Synthesis and Anti-inflammatory Activity of 3-(Benzylideneamino)coumarins in Rodents

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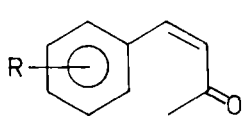
Abstract □ A series of substituted 3-(benzylideneamino)coumarins was synthesized and evaluated for anti-inflammatory activity against carrageenan-induced edema in rats. Halogenated derivatives **4g** and **4c**, at oral doses of 100 mg/kg, showed 75 and 60% antiedematous activity, respectively (phenylbutazone antiedematous activity, 58%). The analgesic activity of **4g** and **4c**, based on inhibition of acetic acid-induced writhing in mice (67 and 62%, respectively, at oral doses of 100 mg/kg) was comparable with that of aspirin (58%). However, these derivatives were devoid of antipyretic activity and showed low activity against adjuvant-induced arthritis.

We have studied[1] the anti-inflammatory activity of phenylbutenones, **1**, synthesized as simple analogue of the potent anti-inflammatory agent curcumin. Like curcumin, these phenylbutenones are potent scavengers of oxygen free radicals, which are implicated as mediators of inflammation.[2,3] Because curcumin and phenylbutenones are derivatives of styryl carbonyls, the study was further extended to include cinnamic acids, **2**. Many cinnamic acids possess appreciable anti-inflammatory activity.[4] These results prompted us to study 3-aminocoumarin derivatives, because the coumarin nucleus incorporates the styryl carbonyl moiety into a rigid framework. Placement of an amino group at the 3-position yields phenylalanine (**3**). An earlier report[5] showed that **3** has good anti-inflammatory activity. The present study describes the synthesis and evaluation of substituted 3-(benzylideneamino)coumarins (**4a–4q**) as rigid analogues of **1**, **2**, and **3**. The compounds that showed good anti-inflammatory activity in the carrageenan-induced edema model were further tested for antiarthritic and antipyretic activities in rats and for analgesic activity in mice.

Experimental Section

Melting points were determined on a Toshniwal melting-point apparatus and are uncorrected. IR spectra (KBr disks) were recorded on a Perkin-Elmer model 157 spectrophotometer, ultraviolet spectra were recorded on a Graphicord UV-240 Shimadzu spectrophotometer in chloroform, and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian A-60 spectrometer with tetramethylsilane as internal standard. The purities of compounds were examined by elemental analysis. Results were within ±0.4% of theoretical values.



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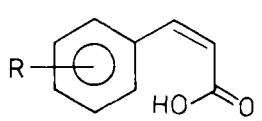

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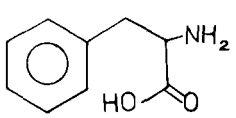

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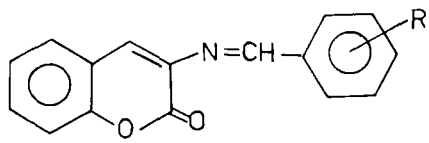
General Procedure for Synthesis of 4a–4q—*Synthesis of 3(3',4'-Dichlorobenzylideneamino)coumarin (4g)*—A mixture of 3-aminocoumarin[6] (0.031 mol, 5 g), 3,4-dichlorobenzaldehyde (0.04 mol, 7 g), and acetic anhydride (0.5 mL) in absolute ethanol (25 mL) was refluxed for 6 h, and the solvent was removed under reduced pressure. The resulting solid was washed with cold water and recrystallized from ethanol to yield 6.5 g of **4g**; retardation factor (R_f, ethyl acetate:benzene, 1:3), 0.67; IR (KBr): 1700 (lactone C=O) and 1520 (C=N) cm^{-1}; NMR ($CDCl_3$) :δ 7.3–8.1 (8H, m, ArH and ArCH=), and 9.3 (1H, s, –CH=N–).

Other compounds were synthesized by a similar procedure. In all cases, elemental analysis and IR, and NMR spectra were in accordance with the proposed structures (Table I).

