g, 74.6%), mp 78–80.5 °C. Anal. ($C_{23}H_{28}FN_3O_4$) C, H, N.

2-[4-(2-Aminoanilino)piperidin-1-ylpropyl]-2-(4-fluorophenyl)-1,3-dioxolane (3). A mixture of **2** (4 g, 9.3 mmol) and Raney nickel (4 mL) in MeOH (100 mL) was submitted to catalytic hydrogenation at ordinary temperature and pressure. After the theoretical amount of H_2 was absorbed, the catalyst and the solvent were removed. The residue was crystallized from Et_2O to give needles of **3** (3.18 g, 85.4%), mp 111–113 °C. Anal. ($C_{23}H_{30}FN_3O_2$) C, H, N.

Pharmacological Methods. Use was made of male mice of the STD-ddY strain weighing 20–28 g and male albino rats of the Wistar strain weighing 110–220 g. Test compounds were suspended in 0.5% CMC and given orally in a volume of 10 mL/kg to mice and 2 mL/kg to rats. ED_{50} values with 95% fiducial limits were calculated according to the Litchfield–Wilcoxon method.[4]

(a) **Spontaneous Motor Activity (SMA) Test.** Ten mice were used in each group. One hour after the administration of test compounds, the mouse was placed in the wheel cage, 210 mm in diameter and 40 mm in width (Kishimoto IKa Co., Kyoto). The number of revolutions of the cage which the mouse rotated for 5 min was taken as an index for spontaneous motor activity. ED_{50}, the dose which decreased the number of revolutions by 50%, was determined.

(b) **Antagonism to Methamphetamine Group Toxicity (MGT).** The experiment was carried out by a modification of method of Sharma et al.[5] Twenty mice were used in each group. The animals were kept aggregated in a metallic cage (22 × 32 × 10.5 cm). Each group was treated with test compounds 1 h before subcutaneous injection of 25 mg/kg of methamphetamine hydrochloride. ED_{50}, the dose required to protect 50% of the animals from death due to methamphetamine, was determined from mortality of the animals at 24 h after methamphetamine injection.

(c) **Antagonism to Methamphetamine-Induced Stereotyped Behavior.** Rats were separately kept in individual cages (26 × 42.5 × 15 cm) made of plastic. Each group of five rats was administered test compounds 2 h before the treatment with methamphetamine hydrochloride (10 mg/kg ip). Stereotyped behavior induced by methamphetamine hydrochloride was scored 1, 2, and 3 h after methamphetamine injection as 0 (sleeping), 1 (squating), 2 (looking about), 3 (preening and grooming), 4 (ambulating), 5 (rearing), 6 (sniffing), 7 (neck shaking), 8 (licking, biting, and gnawing), 9 (body shaking, walking back, and rotating), and 10 (hard ataxia and death). ED_{50}, the dose which reduced the total scores by 50%, was determined.

(d) **Antagonism to Apomorphine-Induced Stereotyped Behavior.** The experiment was carried out by a modification of the method of Janssen et al.[2] Rats were kept in the same manner as described in the preceding item (c). Each group of six rats was administered test compounds 2 h before the treatment with apomorphine hydrochloride (1.25 mg/kg iv). The effect against apomorphine was taken as positive when, 20 min after apomorphine injection, gnawing behavior was not observed during an observation period of 1 min. ED_{50}, the dose which produced a positive effect in 50% of the animals, was determined.

(e) **Catalepsy Test.** The test was carried out according to the method of Costall et al.[6] Six rats were used in each group. The animals were subjected to the catalepsy test 8 h after medication by placing both front limbs on a horizontal bar set up at a height of 12 cm from the floor. When the animals showed catalepsy for more than 1 min, the cataleptic syndrome was regarded as positive. ED_{50}, the dose which produced a positive effect in 50% of the animals, was determined.

(f) LD_{50}. Ten mice were used for each dose level. LD_{50} values were determined from 7-day mortality.

Acknowledgment. We are indebted to Dr. G. Ohta, director of this institute, for his support and encouragement and Dr. R. Dohmori for his valuable advice. Thanks are also due to the members of the analytical section of this institute for the elemental analyses and the mass spectra.

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Benzopyrones.[1] 14. Synthesis and Antiallergic Properties of Some *N*-Tetrazolylcarboxamides and Related Compounds

G. P. Ellis,* G. J. P. Becket, D. Shaw, H. K. Wilson,

Department of Chemistry, University of Wales Institute of Science and Technology, Cardiff CF1 3NU, United Kingdom

C. J. Vardey, and I. F. Skidmore*

Biochemistry Department, Allen and Hanburys Research Limited, Ware, Herts SG12 ODJ, United Kingdom.
Received April 4, 1978

A series of chromones containing an acidic group has been synthesized and screened for the ability to inhibit passive cutaneous anaphylaxis and the release of histamine from mast cells of the rat. Many of the chromones contain the *N*-(5-tetrazolyl)carboxamido group, a novel source of acidity. Others contain a carboxyl, *C*-(5-tetrazolyl), 5-(4*H*)-oxotetrazolinyl, or *N*-(5-tetrazolyl)sulfonamido function. The compounds were compared with cromolyn sodium (sodium cromoglycate) and many were found to be powerful inhibitors of anaphylaxis. The most potent was 7-methoxy-4-oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide (**15**). Structure–activity relationships among the chromones and also some related compounds are discussed.

