ± 0.3	1.5	60 ± 9	108 ± 14	1.0	13.5
		375 i.d.	0.1 ± 0.04	4.1	0	46[a]	1.5	15.5
III	690	25 s.c.	4.3 ± 0.5	1.2	70 ± 10	114 ± 8	8.0	37.0
	(627–759)	50 s.c.	3.8 ± 0.9	1.7	26 ± 7	77 ± 7	6.0	37.0
		125 s.c.	3.6 ± 0.6	1.3	52 ± 8	106 ± 7	3.5	13.5
		250 s.c.	1.6 ± 0.5	3.2	0	52 ± 10	0	1.5
		500 s.c.	1.9 ± 0.5	1.6	19 ± 11	110 ± 13	2.5	14.5
IV	780	50 s.c.	4.9 ± 0.6	1.6	52 ± 7	100 ± 6	5.0	38.0
	(685–890)	125 s.c.	3.1 ± 0.8	1.7	46 ± 10	100 ± 8	3.5	29.5
		250 s.c.	2.0 ± 0.9	1.9	37 ± 7	92 ± 7	1.0	10.0
V	800	200 s.c.	2.8 ± 0.5	1.6	45 ± 9	100 ± 6	6.0	20.5
	(725–880)	400 s.c.	0.7 ± 0.2	3.1	13 ± 11	120 ± 14	0	9.0
VI	>2000	200 s.c.	2.6 ± 0.7	2.0	36 ± 6	104 ± 9	4.0	10.5
		400 s.c.	1.6 ± 0.2	1.6	32 ± 11	116 ± 21	4.0	15.0
VII	1600	100 s.c.	1.2 ± 0.8	3.1	5 ± 2	81 ± 6	2.5	15.0
	(1430–1795)	200 s.c.	1.9 ± 0.8	2.2	20 ± 7	70 ± 12	1.5	14.0
		400 s.c.	3.4 ± 0.7	1.4	47 ± 13	110 ± 8	5.0	28.5
Atropine	198	25 s.c.	0.5 ± 0.04	4.5	0	20[a]	1.0	4.5
	(190–203)	50 s.c.	0.6 ± 0.05	6.5	0	17[a]	1.5	5.5
Phenobarbital	250	50 s.c.	5.4 ± 0.5	1.2	71 ± 10.9	126 ± 13.2	7.5	37.5
	(236–265)	100 s.c.	0.7 ± 0.1	5.6	0	76 ± 9.8	3.5	14.5
Meprobamate	660	200 s.c.	3.2 ± 0.5	1.3	54 ± 3.1	122 ± 8.2	7.5	30.0
	(600–726)	400 s.c.	0.6 ± 0.9	2.6	0	83 ± 6.7	3.0	7.0
Phenformin	100 LD min.[3]	100 s.c.	2.5 ± 0.4	2.9	18 ± 6	79 ± 5	2.8	15.8

[a] Pooled sample, no S.E.M. calcd.

substituted on the *para* position of the benzene ring, were less effective.

All derivatives decreased incidence of ulcer formation as assessed by either macroscopic or microscopic procedures. As previously observed,[4] it was not possible to correlate the inhibition of ulcer formation, gastric volume, and acid concentration. Phenethylbiuret (II) gave maximum protection against ulcer formation over the dose levels tested, and was most effective when administered by the intraduodenal route. Phenyl-

Table III

Action of Compounds Against Clonic Convulsions in the Mouse

Number	Dose, mg./kg.	Strychnine CD_{50}, mg./kg.	Nicotine CD_{50}, mg./kg.	Pentylene tetrazole CD_{50}, mg./kg.	Picrotoxin CD_{50}, mg./kg.
Control	..	1.37 (1.29–1.46)[a]	2.05 (1.43–2.81)	40.0 (36.4–44.0)	4.15 (3.79–4.54)
I	200	No effect (reduced severity)	No effect	No effect (reduced severity)	No effect (reduced severity)
II	200	No effect (reduced severity)	4.0 (3.28–4.87)	60.5 (56.5–64.9)	No effect (reduced severity)
III	200	1.58 (1.49–1.68)	No effect	13.5 (7.3–25.0)	3.35 (2.88–3.88)
IV	200	No effect (reduced severity)	No effect	14.0 (7.8–25.2)	3.20 (2.78–3.71)
V	200	No effect (reduced severity)	No effect	60.0 (55.0–65.5)	No effect
VI	200	No effect	No effect	No effect	No effect
VII	200	No effect	No effect	No effect	No effect

[a] 95% confidence limits.