Pharmacological Tests—Male, albino Charles–Foster rats (150–180 g) and albino mice of either sex (18–25 g) from the animal house of the College of Pharmaceutical Sciences were used. The compounds were given orally as a homogenized suspension in 5% gum acacia with a feeding tube.

Carrageenan-Induced Edema—The method used is based on the method of Winter et al.[7] Groups of four rats were dosed (100 mg/kg) orally with the test compound 1 h before injection of 0.05 mL of a 1% suspension of carrageenan (Sigma) into the subplantar region of the right hind paw. Paw volume was measured by a plethysmograph

Table I—Physical Data for 3-(Benzylideneamino)coumarins



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Compound	R	mp, °C[a]	Yield, %	Formula[b]
4a	H	150–152	65	$C_{16}H_{11}NO_2$
4b	4–F	199–200	56	$C_{16}H_{10}FNO_2$
4c	4–Cl	178–179	65	$C_{16}H_{10}ClNO_2$
4d	4–Br	192	64	$C_{16}H_{10}BrNO_2$
4e	3–Cl	202–203	57	$C_{16}H_{10}ClNO_2$
4f	2–Cl	164	46	$C_{16}H_{10}ClNO_2$
4g	3,4–Cl_2	207–208	65	$C_{16}H_9Cl_2NO_2$
4h	2,6–Cl_2	200–201	73	$C_{16}H_9Cl_2NO_2$
4i	4–Me	131–132	62	$C_{17}H_{13}NO_2$
4j	4–OH	75–78	31	$C_{16}H_{11}NO_3$
4k	4–OMe	123–124	58	$C_{17}H_{13}NO_3$
4l	4–NO_2	185	58	$C_{16}H_{10}N_2O_4$
4m	4–NMe_2	68–70	50	$C_{18}H_{16}N_2O_2$
4n	4–NAc	237	63	$C_{18}H_{16}N_2O_3$
4o	2–OH	183–185	61	$C_{16}H_{11}NO_3$
4p	4–OH, 3–OMe	103–105	76	$C_{17}H_{13}NO_4$
4q	3–OH, 3–OEt	65–67	61	$C_{18}H_{15}NO_4$

[a] Recrystallized from ethanol. [b] All compounds were analyzed for C, H, and N, and the results are within ±0.4% of the theoretical values.

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Table II—Anti-Inflammatory Activity of Substituted 3-(Benzylideneamino)coumarins in Rats

Compound	Edema Inhibition (SE), %[a]
4a	12.5 (17)
4b	27.4 (2.1)
4c	60.4 (8.5)[b]
4d	55.0 (11.6)
4e	11.7 (5)
4f	35.3 (2)[b]
4g	74.9 (6.8)[b]
4h	53.0 (8.5)[b]
4i	Not active
4j	47.9 (1.7)[b]
4k	39.6 (3.4)[b]
4l	9.8 (5)
4m	35.4 (1.7)[b]
4n	27.1 (15.3)
4o	20.8 (3.4)
4p	10.8 (2.2)
4q	39.1 (2.4)[b]
3-Aminocoumarin	16.6 (8.3)
Phenylbutazone	58.4 (4.2)[b]

[a] At oral dose of 100 mg/kg; activity calculated as percent edema inhibition compared with control animals, standard errors in parentheses. [b] Statistically significant ($p < 0.05$, Mann–Whitney test, $n = 4$).

immediately and after 3 h. The change in paw volume was compared with that in vehicle-treated control animals and expressed as percent edema inhibition.

Adjuvant Arthritis[8]—Adjuvant arthritis was induced in groups of four rats by subcutaneous injection of 0.125 mg of dead tubercle bacillus homogenized in liquid paraffin (Sigma) into the plantar surface of the right hind paw. The course of subsequent inflammation was monitored over 18 days by measurement of hind paw volume with a plethysmograph. Compounds **4c**, **4d**, **4g**, **4h**, or phenylbutazone were given orally in 14 daily doses (33 mg/kg) beginning on the day before adjuvant injection. The primary lesion on the injected paw was measured on day 8, and the secondary lesions on both injected and uninjected paws were measured on day 18. Joint mobility was measured by the angle through which the injected paw could be moved easily. Results are expressed with respect to the untreated control animals.