Many derivatives of chromones have been shown to inhibit the release of spasmogens which usually follows an antigen–antibody interaction.[2–5] Although the mechanism of action of these compounds is not fully understood, the presence of an acidic group at C-2 or C-3 is characteristic of most of the active chromones. The majority of these contain a carboxyl group,[2,5,6] but its replacement by a 5-tetrazolyl ring has been shown to result in some com-

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pounds with increased pharmacological activity.[4] Such tetrazolylchromones have acid strengths comparable with those of the carboxylic acids, although the pK_a values of only a few tetrazolylchromones have been determined.[4,7]

We wish to describe the synthesis and pharmacological study of a number of chromones containing a tetrazole ring in which the two-ring systems are separated by a carbamoyl group, -CONH-. A number of related compounds are also included. The characteristic of most of these compounds is the *N*-(5-tetrazolyl)carboxamide group, as in the simplest member of the series, 4-oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide (I, R = H). These

I

and similar compounds are assumed to be 5(1*H*)-tetrazolyl derivatives unless otherwise stated. Among the variations that were made on this structure were replacement of the tetrazolyl ring by other cyclic and acyclic substituents, attachment of the *N*-(5-tetrazolyl)carboxamide at other positions of the chromone and to different heterocycles, and replacement of the amide carbonyl by a sulfonyl group. Some of these compounds were potent inhibitors of the release of spasmogens.

Chemistry. *N*-(5-Tetrazolyl)carboxamides such as I were synthesized by one of three methods: condensation of 5-aminotetrazole with (a) a carboxylic acid chloride, (b) a carboxylic acid in the presence of *N*,*N'*-dicyclohexylcarbodiimide, or (c) a carboxylic acid in the presence of *N*,*N'*-carbonyldiimidazole. Most of the tetrazolylcarboxamides were very high melting solids which were almost insoluble in common solvents; DMF alone or with another solvent was often employed for recrystallization. These and other chromonecarboxamides substituted on nitrogen by various groups were similarly prepared and are listed in Table I. Chromones used as intermediates, as well as some containing a tetrazolyl group at the end of a side chain, are listed in Table II. Table III contains various derivatives of chromone, isocoumarin, and acridine.

Pharmacology. The pathophysiological basis of the immediate hypersensitivity which characterizes allergic asthma, rhinitis, and urticaria is the anaphylactic reaction.[8a] Compounds which inhibit anaphylaxis are thus potentially useful for these conditions. Compounds were tested against two anaphylactic reactions: passive cutaneous anaphylaxis (PCA) in the rat and anaphylactic release of histamine from a mixed population of passively sensitized peritoneal and pleural cells from the rat and from fragments of the human lung (Tables IV and V).

Results and Discussion

The ability of chromone-2-carboxylic acids and 2-(5-tetrazolyl)chromones to inhibit passive cutaneous anaphylaxis is well-known.[2,4-7] The corresponding primary carboxamides possess little activity and some of these are too insoluble to be tested. Compounds **4–6** contain a carboxyl group at the end of a chain attached to C-2 but show negligible activity. A source of acidity nearer to the ring therefore appears desirable. A new type of group which provides this is the *N*-(5-tetrazolyl)carboxamido function. Attachment of this at C-2 gave compound **10**, which is a potent inhibitor of the PCA reaction and of anaphylaxis in rat mast cells. Ring-substituted derivatives (I) of this compound were synthesized and show a wide variation in activity (Table I, compounds **11–28**). In the

Chart I

62, R = OH

63, R = NH-tetrazolyl

64, R =

65, R =

66, R =

67, R =

68, R =

69, R =

70

71

mast cell test, the 7-methoxy (**15**) and the 6-methyl (**11**) derivatives were more potent than the parent compound (**10**), but the PCA test showed that **18**, **22**, **23**, **25**, and **26** were also very potent. In the chromone-2-carboxylic acids, the 2-tetrazolylchromones, and the present series, substitution by chlorine or methyl at C-3 lowered activity,[4,7] but a basic side chain at C-3 gave compounds **22**, **23**, and **25**, which retained high activity in the PCA test although not in the mast cell screen.

Replacement of the tetrazole ring by thiazole (**29**), 1,2,4-triazole (**30**), or substituted piperazines (**35** and **36**) resulted in almost complete loss of activity. The *N*-tetrazolylcarboxamido group contains two hydrogens, that on the ring nitrogen probably being the more acidic. The importance of these two hydrogens in conferring antiallergic activity on the compound was investigated by replacing each, in turn, by a methyl group. The resulting compounds **42** and **43**, as well as the isomeric *N*-(2-methyl-5-tetrazolyl)carboxamide (**44**), are almost devoid of activity.

When the tetrazolylcarboxamide group was attached at C-3 or C-6 of the chromone system, compounds **38** and **40** have low activity, but inclusion of an acidic tetrazolyl group at C-2 in the latter gives a very active compound, **41**, which is considerably better than the isomer **19**, in which the substituents were interchanged. Chromones containing a sulfonamide group at C-2 or C-3 are unknown, but a 6-tetrazolylsulfonamide **61** is almost inactive as expected by comparison with the corresponding carboxamide **40**.

Tetrazolylcarboxamides **64** and **65** of the isomeric isocoumarin series are inactive, but 9-oxo-*N*-(5-tetrazolyl)-acridine-4-carboxamide (**66**) shows good activity in the PCA test (see Chart I).

