Synthesis and Structure–Activity Relationships of Anti-inflammatory 9,10-Dihydro-9-oxo-2-Acridine-alkanoic Acids and 4-(2-Carboxyphenyl)aminobenzenealkanoic Acids

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Abstract □ Eighteen test compounds, in three chemical series, were prepared as potential anti-inflammatory agents and evaluated by the rat hindpaw carrageenan-induced edema assay. The compounds, isosteric with known anti-inflammatory and antiallergic cyclo-oxygenase and lipoxygenase inhibitors, are 10-methyl-9,10-dihydro-9-oxo-2-acridinealkanoic acids, 9,10-dihydro-9-oxo-2-acridinealkanoic acids, and 4-(2-carboxyphenyl)aminobenzenealkanoic acids. Compounds within each of these series differ in the structure of the alkanoic acid side chain. Compounds containing the acetic acid and the branched 2-propionic acid side chain showed inhibition of carrageenan-induced edema. The activity of compounds with these side chains and the inactivity of those with carboxy, oxyacetic, thioacetic, and 3-propionic acid side chains is in accordance with the proposed template model of Appleton and Brown for the active site of cyclo-oxygenase, rather than with the alternative active site model proposed by Gund and Shen. One compound, (±)-2-[*N*-(2-carboxyphenyl)-4-aminophenyl]propionic acid (**3c**), showed edema inhibition at 50 mg/kg po, comparable to that of an equivalent dose level of (+)-naproxen. Compounds **4a** and **5a**, which contain a carboxylic acid side chain, exhibited inhibition of soybean 12-lipoxygenase with IC_{50} values of 17.2 and 8.4 μM, respectively. The inhibition observed for the control drug, naproxen, was 24 μM.

Anti-inflammatory activity is exhibited by a variety of arylalkanoic acids and heteroarylalkanoic acids.[1,2] Such nonsteroidal anti-inflammatory agents are believed to act by inhibition of the enzyme cyclo-oxygenase (prostaglandin H synthase), which is responsible for the conversion of fatty acid substrates, such as arachidonic acid (5,8,11,14-eicosatetraenoic acid), into prostaglandins and thromboxanes, chemical mediators in inflammation processes.[3] The closely related and competitive 5-lipoxygenase system converts the same substrate, arachidonic acid, into the bronchoconstrictory leukotrienes which may play important roles in the symptomology of bronchial asthma and related allergic disorders.[4] Since these two enzyme systems have been shown to be very similar, both structurally and mechanistically,[5,6] the structures of their respective inhibitors could be similar as well. In fact, it has been shown that nonsteroidal anti-inflammatory drugs (NSAIDs) such as benoxaprofen [2-(4-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid] and naproxen [(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid] inhibit both cyclo-oxygenase and lipoxygenase.[7] More recently, it has been demonstrated that the fenamic acid drug tolfenamic acid also is a dual inhibitor of these enzymes.[8]

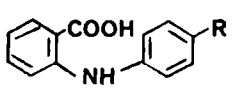
In addition, very closely related nonsteroidal chemical structures are known to have either nonsteroidal anti-inflammatory or antiallergic activity. For example, a series of xanthone-2-carboxylic acids has been reported to be antiallergic agents,[9,10] while the homologous xanthone-2-acetic acids have been shown, in a patent by a different group, to be anti-inflammatory agents.[11] Similarly, 9,10-dihydro-10-methyl-9-oxo-acridine-2-acetic acids show anti-inflammatory properties,[12] while 9,10-dihydro-10-methyl-9-oxo-acridine-2-carboxylic acids have antiallergic and bronchodilatory activities.[13] Anti-inflammatory activity is also reported in various aryl- and heteroarylalkanoic acids with 2-propionic acid, 3-propionic acid, or oxyacetic side chains[14] and thioacetic acid side chains.[15]

The abovementioned facts prompted us to investigate the role of the various side chains in imparting anti-inflammatory activity to compounds with the tricyclic 9,10-dihydro-9-oxo-acridine nucleus or the structurally analogous 4-(2-carboxyphenyl)aminobenzene nucleus. We report here the synthesis, anti-inflammatory activity, and qualitative structure–activity relationships of a series of 4-(2-carboxyphenyl)-aminobenzenealkanoic acids (**3a–3f**), 9,10-dihydro-9-oxo-acridine-2-alkanoic acids (**4a–4f**), and 9,10-dihydro-10-methyl-9-oxo-acridine-2-alkanoic acids (**5a–5f**); the six compounds in each group differ in the nature of the side chain alkanoic acid group, R (Tables I–III). The 4-(2-carboxyphenyl)aminobenzenealkanoic acids are not only convenient synthetic intermediates for the 9,10-dihydro-9-oxo-acridines, but are also structurally related to the clinically used fenamic acid class of NSAIDs. The reported compounds in this series serve as a model to test the validity of the Appleton and Brown template model[16,17] for the action of NSAIDs as cyclo-oxygenase inhibitors versus the alternative model of Gund and Shen.[18]

