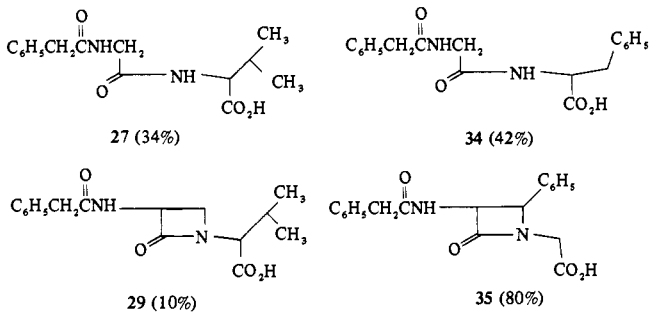
Chart II. Comparison of Inhibition Produced by Compounds Containing Regions A and C (27 and 29) with That of Compounds Possessing A, C, and D (34 and 35)



27 (34%) 34 (42%) 29 (10%) 35 (80%)

10%) *vs.* 1-(carboxymethyl)-3-phenylacetamido-4-phenyl-2-azetidinone (**35**, 80%). In each comparison between structurally similar compounds, the compound bearing a phenyl group is the better inhibitor.

All the inhibition values reported herein were determined at an inhibitor/substrate ratio of 90. The overall inhibition was disappointing; even compounds which are markedly similar to penicillin G, for example, dethiobenzylpenicillin (**29**) and *N*-phenaceturoyl-*S*-methyl-DL-penicillamine (**33**), are bound poorly by penicillinase derived from *B. cereus* 569/H.

Experimental Section

The penicillinase (Nutritional Biochemicals Corp., Cleveland, Ohio) used in this study was a purified from *B. cereus* 569/H. A solution of the enzyme was prepared by dissolving a 50,000-unit sample of the enzyme in 5 ml of 0.5% aqueous gelatin (Pharmagel B, Pharmagel Corp., N. Y.), which imparts stability to the enzyme solution.[7]

This solution was refrigerated at all times. Enzyme stock solutions were prepared fresh daily by diluting 0.25 ml of the above solution with 500 ml of 0.5% gelatin solution. The substrate stock solution, prepared fresh daily, was a 0.188 *M* aqueous solution of the potassium salt of penicillin G (Sigma Chemical Co., St. Louis, Mo.). All inhibitor stock solutions were adjusted to pH 7 with dilute sodium hydroxide and were 0.424 *M* in inhibitor. All inhibitors except *N*-phenaceturoyl-DL-phenylalanine (34) have been previously described and were either commercially available or prepared by conventional methods. We are grateful to Dr. J. N. Wells of Purdue University for the sample of 1-(carboxymethyl)-3-phenylacetamido-4-phenyl-2-azetidinone, whose synthesis has been previously described.[8] The phenaceturoyl derivatives were synthesized by condensation of phenaceturoyl azide with the corresponding amino acid as illustrated in the following procedure. A pH-stat method similar to that reported by Zyk and Citri[9] was employed in this work. The pH-stat titrations were carried out at 25° at pH 6.93 using a Radiometer (Copenhagen, Denmark) Titrator II in conjunction with the following Radiometer equipment: Auto-Burette Type ABU 1b, pH Meter 26, and Titrigraph SBR2c. All inhibition studies were performed in the same manner. Enzyme solution (10 ml) and 2 ml of inhibitor solution, or water in the case of the standardization assay, were pipetted into the reaction vessel. After this solution had been adjusted to pH 7.00, 0.050 ml of substrate solution was added; thus, the ratio of inhibitor to substrate was 90. The pH-stat titration was then carried out with 0.0100 *M* sodium hydroxide up to 0.25 ml of base, so that the hydrolysis of penicillin was monitored up to 27% of completion. Under these conditions, base uptake gave a linear plot against time, and no product inhibition was detected. By this method the stoichemistry of the hydrolysis reaction was within 3% of the theoretical value; the reproducibility of the inhibition results was uniformly better. Substrate activity could not be detected in any of the inhibitors tested.

N-Phenaceturoyl-DL-phenylalanine (34). To a solution of 1.98 g (12 mmol) of sodium hydroxide was added 2.65 g (12 mmol) of phenaceturoyl azide. The mixture was stirred for 6 hr at room temperature and then filtered. The filtrate was acidified with hydrochloric acid and again filtered to give 1.6 g (39%) of a solid, mp 166–169°. Recrystallization from acetone yielded *N*-phenaceturoyl-DL-phenylalanine (34) as a white solid, mp 178–180°.

Anal. Calcd for $C_{19}H_{20}N_2O_4$: C, 67.04; H, 5.92; N, 8.23. Found: C, 66.85; H, 6.16; N, 8.31.

Acknowledgments. The authors wish to acknowledge the technical assistance of Linda Riggs, Carol Claywell, and Sharon Rowland.