Compound **10** was chosen for further evaluation as an inhibitor of anaphylaxis. The results given in Table IV show that this compound is also active when given orally to rats, but its activity against reversed anaphylaxis in fragments of human lung, although dose related, was weak in comparison with that in the rat mast cell. Clinical

Table I. Chromonecarboxamides[a]

(structure: R¹ on the chromone ring; CONR²R³; O; O)

no.	R¹	CONR²R³	method of prepn[b]	yield, %	mp, °C	formula[c]	recrystn solvent[d]	rat mast cell		rat PCA[g]
								EC$_{50}$[e]	R.P.[f]	
1	6-NH$_2$	2-CONH$_2$	A	91	305–307 dec	$C_{10}H_8N_2O_3$	A	>18	<0.005	10
2	8-NH$_2$-7-OH	2-CONH$_2$	B	92	314–315 dec	$C_{10}H_8N_2O_4$	B	>18	<0.005	15
3	7-OAc	2-CONH$_2$	A	84	299–300 dec	$C_{12}H_9NO_5$	A	*h*	*h*	*h*
4		2-CONH(CH$_2$)$_2$COOH	C	57	217–218 dec	$C_{13}H_{11}NO_5$	C	>18	<0.005	0
5		2-CONH(CH$_2$)$_3$COOH	C	90	185–186 dec	$C_{14}H_{13}NO_5$	C	>18	<0.005	0
6		2-CONH(CH$_2$)$_5$COOH	C	83	156–157 dec	$C_{16}H_{17}NO_5$	C	>18	<0.005	0
7		2-CONHC$_{10}$H$_{15}$[i]	C	95	156–157 dec	$C_{20}H_{21}NO_3$	A	*h*	*h*	*h*
8		2-CONMePh	C	72	115–116	$C_{17}H_{13}NO_3$	B	*h*	*h*	*h*
9		2-CONHC$_6$H$_4$-4-CN	C	71	305–306 dec	$C_{17}H_{10}N_2O_3$	A	*h*	*h*	*h*
10		2-CONHtet[j]	C, D	54[k]	289–290 dec	$C_{11}H_7N_5O_3$	A	0.08	1.0	100
11	6-Me	2-CONHtet	C	51[k]	300–301 dec	$C_{12}H_9N_5O_3$	D	0.075	1.2	75
12	7-Me	2-CONHtet	E	47[k]	290–291 dec	$C_{12}H_9N_5O_3$	A	0.58	0.19	75
13	7-Me	2-CONHtet[l]	F	80	175–185			0.16	0.75	80
14	7-OH	2-CONHtet	D	55	300 dec	$C_{11}H_7N_5O_4$	A	0.11	0.77	65
15	7-OMe	2-CONHtet	D	47	298 dec	$C_{12}H_9N_5O_4$	A	0.048	1.8	75
16	6-Cl	2-CONHtet	D	49	264 dec	$C_{11}H_6ClN_5O_3$	A	0.75	0.12	65
17	6-NO$_2$	2-CONHtet	D	52	290 dec	$C_{11}H_6N_6O_5$	A	5.5	0.01	15
18	6-CN	2-CONHtet	C	77	289–290 dec	$C_{12}H_6N_6O_3$	A	2.3	0.03	100
19	6-tet	2-CONHtet	G	67	>330 dec	$C_{12}H_7N_9O_3$	D	0.1	0.85	70
20	3-Me	2-CONHtet	D	43	270–272 dec	$C_{12}H_9N_5O_3$	A	0.4	1.0	70
21	3-Cl	2-CONHtet	D	49	263–264 dec	$C_{11}H_6ClN_5O_3$	A	0.8	0.11	75
22	3-NH(CH$_2$)$_2$OH	2-CONHtet	H	23	184	$C_{15}H_{19}N_7O_5$	B	0.085	0.18	100
23	3-NH(CH$_2$)$_3$OH	2-CONHtet	H	43	174–175[m] dec	$C_{17}H_{23}N_7O_5$	B	0.024	0.67	100
24	3-c-N(CH$_2$CH$_2$)$_2$O	2-CONHtet	H	38	209 dec	$C_{15}H_{14}N_6O_4 \cdot 0.5H_2O$	F	>18	<0.005	10
25	3-NHMe	2-CONHtet	H	70	210–211 dec	$C_{12}H_{10}N_6O_3$	G	0.4	0.04	100
26	6-CONHtet	2-CONHtet	C	68[k]	>340	$C_{13}H_8N_{10}O_4$	D	0.55	0.1	100
27	6-Me-3-Ph	2-CONHtet	D	43	263–264 dec	$C_{18}H_{13}N_5O_3$	B	8	0.005	50
28	7-OMe-3-Ph	2-CONHtet	C	39[k]	258–260	$C_{18}H_{13}N_5O_4$	B	>18	<0.005	25
29		2-CONH– (ring: S, N)	C	75	268–269 dec	$C_{13}H_8N_2O_3S$	A	>18	<0.005	15
30		2-CONH– (ring: N–NH, N)	C	74	322–324 dec	$C_{12}H_8N_4O_3$	A	5	0.002	0
31		2-CONH– (ring: S, N, benzo)	C	90	296–298 dec	$C_{18}H_{12}N_2O_4S$	A	*h*	*h*	*h*
32	6-CONHC$_6$H$_4$-4-Me	2-CONHC$_6$H$_4$-4-Me	C	73	>350 dec	$C_{22}H_{20}N_2O_4$	A	*h*	*h*	*h*
33	6-CONHCH$_2$COOH	2-CONHCH$_2$COOH	C	46	270–272 dec	$C_{15}H_{12}N_2O_8$	A	>18	<0.005	10
34	6-CONH(CH$_2$)$_2$OH	2-CONH(CH$_2$)$_2$OH	C	44	234–235 dec	$C_{15}H_{16}N_2O_6$	B	>18	<0.005	15
35		2-CO-c-N(CH$_2$CH$_2$)$_2$N-Me·HCl	C	79	230–235 dec	$C_{15}H_{16}N_2O_3 \cdot HCl$	B	>18	<0.005	0
36		2-CO-c-N(CH$_2$CH$_2$)$_2$N-COOEt	C	98	91	$C_{17}H_{18}N_2O_5$	E	*h*	*h*	*h*
37		2-CON(C$_6$H$_{11}$)CONHC$_6$H$_{11}$	I	47	168–170	$C_{23}H_{28}N_2O_4$	B	>18	<0.005	0
38		3-CONHtet	C	30	260–261 dec	$C_{11}H_7N_5O_3$	A	0.033	0.48	65
39		3-CO-c-N(CH$_2$CH$_2$)$_2$N-Me·HCl	C	78	215–220 dec	$C_{15}H_{16}N_2O_3 \cdot HCl$	E	14	0.007	20