Analgesic Activity[9]—This method was based on acetic acid-induced writhing in mice. Groups of 10 mice (18–25 g) were dosed orally with the drug (100 mg/kg) 30 min before intraperitoneal injection of 0.6% acetic acid (10 mL/kg). Mice were observed for the total number of writhes for 20 min immediately after acetic acid injection. The mean values for each group were calculated and compared with those of the control.

Yeast-Induced Pyrexia[10]—Male rats were injected subcutaneously with a 20% (10 mL/kg) aqueous suspension of dried yeast. Rats developing satisfactory pyrexia were divided into groups of six and dosed orally with **4c**, **4d**, **4g**, and **4h** (100 mg/kg) 18 h after the yeast injection. Rectal temperature was recorded for 8 h and compared with control values.

Acute Toxicity—The approximate dose that is lethal in 50% of the cases (LD_{50}) was determined in mice (18–25 g) by giving the compounds orally and intraperitoneally at a maximum dose of 1000 mg/kg. Lethality was observed over a period of 7 days.

Table IV—Effects of 4c, 4d, 4g, 4h, and Aspirin on Acetic Acid-Induced Writhing in Mice[a]

Compound	Number of Writhes in 20 min[b]	% Reduction from Control
4c	26.0 ± 8.2[c]	62.2
4d	49.1 ± 3.8[c]	28.6
4g	22.6 ± 4.8[c]	67.1
4h	57.9 ± 4.9	15.7
Aspirin	29.0 ± 6.13[c]	57.8
Control	68.7 ± 3.8	—[d]

[a] Compounds administered at oral doses of 100 mg/kg. [b] Results expressed as mean ± standard error. [c] Statistically significant ($p < 0.05$, t test, $n = 10$). [d] Not determined.

Statistical Analysis—All values are expressed as mean ± standard error. The significance of the data was analyzed by the Mann–Whitney method or the t test.[11]

Results and Discussion

Condensation of 3-aminocoumarin[6] with substituted benzaldehydes gave the title compounds **4a–4q** in good yield (Table I). The compounds were characterized by elemental analysis, and UV, IR, and ^1H NMR spectroscopy. The anti-inflammatory activities of **4a–4q** were determined in terms of their ability to inhibit carrageenan-induced edema (Table II); some compounds showed significant ($p < 0.05$) activity that is comparable with or higher than that of the standard drug phenylbutazone. Compounds substituted at the *para* position with chloro or bromo groups (**4c** and **4d**) showed activity (60.4 and 55%, respectively) comparable with that of phenylbutazone (58.4%). Maximal activity (74.9%) was exhibited by the 3,5-dichloro derivative **4g**, whereas substitution of a chloro group at the *ortho* or *meta* positions decreased activity. Significantly, 3-aminocoumarin was not active, although it incorporates all the important features of **1**, **2**, and **3** in a rigid framework.

Compounds **4c**, **4d**, **4g**, and **4h** were further tested in a chronic model of adjuvant-induced arthritis (Table III). The primary lesion was reduced by **4g** and **4c** at 33 mg/kg/day to a greater extent than by phenylbutazone at the same dose, but **4g** and **4c** were less effective than phenylbutazone in reducing the secondary lesion. The improvement in joint mobility due to **4g** and **4h** was comparable to that after exposure to phenylbutazone. Compound **4d** was pro-inflammatory with respect to the secondary lesion. The analgesic activity was tested at a dose of 100 mg/kg dose by the acetic acid-induced writhing test (Table IV). Compounds **4g** and **4c** show good analgesic activity (67 and 62.2%, respectively) and were significantly more potent than aspirin (57.8%); **4d** was less active, and **4h** was the least active. Antipyretic activity against yeast-induced pyrexia was tested. All the compounds tested (**4c**, **4d**, **4g**, and **4h**) were inactive (data not shown). Acute toxicity studies showed that **4c**, **4d**, **4g**, and **4h** are nontoxic (oral or intraperitonial) at a maximum dose of 1000 mg/kg.