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Benzopyrones. 9.[1] Synthesis and Pharmacology of Some Novel Bischromones

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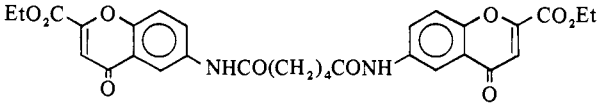
Received July 10, 1972

The antiallergic action of bischromones is of longer duration than that of their simpler analogs.[2] A number of such compounds in which the two chromone moieties are joined together through an alkyl or alkoxy linkage are described in the literature.[3,4] We now report the synthesis of several bischromones in which the two units are linked through amide groups attached to C-2, C-6, or C-8 and one in which the internuclear bridge contains an amide and an ether linkage. Both kinds of linkages are resistant to cleavage and therefore would be expected to promote a long duration of action.

Chemistry. Compounds **1** and **2** in which the amino group



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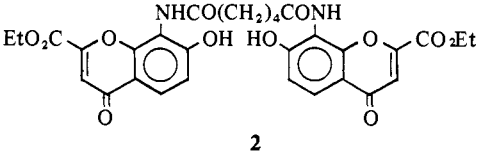


2

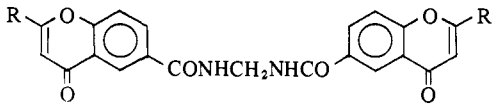
is attached to the chromone ring were prepared by reacting adipoyl chloride with ethyl 6-amino-4-oxo-4*H*-1-benzopyran-2-carboxylate[5] and ethyl 8-amino-7-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylate,[6] respectively. Both products are high-melting compounds of very low solubility in commonly used solvents and could not be recrystallized. Their insolubility also precluded their hydrolysis to the carboxylic

Table I. Bischromones

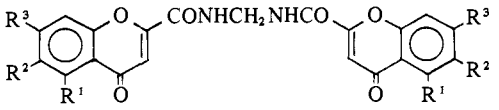
Compd	Mp,[a] °C	Yield, %	Molecular formula[b]
3	283–284	91	$C_{27}H_{22}N_2O_{10}$
4	305–306	84	$C_{23}H_{14}N_2O_{10}$
5	345–346	77	$C_{23}H_{18}N_2O_6$
6	352–354	86	$C_{21}H_{14}N_2O_6$
7	298–299	81	$C_{25}H_{22}N_2O_6$
8	317–318	81	$C_{23}H_{18}N_2O_6$
9	300–302	89	$C_{23}H_{18}N_2O_6$
10	312–313	90	$C_{27}H_{22}N_2O_{10}$

[a] Recrystallizing solvent, DMF–EtOH. [b] Satisfactory analyses (within ±0.4% of the theoretical values) were obtained for C, H, and N.

acids. After this work was completed, a German patent application described the synthesis of the carboxylic acid corresponding to **1** from 5-amino-2-hydroxyacetophenone and the diacyl chloride.[7] In compounds **3–10** (Table I), the amide groups are reversed with respect to the chromone nucleus. These bischromones were prepared from the appropriate chromonecarbonitrile[1,8] and formaldehyde. Attempts to convert the ester **10** to the more water-soluble carboxylic acid or its sodium salt without cleaving the bridge gave an unidentified product. The possibility that compounds **6–10** would break down *in vivo* to the pharmacologically more active 2-carboxylic acids did not materialize (see biological results below).



3, R = CO_2Et
4, R = CO_2H
5, R = Me



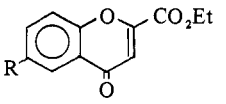
6, $R^1 = R^2 = R^3 = H$
7, $R^1 = R^3 = Me$; $R^2 = H$
8, $R^1 = R^3 = H$; $R^2 = Me$
9, $R^1 = R^2 = H$; $R^3 = Me$
10, $R^1 = R^3 = H$; $R^2 = CO_2Et$

The above bischromones and almost all those described elsewhere consist of two identical chromones symmetrically linked together. Condensation of ethyl 6-chloroacetamido-4-oxo-4*H*-1-benzopyran-2-carboxylate (**11**) with ethyl 7-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylate gave the unsymmetrical bischromone **13**. The chloroacetamide **11** was similarly condensed with 4-chloro-3,5-dimethylphenol to give the chromone **14** which, like the 6-dichloroacetamidochromone (**12**), was of interest as a potential antimicrobial compound.

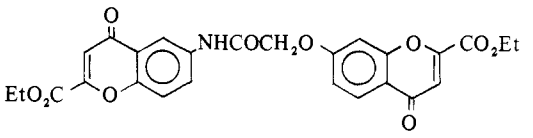
Biology. The compounds were tested for antiallergic activity as previously described.[8] Compound **4** showed a level of activity comparable with disodium cromoglycate while slight activity was exhibited by **1**, **6**, **10**, and **14**. The low solubility of all the bischromones (except **4**) may have an adverse effect on their activity. The compounds were not sufficiently promising to justify an investigation of their duration of action. None of the compounds showed a pronounced effect on the CNS; compounds **12** and **14** possessed no antibacterial action.