40		6-CONHtet	D	45	310-312	$C_{11}H_7N_5O_3$	A	0.7	0.08	35
41	2-tet	6-CONHtet	D	42	>340 dec	$C_{12}H_7N_9O_3$	D	0.055	1.0	70
42		2-CONMetet	C	30	263-265 dec	$C_{12}H_9N_5O_3$	A	>18	<0.005	0
43		2-CONH–(Me; N–N; N; N)	C	36	264-265	$C_{12}H_9N_5O_3$	H	>18	<0.005	0
44		2-CONH–(N–NMe; N=N)	C	70	258-259	$C_{12}H_9N_5O_3\cdot{}^1/_3H_2O$	H	*h*	*h*	25
disodium cromoglycate (cromolyn sodium)								2.55	0.02	40

[a] R^1 = H unless otherwise stated. [b] A, ethyl ester treated with gaseous NH_3 (ref 4a); B, reduction of the nitro compound; C, from the acid chloride and the amine; D, from the carboxylic acid, amine, and *N,N'*-dicyclohexylcarbodiimide; E, from the carboxylic acid, amine, and *N,N'*-carbonyldiimidazole; F, from the amide and $Me_2N(CH_2)_2OH$ in MeOH (EtOAc added to precipitate the product); G, from compound **18** and NaN_3 (ref 4a); H, see text; I, by-product in the synthesis of **10** by method D. [c] Compounds gave correct (±0.4%) analyses for C and H (and N where present). [d] A, DMF-EtOH; B, EtOH; C, purified by dissolving in aqueous $NaHCO_3$ and acidification; D, DMF; E, PhH-petroleum ether; F, MeOH; G, washed with EtOH; H, aqueous DMF. [e] μg/mL. [f] Relative potency compared with compound **10** = 1.0. [g] Percentage inhibition at 1 mg/kg given intravenously. [h] This compound was not tested because of its very low solubility. [i] Adamantyl. [j] tet = 5(1*H*)-tetrazolyl. [k] Yield from carboxylic acid. [l] 2-Hydroxyethylammonium salt. [m] 3-Hydroxypropylammonium salt.

Table II. Other Chromone Derivatives

(structure: chromone ring with O, R^1, R^2, R^3, O)

no.	R^1	R^2	R^3	method of prepn[a]	yield, %	mp, °C	formula[b]	recrystn solvent[c]	rat mast cell EC_{50}[d]	rat mast cell R.P.[e]	rat PCA[f]
45			CN	A	55	181-186	$C_{10}H_5NO_2$	A	*g*	*g*	*g*
46			COOH	B	60	>340 dec	$C_{10}H_6O_4$	B	>18	<0.005	0
47			COOEt	C	92	94-95	$C_{12}H_{10}O_4$	A	*g*	*g*	*g*
48		COOH		B	38	203-204	$C_{10}H_6O_4$	A	>18	<0.005	20
49		COCl		B	82	143-144	$C_{10}H_5ClO_3$	C	*g*	*g*	*g*
50		CH=NOH		D	94	196	$C_{10}H_7NO_3$	A	>18	0.0006	25
51		CHN_4O[h]		B	41	181	$C_{10}H_6N_4O_3$	A	5	0.01	75[i]
52		CH=CHCOCl		B	85	178-179	$C_{12}H_7ClO_3$	C	*g*	*g*	*g*
53		CH=CHCONHtet[j]		E	42	285	$C_{13}H_9N_5O_3$	D	6	<0.005	65
54		$CH=CHCO\text{-}c\text{-}N(CH_2CH_2)_2N\text{-}Me$		F	73	151-152	$C_{17}H_{18}N_2O_3$	C	>18	<0.005	0
55		$CH=C(CN)_2$		B	29	218	$C_{13}H_6N_2O_2$	A	*g*	*g*	*g*
56		$NHCOCH_2$tet		F	69	275-277	$C_{12}H_9N_5O_3$	D	>18	<0.005	0[i]
57		NHCOCOOEt		F	63	148-149	$C_{13}H_{11}NO_5$	A	*g*	*g*	*g*
58	COOEt		NHCOCOOEt	F	91	230-231	$C_{16}H_{15}NO_7$	A	12	0.005	65
59	COOEt		$NHCOCH_2$tet	E	82	274-275	$C_{15}H_{13}N_5O_5$	D	11	0.005	65
60	COOH	Ph	Me	B	75	217 dec	$C_{17}H_{12}O_4$	A	>18	<0.005	0
61	Me	Me	SO_2NHtet	F	41	154	$C_{12}H_{11}N_5O_4S$	D	>18	<0.005	20

[a] A, decarboxylation of the 2-carboxylic acid (ref 4a); B, see text; C, from the carboxylic acid, EtOH, and HCl gas; D, from the aldehyde and NH_2OH; E, see Table I, footnote *b*, method D; F, see Table I, footnote *b*, method C. [b] Compounds gave correct (±0.4%) analyses for C and H (and N where present). [c] A, EtOH; B, DMF; C, PhH-petroleum ether (bp 60-80 °C); D, DMF-EtOH. [d] See Table I, footnote *e*. [e] See Table I, footnote *f*. [f] See Table I, footnote *g*. [g] This compound was not tested because of its very low solubility. [h] 5(4*H*)-Oxotetrazolin-1-yl. [i] Tested at 5 mg/kg. [j] tet = 5(1*H*)-tetrazolyl.