Table IV

Effect of Phenethylbiuret on Various Tests

Procedure	Dose, mg./kg., and route	Species	Observations
Antichromodacryorrhea[11]	256–512 i.p.	Rat	No protection (15 min. pretreatment, 60 min. observation, 512 mg./kg. dose in lethal range)
Intestinal motility[12]	200–400 s.c.	Rat	No inhibition (1 hr. pretreatment)
Potentiation of loss of righting reflex			
(a) Hexobarbital	100 i.p.	Mouse	Potentiation (17.5 ± 1.8 min. Control *vs.* 23.9 ± 2.3 treated)
(b) Ether	100 i.p.	Rat	Potentiation (2.2 ± 1.5 min. Control *vs.* 22.1 ± 3.4 treated)
Conditioned avoidance[13]	30–150 i.p.	Rat	Antagonism (24% at 100 mg./kg. $P = 0.05$) neurological deficit at 150 mg./kg.
Histamine induced[14] gastric secretion	100 i.v.	Cat	Antagonism (50% decrease three of four cats 100 min. after injection; no effect on free or total HCl)

propylbiuret (III) and phenylbutylbiuret (IV) were effective at the higher doses, but were not as potent as phenethylbiuret at the lower levels.

Action of substituted birets against clonic seizures produced by four chemical convulsants is summarized in Table III. Of the series only phenethylbiuret (II) showed significant antagonism against nicotine. Against strychnine convulsions, while the severity was decreased by most derivatives, only phenylpropylbiuret (III) showed any significant antagonism. Pentylenetetrazole convulsions were antagonized by phenethylbiuret, while compounds I, VI, and VII were inactive. The remainder facilitated pentylenetetrazole-induced convulsions. Similarly with picrotoxin, facilitation was observed with three analogs (III, IV, VII) while the remainder reduced severity but did not modify the ED_{50} values.

Since phenethylbiuret was the most active against ulcer formation and as an anticonvulsant, it was investigated for ancillary pharmacological properties (Table IV). In the lightly anesthetized cat, in which gastric secretion was stimulated by continuous infusion of histamine (0.1 γ/kg./min.), 100 mg./kg. intravenously of the compound produced a 50% decrease in three out of four cats. There was no effect on the free or total hydrochloric acid concentration of gastric secretion.

The insoluble nature of phenethylbiuret precluded the use of many *in vivo* and *in vitro* procedures. By the antichromodacryorrhea test in the rat, doses of 260–512 mg./kg. i.p. failed to demonstrate any anticholinergic activity of the compound. Intravenous injection of a 1% suspension of phenethylbiuret (100 mg./kg.) in the anesthetized dog produced a slight transient depression of blood pressure and did not modify the action of injected methacholine. Similarly, intestinal motility measured by the passage of charcoal along the intestinal tract (charcoal meal test) was not influenced by subcutaneous doses of 200–400 mg./kg.

Phenethylbiuret demonstrated some effect on the central nervous system. Conditioned avoidance response in the rat was significantly inhibited at a dose of 100 mg./kg., but higher doses produced a neurological deficit. The compound inhibited both polysynaptic reflexes and monosynaptic reflexes. ED_{50} values were determined to be 41 (29–56) mg./kg. and

35 (23–52) mg./kg., respectively. Subsequent doses depressed the polysynaptic pathway to a greater degree than the monosynaptic reflex. The duration of the effect was from 40 to 60 min.

Potentiation of the loss of righting reflex induced by hexobarbital (100 mg./kg.) was observed in the mouse while ether-induced loss of righting reflex was much more influenced in the rat. When administered to mice just recovering from hexobarbital hypnosis[15] recurrence of the loss of righting reflex resulted in 100% of the animals tested.