Experimental Section

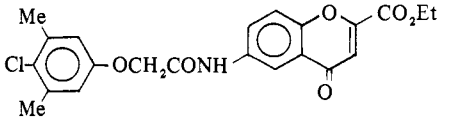
Melting points were determined on a Reichert hot-stage instrument. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of theoretical values.



11, R = $NHCOCH_2Cl$
12, R = $NHCOCHCl_2$



13



14

N,N'-Bis(2-ethoxycarbonylchromon-6-yl)adipamide (1). Ethyl 6-amino-4-oxo-4*H*-1-benzopyran-2-carboxylate[5] (2 g, 8.6 mmol) was boiled under reflux for 2 hr with adipoyl chloride (3.2 g, 17.4 mmol) in dry PhH (50 ml). The solid was filtered off and washed with hot EtOH to give the diamide **1** (2.5 g, 100%), mp 299–300°. *Anal.* ($C_{30}H_{28}N_2O_{10}$) C, H, N.

N,N'-Bis(2-ethoxycarbonyl-7-hydroxychromon-8-yl)adipamide (**2**). Ethyl 8-amino-7-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylate[6] (1.25 g, 5.0 mmol) was treated as above to give the diamide **2** (1.25 g, 83%), mp 267–268°. *Anal.* ($C_{30}H_{28}N_2O_{12}$) C, H, N.

Bis(4-oxo-4*H*-1-benzopyran-2- and -6-carboxamido)methanes. Compounds **3–10** listed in Table I were prepared from the corresponding 2- or 6-carbonitrile[8] and 1,3,5-trioxane as described by Magat, Faris, Reith, and Salisbury.[9]

Ethyl 6-Chloroacetamido-4-oxo-4*H*-1-benzopyran-2-carboxylate (**11**). A solution of chloroacetyl chloride (3.5 g, 31.0 mmol) in dry PhH (10 ml) was added to ethyl 6-amino-4-oxo-4*H*-1-benzopyran-2-carboxylate[5] (5 g, 21.5 mmol) in PhH (140 ml). The mixture was heated under reflux for 1 hr and the white solid which precipitated was washed with PhH to give the amide **11** (6.0 g, 90%) as white needles, mp 274–275° (EtOH). *Anal.* ($C_{14}H_{12}ClNO_5$) C, H, N.

Ethyl 6-(Dichloroacetamido)-4-oxo-4*H*-1-benzopyran-2-carboxylate (**12**). Ethyl 6-amino-4-oxo-4*H*-1-benzopyran-2-carboxylate (5 g, 21.5 mmol) was similarly treated with dichloroacetyl chloride (3.8 g, 25.8 mmol) to give the dichloroacetamide **12** (5 g, 74%) as pale yellow crystals, mp 251–252° (EtOH). *Anal.* ($C_{14}H_{11}Cl_2NO_5$) C, H, N.

Ethyl 6-[(2-Ethoxycarbonyl-4-oxo-4*H*-1-benzoypyran-7-yloxy)-acetamido]-4-oxo-4*H*-1-benzopyran-2-carboxylate (**13**). The chloroacetamide **11** (1.0 g, 3.2 mmol), ethyl 7-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylate (0.76 g, 3.2 mmol), anhydrous K_2CO_3 (0.5 g, 3.6 mmol), KI (0.2 g, 1.2 mmol), and dry Me_2CO (150 ml) were heated under reflux for 3.5 hr. The solid which separated was washed with water and crystallized from DMF–EtOH to give white needles of **13** (1.0 g, 61%), mp 254–256°. *Anal.* ($C_{26}H_{21}NO_{10}$) C, H, N.

Ethyl 6-[(4-Chloro-3,5-dimethylphenoxyacetyl)amino]-4-oxo-4*H*-1-benzopyran-2-carboxylate (**14**). The chloroacetamide **11** (3.5 g, 11.2 mmol), 4-chloro-3,5-dimethylphenol (1.5 g, 9.6 mmol), anhydrous K_2CO_3 (1.4 g, 10.1 mmol), and KI (0.3 g, 1.8 mmol) were refluxed in dry Me_2CO (100 ml) for 6 hr. Removal of solids by filtration and evaporation of the filtrate gave the ester-amide **14** (4.0 g, 96%) as white needles, mp 223–224° (DMF–EtOH). *Anal.* ($C_{22}H_{20}ClNO_6$) C, H, N.

Acknowledgment. The authors wish to thank the Research Division of Allen and Hanburys Limited for the pharmacological results. We gratefully acknowledge support of this work by UWIST (to G. B.) and the Science Research Council (to D. S.).