Table III Miscellaneous Compounds

no.	method of prepn[a]	yield %	mp, °C	formula[b]	recrystn solvent[c]	rat mast cell EC_{50}[d]	rat mast cell R.P.[e]	rat PCA[f]
62	A	66	259-260 dec	$C_{20}H_{12}O_6$	A	g	g	g
63	B	33	>320 dec	$C_{21}H_{13}N_5O_5$	B	g	g	g
64	B	49	302 dec	$C_{11}H_7N_5O_3$	B	>18	<0.005	0
65	B	52	240 dec	$C_{11}H_7N_5O_3$	B	>18	<0.005	0
66	B	49	300 dec	$C_{15}H_{10}N_6O_2$	B	0.65	0.14	90
67	C	62	320	$C_{24}H_{18}N_2O_6$	C	g	g	g
68	C	58	308-310	$C_{24}H_{18}N_2O_6$	B	g	g	g
69	C	99	320 dec	$C_{28}H_{22}N_2O_6$	C	g	g	g
70	D	31	188-190	$C_{23}H_{28}N_2O_4$	D	g	g	g
71	E	60	212-214 dec	$C_{14}H_9N_5$	D	g	g	g

[a] A, see text; B, see Table I, footnote b, method D; C, see Table I, footnote b, method C; D, by-product in the synthesis of compound **64**; E, see ref 4a. [b] Compounds gave correct (±0.4%) analyses for C and H (and N where present). [c] A, pentanol; B, DMF; C, DMF-EtOH; D, aqueous EtOH. [d] See footnote e, Table I. [e] See footnote f, Table I. [f] See footnote g, Table I. [g] This compound was not tested because of its low solubility.

Table IV. Antianaphylactic Activity of 4-Oxo-N-(5-tetrazolyl)-4H-1-benzopyran-2-carboxamide (10) in the Rat

test	antianaphylactic act., ED_{50}[a]
rat mast cell	0.063 (0.053-0.075) μg/mL
rat PCA (intravenous)	0.037 (0.014-0.056) mg/kg
rat PCA (oral)[b]	21 (5-35) mg/kg

[a] Dose or concentration necessary to cause 50% inhibition with 95% confidence limits assuming a log linear dose response.[8b] [b] Rats were starved overnight and dosed with an aqueous solution 10 min before challenge with antigen intravenously.

investigation of **10** has shown it to be of very similar activity to that of disodium cromoglycate.

In this series of compounds, antiallergic activity requires an acidic substituent attached to C-2 or C-3 of the chromone nucleus. The active compounds contain an N-(5-tetrazolyl)carboxamido function at C-2 or C-3. When the tetrazolylcarboxamido group is attached to other heterocycles, active compounds may or may not be obtained.

Experimental Section

Melting points were determined on a Reichert hot-stage apparatus. IR spectra were obtained on KBr disks with a Perkin-Elmer Model 157G or 521 spectrophotometer. NMR spectra were recorded on a Perkin-Elmer Model R10 (60 MHz) or R32 (90 MHz) instrument (Me_4Si as internal reference).

***N*-(5-Tetrazolyl)carboxamides. Method A.** Crude acid chloride [prepared in situ from the carboxylic acid (24 mmol), $SOCl_2$ (3.6 mL), and DMF (0.1 mL) in $(CH_2Cl)_2$ (50 mL) by refluxing under N_2 for 4 h and then concentrating the solution under reduced pressure] was added to a stirred suspension of 5-aminotetrazole (2.1 g) and $NaHCO_3$ (3.4 g) in ice-cold water (30 mL). Stirring was continued for another hour. The precipitate was collected, washed thoroughly with aqueous $NaHCO_3$, and crystallized from an appropriate solvent.

Method B. The carboxylic acid (15 mmol) and 5-aminotetrazole (1.75 g, 17 mmol) were dissolved or suspended in dry THF (60 mL). N,N'-Dicyclohexylcarbodiimide (3.3 g, 17 mmol) was added and the mixture was stirred for 6 h at ~18 °C. The resulting precipitate was collected and dicyclohexylurea was removed by heating the mixture with ethanol (100 mL). The insoluble product was washed with hot ethanol and recrystallized.

Method C. N,N'-Carbonyldiimidazole (2.1 g, 13 mmol) was added to the carboxylic acid (13 mmol) in dry DMF (120 mL) and THF (80 mL). The mixture was stirred for 1 h and 5-aminotetrazole (1.32 g, 15 mmol) was added. After further stirring for 72 h, the mixture was filtered and the volume of filtrate reduced to ~20 mL under diminished pressure. Addition of AcOEt gave a white solid which was purified by collection on a funnel, dissolution in DMF, and reprecipitation with AcOEt.