Table III—Effects of 4c, 4d, 4g, 4h, and Phenylbutazone on Adjuvant-Induced Arthritis in Rats[a]

Compound	Reduction of Primary Lesion, %[b]	Reduction of Secondary Lesion, %[c]		Improvement of Joint Mobility, %[d]
		Injected foot	Uninjected foot	
4c	28.5 ± 18.1	19.6 ± 7.6	22.2 ± 15.7	9.5 ± 6.6
4d	11.8 ± 3.6	−30.8 ± 8.0	−16.7 ± 10.1	NT[e]
4g	32.1 ± 9.7	30.6 ± 12.1	33.3 ± 12.8	14.5 ± 7.8
4h	17.7 ± 4.2	23.1 ± 8.1	27.8 ± 9.4	13.8 ± 2.2
Phenylbutazone	17.7 ± 10.5	46.2 ± 7.1	50.0 ± 5.5	13.5 ± 5.6

[a] Compounds administered at oral dose of 33 mg/kg; results expressed as means ± standard errors. [b] Primary lesion on injected foot was measured on day 8. [c] Secondary lesion was measured on day 18. [d] Joint mobility on injected foot was measured on day 18. [e] Not tested.

The present study shows that 3-(benzylideneamino)coumarin derivatives are another class of styryl carbonyl compounds with significant anti-inflammatory activity. Although 3-aminocoumarin incorporates the structural features of **1**, **2**, and **3**, it has little anti-inflammatory activity. However, the Schiff bases of 3-aminocoumarin showed good anti-inflammatory activity.

References and Notes

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BOOK REVIEWS

Biopharmaceutics and Clinical Pharmacokinetics. Fourth Edition. By Milo Gibaldi. Lea and Febiger: Malvern, PA, 1991. 406 pp. 19 × 26 cm. ISBN 0-8121-1346-2. Price not given.

This volume, one of the standard textbooks for undergraduate pharmacy students, provides an excellent foundation in principles of biopharmaceutics and pharmacokinetics and covers a wide range of topics in varying degrees of detail. Emphasis is on explanation of principles with specific examples from the literature. Examples, well-supplemented with figures from primary sources, include basic research, such as a study that evaluated brain distribution of iodoantipyrine in monkeys when administered at slow and fast infusion rates. Also included are numerous citations from clinical studies. References are both relevant and current through 1989. Presentation of equations and mathematics is at an appropriate level for undergraduate students, but not with the rigor required for graduate students.

Approximately 40% of the text is devoted to issues related to absorption, including three chapters on gastrointestinal absorption and separate chapters on bioavailability, prolonged-release medication, and non-oral medication. A brief introduction to pharmacokinetics and a short chapter providing the basics necessary to evaluate compartmental and noncompartmental pharmacokinetics are included. A more detailed discussion (approximately 40% of the text) presents concepts related to drug disposition and factors influencing pharmacokinetic variability. Only elementary coverage is provided on the principles of pharmacodynamics. In the chapter "Drug Concentration and Clinical Response" basic information on pharmacodynamic modeling is provided. A noted omission in this chapter is the lack of any discussion concerning situations where a lag time exists between concentration of drug and response. The text lacks discussion of hysteresis and "effect compartment" modeling. The final chapter, entitled "Individualization and Optimization of Drug Dosing Regimens", presents an overview of specific drugs for which individualization of drug dosing is beneficial. This chapter provides direct relevance to the area of clinical pharmacokinetics and helps to enhance the relevance of this field to undergraduate students. However, there are more complete textbooks that cover the pharmacokinetics of individual drugs in much greater detail.

This fourth edition is an excellent textbook for undergraduate pharmacy students and is also a useful reference text for basic principles in drug absorption and disposition. As such, it is a welcome addition to the library of drug information centers and individual pharmacists practicing or researching in the area of clinical pharmacokinetics. Other texts are more appropriate for graduate students and in situations where a reference text is needed that focuses on the applied pharmacokinetics of individual drugs.

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