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Antimalarials. Some 9-Substituted Amino-6-chloro-2-methoxyacridines

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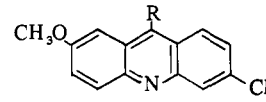
Some time ago[1] we prepared chloroquine analogs incorporating acetylenic and *cis*- and *trans*-ethylenic functions in the side chain which showed interesting antimalarial properties. In two other publications[2,3] we reported many 4-substituted amino-7-chloroquinolines out of which some showed greater antimalarial activity and lower toxicity than chloroquine. In this communication, we report some 9-substituted amino-6-chloro-2-methoxyacridines incorporating these side-chain functions and their comparative antimalarial activity.

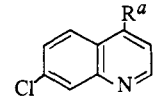
Biological Activity. The compounds were tested for their antimalarial activity against *Plasmodium berghei* in mice according to a procedure already published.[4] The test results are given in Table I. The new compounds show greater activity and less toxicity than the parent drugs. The chloroquine derivatives are found to have greater curative power than the quinacrine derivatives.

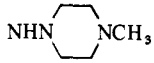
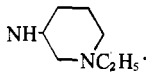
Experimental Section

General Procedure. A mixture of 6,9-dichloro-2-methoxyacridine (0.05 mol) and 75 ml of phenol was heated at 120° for 0.5 hr. It was cooled to 40°, K_2CO_3 (0.1 mol) added, and the mixture stirred for 0.5 hr. The required side-chain amine[1-3] (0.06 mol) was added and the reaction mixture heated at the appropriate temperature (see Table II) for 4 hr. It was cooled and poured into 2 *N* NaOH

Table I. Comparative Antimalarial Activity





Serial no.		Dose, mg/kg	C[b]	TD	T–C	Remarks[c]	C	TD	T–C	Remarks
1	$NHCH(CH_3)C(H)=C(H)CH_2N(C_2H_5)_2 \cdot 2HCl$	20	0	0	?.7		0	0	9.7	Active
		80	0	0	7.5	Active	5	0		Curative
		320	4	0		Curative	4	1		Curative; toxic
		640		5		Toxic	3	2		Curative; toxic
2	$NHCH(CH_3)C(H)=C(H)CH_2N(C_2H_5)_2 \cdot 2HCl$	20	0	0	6.1		2	0		Curative
		80	1	0		Curative	2	0		Curative
		320	3	0		Curative	0	5		Toxic
		640	2	3		Curative; toxic	0	5		Toxic
3	$NHCH(CH_3)C{\equiv}CCH_2N(C_2H_5)_2$	20	0	0	0.7		0	0	8.5	Active
		80	0	0	4.3		0	0	13.5	Active
		320	0	0	11.7	Active	5	0		Curative
		640	0	0	15.1	Active	5	0		Curative
4	$NHNCH_2CH_2N(CH_3)_2$	20	0	0	3.2		0	0	5.3	
		80	0	0	10.9	Active	0	0	9.0	Active
		320	5	0		Curative	1	0		Curative
		640					4	0		Curative
5	$NHN(CH_2)_3N(CH_3)_2 \cdot 2HCl$	20	0	0	4.5		0	0	2.5	
		80	0	0	8.7	Active	0	0	4.3	
		320	1	0		Curative	4	0		Curative
		640	3	0		Curative	5	0		Curative
6	$NHNCH_2CH_2N(CH_3)_2 \cdot 2HCl$	20	0	0	0.7		0	0	3.5	
		80	0	0	6.5		0	0	7.3	Active
		320	0	0	10.7	Active	4	0		Curative
		640	3	0		Curative	5	0		Curative
7		20	0	0	0.3		3	0		Curative
		80	0	0	3.5		3	0		Curative
		320	0	0	8.5	Active	5	0		Curative
		640	2	0		Curative	5	0		Curative
8		20	0	0	3.3		0	0	9.7	Active
		80	4	0		Curative	3	0		Curative
		320	5	0		Curative	5	0		Curative
		640					5	0		Curative
9	$NHCH(CH_2)_3N(C_2H_5)_2$			Quinacrine				Chloroquine		
		20	0	0	0.7		0	0	6.5	
		80	0	0	1.5		0	1	8.9	Active; toxic
		320	0	0	7.3	Active	0	5		Toxic
		640	0	0	16.3	Active				

[a] Preparation and test results of these compounds have also been reported earlier.[1-3] [b] C, cures; TD, toxic deaths when mice die in 2–5 days post infection, attributed to drug toxicity; T–C, increase in mean survival time of the treated mice over the control group. [c] A compound is active if the T–C exceeds 6.1 days and curative if one or more mice live for 60 days or more post infection. Five mice were used in each test.

†Formerly Tara Singh.