3-(2-Hydroxyethylamino)-4-oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide 2-Hydroxyethylammonium Salt (22). 3-Chloro-4-oxo-N-(5-tetrazolyl)-4H-1-benzopyran-2-carboxamide (0.5 g), 2-aminoethanol (0.31 g), ethanol (5 mL), and water (5 mL) were stirred at room temperature for 17 h. The yellow solid was filtered off and crystallized.

The **3-(3-hydroxypropylamino)-4-oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide 3-hydroxypropylammonium salt (23)** was obtained similarly.

3-(4-Morpholinyl)-4-oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide (24). 3-Chloro-4-oxo-N-(5-tetrazolyl)-4H-1-benzopyran-2-carboxamide (1.0 g), morpholine (1.8 g), ethanol (10 mL), and water (10 mL) were stirred for 24 h at room temperature. The mixture was evaporated and the residue, in water, was acidified with 2 N HCl. The solid was collected and crystallized.

3-Methylamino-4-oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide (25). 3-Chloro-4-oxo-N-(5-tetrazolyl)-4H-1-benzopyran-2-carboxamide (0.5 g), methylamine (0.69 mL, 25% solution in water), ethanol (5 mL), and water (5 mL) were stirred at room temperature for 27 h. The yellow solid was filtered off, washed with ethanol, and dried.

***N*-Methyl-4-oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide (42).** 4-Oxo-4H-1-benzopyran-2-carbonyl chloride (1 g) and 5-methylamino-1H-tetrazole (0.5 g) in dry pyridine (15 mL) were heated for 3 h on a steam bath. The solution was poured into 2 N HCl (100 mL). The solid was collected and crystallized.

***N*-[1-Methyl-5(1*H*)-tetrazolyl]-4-oxo-4*H*-1-benzopyran-2-carboxamide (43).** 4-Oxo-4H-1-benzopyran-2-carbonyl chloride (1.5 g) and 5-amino-1-methyl-1H-tetrazole (0.65 g) in dry pyridine (20 mL) were heated on a steam bath for 4.5 h. The solution was cooled and poured into 2 N HCl (200 mL), and the solid was collected and crystallized.

***N*-[2-Methyl-5(2*H*)-tetrazolyl]-4-oxo-4*H*-1-benzopyran-2-carboxamide (44).** 4-Oxo-4H-1-benzopyran-2-carbonyl chloride (1.5 g) and 5-amino-2-methyl-2H-tetrazole (0.65 g) in dry pyridine (15 mL) were heated on a steam bath for 4.5 h. The solution was cooled and poured into 2 N HCl (150 mL) to give the product.

4-Oxo-4*H*-1-benzopyran-6-carboxylic Acid (46). The nitrile **45** (1.5 g) was refluxed with AcOH (15 mL) and HCl (7.5 mL) for 3 h. On pouring the mixture into H_2O, the acid **46** (1 g, 60%) was obtained: IR ν_{max} 3200-2300, 1700, 1660, 1630, 1590 cm^{-1}.

4-Oxo-4*H*-1-benzopyran-3-carboxylic Acid (48) and Its Acyl Chloride 49. 4-Oxo-4H-1-benzopyran-3-carboxaldehyde[9] (8.0 g) was added over 5 min to a vigorously stirred solution, at 10-15 °C, of $K_2Cr_2O_7$ (50 g) in H_2O (1 L) and concentrated H_2SO_4 (250 mL). Stirring was maintained for 2 h. The precipitate was removed and dissolved in cold aqueous $NaHCO_3$. The solution was filtered and acidified with HCl to give the acid (3.3 g, 38%): IR ν_{max} 3080, 2800-2400, 1740, 1615, 1578 cm^{-1}. The acid (2.0 g), $SOCl_2$ (1 mL), DMF (0.2 mL), and $(CH_2Cl)_2$ (12 mL) were refluxed under N_2 for 1 h. Removal of the solvent, addition of more $(CH_2Cl)_2$ (12 mL), and distillation of this gave the acid chloride as a solid residue which was used without purification.

3-[5(4*H*)-Oxotetrazolin-1-yl]-4*H*-1-benzopyran-4-one (51). The method of Horowitz, Fisher, and Tomasewski[10] was applied

Table V. Antianaphylactic Activity of 4-Oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide (10) in Fragments of Human Lung[a]

concn of compd, μg/mL	0.18	1.8	18	180
mean % inhibn of release of histamine[b] ± SEM	5.0 ± 16.5	46.4 ± 18.0	43.3 ± 17.7	61.6 ± 9.3

[a] Fragments of human lung were incubated in Tyrodes solution challenged with sheep anti-human IgE. The compound was incubated with tissue fragments for 10 min before challenge. Fragments were incubated for 15 min after challenge. Free and residual histamine was measured, and percentage release and percentage inhibition were calculated as for the case of the rat mast cell. [b] Percentage inhibitions are the means of three separate experiments performed in duplicate. Due to large variations within groups, it was impossible to estimate an ED_{50} with 95% confidence limits.

to 4-oxo-4*H*-1-benzopyran-3-carbonyl chloride (2.0 g, 9.5 mmol), NaN_3 (1.9 g, 30 mmol), and anhydrous $AlCl_3$ (1.3 g, 10 mmol) in anhydrous THF. The product absorbed at ν_{max} 3308, 2180, 1705, 1651, 1618, and 1540 cm^{-1}.

3-(4-Oxo-4*H*-1-benzopyran-3-yl)propenoyl Chloride (52). A mixture of the carboxylic acid[11] (18 mmol) and $SOCl_2$ (130 mmol) was refluxed under N_2 for 30 min. Removal of the excess $SOCl_2$ gave the acid chloride as a light yellow solid which was used without further purification: IR ν_{max} 3070, 1750, 1660, 1615, 1564 cm^{-1}.