Discussion

The exact mode of action of these substituted biurets on inhibition of gastric secretion and incidence of ulcer formation is not clear. Various classes of compounds will inhibit these gastric parameters in the ligated rat (*i.e.*, anticholinergics, antispasmodics, carbonic anhydrase inhibitors, CNS depressants). While no data are available on possible inhibition of carbonic anhydrase, no evidence of diuresis (qualitative observed with acetazolamide) was noted at near-lethal doses. Phenethylbiuret did not demonstrate properties of anticholinergic or antispasmodic agents as tested by the antichromodacryorrhea and charcoal meal test in animals. It possesses some action at the level of the central nervous system as evidenced by anticonvulsant activity, inhibition of poly- and monosynaptic reflexes, and conditioned avoidance response. The potentiation and recurrence of hexobarbital-induced loss of righting reflex is further evidence of a central effect, according to the interpretation of Brodie, *et al.*[15] However, by comparison with other central nervous system agents, such as phenobarbital and meprobamate, these effects are relatively weak. Doses of phenobarbital and meprobamate required to produce comparable protection against ulcer formation produced signs of neurological depression not observed with phenethylbiuret.

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Bacteriostats. VI.[1a] Bacteriostatic Activities of Some Substituted Guanidines[1b]

A. F. McKAY, D. L. GARMAISE, H. A. BAKER, L. R. HAWKINS, V. FALTA, R. GAUDRY, AND G. Y. PARIS

Monsanto Canada Limited, Montreal, Quebec, and Ayerst, McKenna and Harrison Limited, Montreal, Quebec

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A number of guanidine derivatives were prepared for screening for antibacterial activity. Some of these derivatives were quite active against both Gram-positive and Gram-negative bacteria. The bacteriostatic activity of 1,10-di(3,4-dichlorobenzylguanidino)decane dihydrochloride was comparable with Hibitane.

The performance of 1,6-di(4-chlorophenyldiguanido)-hexane (Hibitane)[2] as a bacteriostat prompted the synthesis of a number of guanidines for screening as antibacterial agents. Although it was reported[2] that 1,6-di(4-chlorophenylguanidino)hexane possessed $^1/_{30}$ the antibacterial activity of Hibitane, the benzylguanidine derivatives were expected[3] to exhibit higher bacteriostatic activities than the corresponding phenylguanidines.

The guanidine derivatives generally were prepared by condensing amines with the corresponding S-methylisothiuronium iodides. New thioureas and isothiuronium salts prepared during this investigation are listed in Tables I and II, respectivly. Tables III to VII, inclusive, describe the properties of the substituted guanidines.

TABLE I

THIOUREAS, $(RNH)_2CS$

R	M.p., °C.	Yield, %	Empirical formula	Analyses, % C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found	S Calcd.	S Found
4-Methylbenzyl	135–136[a]	100	$C_{17}H_{20}N_2S$	71.80	72.02	7.09	7.33	9.85	9.81	11.28	11.26
3,4-Dimethylbenzyl	98–99[b]	99	$C_{19}H_{24}N_2S$	73.03	72.87	7.74	7.66	8.97	8.78	10.26	10.36
3,4-Dichlorophenethyl	124–125[c]	82	$C_{17}H_{16}Cl_4N_2S$[d]	48.36	48.54	3.82	3.96	6.64	6.82	7.59	7.58
α-Naphthylmethyl	168–169[e]	70	$C_{23}H_{20}N_2S$	77.48	77.34	5.65	5.62	7.86	7.35	8.99	8.60
3,4-Dimethoxyphenethyl	135–136[a]	93	$C_{21}H_{28}N_2O_4S$	62.35	62.20	6.98	6.90	6.93	7.22	7.93	7.98
2,5-Dichlorobenzyl	169–170[f]	76	$C_{15}H_{12}Cl_4N_2S$	45.71	46.08	3.07	3.13	7.11	7.39	8.13	8.05
Furfuryl	83–85[b]	88	$C_{11}H_{12}N_2O_2S$	55.90	55.51	5.12	5.33	11.86	11.60	13.57	13.35

[a] Crystallized from ethanol. [b] From aqueous ethanol. [c] From ether–hexane. [d] Calcd.: Cl, 33.86. Found: Cl, 33.60. [e] Crystallized from aqueous dimethylformamide. [f] From acetone. [g] Calcd.: Cl, 35.98. Found: Cl, 36.06.

In the disubstituted guanidine series, *n*-nonyl, *n*-decyl, *n*-undecyl, and *n*-dodecyl derivatives of 4-chlorobenzyl- and 3,4-dichlorobenzylguanidines displayed the highest bacteriostatic activities while in the benzylguanidine series, the undecyl and dodecyl derivatives were the most active (see Table VIII). N-Nonyl-N′-3,4-dichlorobenzylguanidine hydrochloride exhibited

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