3-(1,1-Dicyanoethenyl)-4*H*-1-benzopyran-4-one (55). To a solution of 4-oxo-4*H*-1-benzopyran-3-carboxaldehyde (2.0 g, 11 mmol) in pyridine (5 mL) was added malononitrile (2.0 g, 30 mmol). The temperature rose to about 42 °C and a deep reddish brown color developed. After 10 min, the solution was poured into ice (100 g) and HCl (10 mL). The oil solidified on stirring.

6-Methyl-4-oxo-3-phenyl-4*H*-1-benzopyran-2-carboxylic Acid (60). 2′-Hydroxy-5′-methyl-2-phenylacetophenone[11] (10 g, 44 mmol) and ethyloxalyl chloride (8 g, 59 mmol) were refluxed in dry pyridine (50 mL) for 1 h. After cooling and pouring into dilute AcOH, the product was extracted into PhH. Removal of the solvent gave a crude solid which was refluxed for 2 h in HCl (20 mL) and AcOH (20 mL). On pouring this mixture into ice water, a solid separated and, on recrystallization, gave the carboxylic acid **60**: IR ν_{max} 3200–2700, 1740, 1610, 1585, 1480 cm^{-1}.

Bis(4-oxo-4*H*-1-benzopyran-3-yl)acetic Acid (62). Bis-(4-oxo-4*H*-1-benzopyran-3-yl)acetonitrile[9] (2.0 g, 11.4 mmol), HCl (90 mL), water (30 mL), and NaCl (5.0 g, 85 mmol) were refluxed for 2 h. During this time the clear solution precipitated a white solid which was collected after cooling the mixture. Extraction with aqueous $NaHCO_3$, followed by acidification (HCl), gave the acid: IR ν_{max} 3400–2700, 1700, 1640, 1579 cm^{-1}.

Attempts to hydrolyze the nitrile by heating for 1 h with either HCl or 75% H_2SO_4 and NaCl failed.

Synthesis of Precursors. The carboxylic acid or acyl chloride precursors of the amides of Tables I–III were prepared by published methods: 3-methyl-,[12] 6-methyl-,[13] 7-methyl-,[4a] 7-hydroxy-,[14] 7-methoxy-,[15] 6-chloro-,[15] 6-nitro-,[14] and 7-methoxy-3-phenyl-4-oxo-4*H*-1-benzopyran-2-carboxylic acids;[16] 4-oxo-4*H*-1-benzopyran-2,6-dicarboxylic acid,[4a] 2,3-dimethyl-4-oxo-4*H*-1-benzopyran-6-sulfonyl chloride,[17] 1-oxo-1*H*-2-benzopyran-3-carboxylic acid,[18] 1-oxo-1*H*-2-benzopyran-4-carboxylic acid,[19] and acridone-4-carboxylic acid.[20] 9-Cyanoacridine, the precursor of **71**, was synthesized from acridine and KCN.[21]

Pharmacology. Reaginic antisera to dinitrophenylated ovalbumin (DNP–Oa) were raised in male outbred hooded rats using *Bordetella pertussis* as adjuvant.[22]

Passive Cutaneous Anaphylaxis. Groups of four female albino rats were shaved and sensitized dorsally with an intradermal injection of 0.1 mL of diluted reaginic serum. The animals were challenged intravenously 48 h later with 2 mg of DNP–Oa in 0.5 mL of 1% Evans blue dye in isotonic saline. Compounds (0.1-mL aqueous solution) were injected intravenously simultaneously with the antigen; control animals received aqueous vehicle. After 30 min the animals were killed and the blue weals on the inner aspect of the dorsal skin were assessed visually for size and density of color on a five-point scale. A percentage inhibition was calculated by comparison with controls. Compounds were tested at a single dose (usually 1.0 mg/kg).

Anaphylaxis in Rat Mast Cells. Peritoneal and pleural cells were harvested from decapitated exsanguinated male albino rats and washed three times by centrifugation at 200g for 5 min. The final suspension contained 2.5–3.0 × 10^5 mast cells/mL. These cells were sensitized with a 1:10 dilution of rat reaginic serum against DNP–Oa for 2 h at 37 °C, washed twice, and resuspended in a final concentration of 1.2–1.7 × 10^5 mast cells/mL. Reaction mixtures contained 0.5 mol of this suspension, 50 μL of antigen, and 10 μL of the compound in aqueous solution. Control incubations contained aqueous vehicle. Compound and antigen were added to the cells simultaneously and incubation was continued at 37 °C for 15 min. The cells were centrifuged, the supernatant was assayed for histamine (free histamine, *F*), and the cells were lysed with 1.0 mL of distilled water, followed by freezing, thawing, and assay for histamine (residual histamine, *R*). Histamine was assayed by an automated superfusion bioassay using guinea pig atropine-treated ileum and bracketing standards. Histamine release was calculated as a percentage of total histamine: % release (% R) = $F/(F + R) \times 100$. Net histamine release (NHR) was calculated as % R on challenge − % R in the absence of antigen. The percentage of inhibition of histamine release was calculated as

$$\frac{\text{NHR(compd-free control)} - \text{NHR(plus compd)}}{\text{NHR(compd-free control)}} \times 100$$

All compounds were compared with compound **10** [4-oxo-*N*-(5-tetrazolyl)-4*H*-1-benzopyran-2-carboxamide] in a dose-ranging experiment, and both absolute activity (EC_{50}) and potency relative to **10** are quoted in Tables I–III.

Acknowledgment. Financial support for this work by Allen and Hanburys Limited and the Science Research Council is gratefully acknowledged. We also thank D. Jervis for the spectroscopic and analytical results and our colleagues at Allen and Hanburys Research Limited for the following compounds: J. M. S. Paton (**22–25**), Dr. I. Collins (**42**), and Dr. C. J. Wallis (**38, 43, and 44**). We thank S. A. Selway and V. Burt for preliminary biological results and A. T. Nials for expert technical assistance. We are grateful to Dr. D. C. Bishop for valuable advice and discussion during this work.

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Molecular Properties of the Adrenergic α Receptor. 2. Optimum Covalent Inhibition by Two Different Prototypes of Polyamine Disulfides

Carlo Melchiorre,[1] Man Sen Yong, Bruno G. Benfey, and Bernard Belleau*

Department of Chemistry and Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, H3A 2K6, Canada.

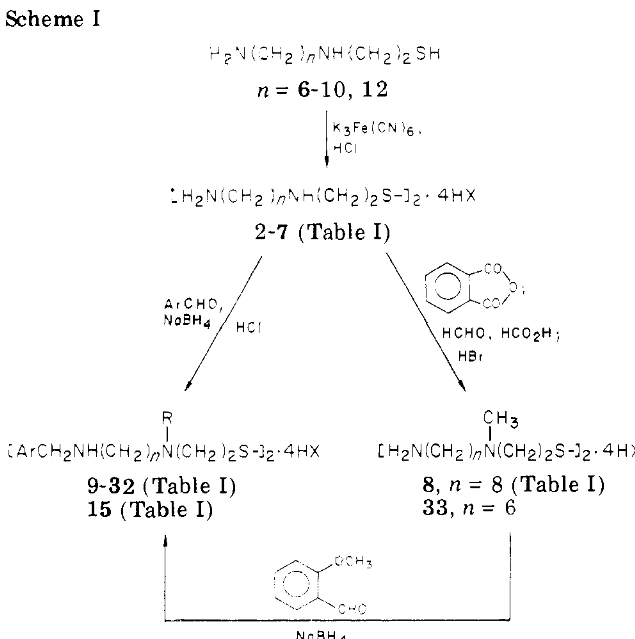
Received March 31, 1978

In order to further improve the α-adrenoreceptor blocking activity of analogues of *N,N'*-bis(5-aminopentyl)cystamine (APC), the effect of distance between the nitrogens of the all-carbon chains together with the effects of benzyl substituents on the terminal nitrogens was studied. It was discovered that with *o*-methoxybenzyl substituents on the terminal nitrogen, optimum α-blocking activity is associated with a six-carbon chain (structure **12**), whereas, in the absence of substituents, the eight-carbon analogue **4** had optimum activity. Evidence is given that these two prototype antagonists involve two distinct binding-site topographies on the α receptor. The effects of N-methylation of the common cystamine segments of **4** and **12** on blocking potency support this conclusion. Polyamine disulfides **4** and **12** (BHC) are shown to be specific irreversible blockers of the α receptor. Complete inactivation of the latter by either one of the inhibitors left intact tissue responses (rat vas deferens and rabbit aortic tissue) elicited by 5-hydroxytryptamine and histamine. However, the response of guinea pig ileum to acetylcholine was weakly inhibited but in a completely reversible manner in contrast to the α receptor. The receptor saturation mechanism for **4** and especially **12** may involve cooperative interactions. The more potent α blocker **12** displayed an affinity and specificity reminiscent of the neurotoxin class of nicotinic receptor inhibitors. The results of receptor protection experiments by norepinephrine (NE) were unusual in that at a ratio as low as 1:10 of BHC to NE, complete protection was achieved. When blockade of the α receptor by **12** was monitored with epinephrine (E) instead of NE, it was observed that the effectiveness of the antagonist was reduced to one-third on rat vas deferens and one-tenth on aortic tissue. This discriminatory power of **12** against NE was not shared by **4** or some close analogues of **12**. It is concluded that **12** is a novel, powerful, and selective pharmacological tool.

In part 1 of this series[2] we have shown that, among a variety of *N,N'*-bis(5-aminopentyl)cystamine (APC) derivatives and analogues, optimum α-adrenoreceptor blocking activity was obtained when the terminal nitrogens of APC carried benzyl or substituted benzyl groups. In order to further improve α-blocking activity, it was necessary to study the effect of distance between the nitrogens of the all-carbon chains together with the effects of benzyl substituents on the terminal nitrogens. It was previously inferred by others[3] that optimum activity was associated with a five-carbon chain, but this conclusion was based on the biological evaluation of the corresponding diaminothiol *S*-phosphate salts. Disulfides other than APC were not reported and since we have already shown that it is the disulfides that constitute the active species at the receptor level,[4] it was essential to make the other homologous tetramine disulfides available for testing. In addition, each new homologue was substituted on the terminal nitrogens by benzyl groups carrying various substituents on the aromatic ring. Finally, the all-carbon analogues of the best prototype antagonists were synthesized in order to clarify the mechanism of α-receptor inactivation by polyamine disulfides.

Chemistry. The structures of the compounds synthesized are given in Table I. They were prepared according to Schemes I and II. The *N*-(ω-aminoalkyl)cysteamines (Table I, n = 6–10, 12) were obtained in 50–55% yield by the procedure already reported[2] for n = 6 and were easily converted to the corresponding disulfides (**2–7**, Table I) in 80–85% yields by potassium ferricyanide

Scheme I



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