Results

Chemistry—Of the test compounds reported here, **3a**, **4a**, and **5a** have been reported in patent literature[13] as having antiallergic activity; **4b** has been synthesized and reported by the present authors for an unrelated application.[19] Compound **5b** is claimed in a Japanese patent,[12] with no data provided,

Table I—4-(2-carboxyphenyl)aminobenzenealkanoic Acids



Compound	R	Yield, %	mp, °C	Formula
Test Compound				
3a	COOH	79	288–289	$C_{14}H_{11}NO_4$
3b	CH_2COOH	69	207–209	$C_{15}H_{13}NO_4$
3c	$CH(CH_3)COOH$	43	188–189	$C_{16}H_{15}NO_4$
3d	CH_2CH_2COOH	38	149–152	$C_{16}H_{15}NO_4$
3e	OCH_2COOH	31	156–157	$C_{15}H_{13}NO_5$
3f	SCH_2COOH	56	194–195	$C_{15}H_{13}NO_4S$
Synthetic Intermediate				
3g	$COCH_2CH_3$	54	117–119	$C_{16}H_{15}NO_3$

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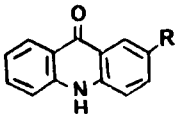
Table II—9,10-Dihydro-9-oxo-2-acridinealkanoic Acids



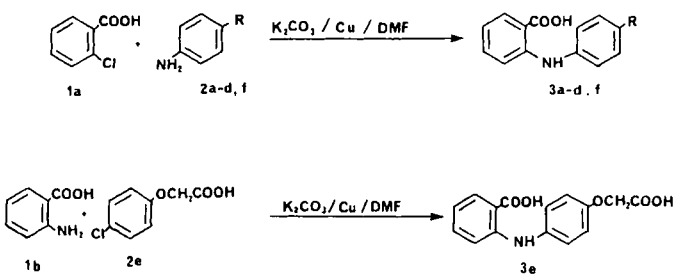
Compound	R	Yield, %	mp, °C	Formula
Test Compound				
4a	COOH	91	>360	$C_{14}H_{19}NO_3 \cdot H_2O$
4b	CH_2COOH	89	299–301	$C_{15}H_{11}NO_3$
4c	$CH(CH_3)COOH$	84	279–280	$C_{16}H_{13}NO_3$
4d	CH_2CH_2COOH	41	192–195	$C_{16}H_{13}NO_3 \cdot H_2O$
4e	OCH_2COOH	64	202–205	$C_{15}H_{11}NO_4 \cdot H_2O$
4f	SCH_2COOH	65	288–291	$C_{15}H_{11}NO_3S$
Intermediate				
4g	$COCH_2CH_3$		270–272	$C_{16}H_{13}NO_2$
4i	$OCNMe_2$		219–222	$C_{16}H_{14}N_2O_2S$
4j	$SCNMe_2$		264–268	$C_{16}H_{14}N_2O_2S$
4k	SH		289–291	$C_{13}H_9NOS$

to have anti-inflammatory activity.

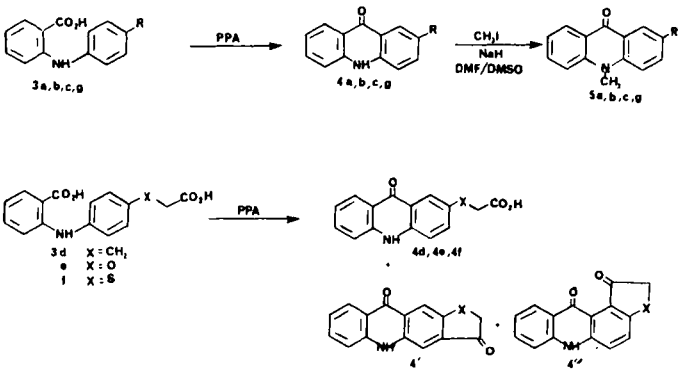
The 4-(2-carboxyphenyl)aminobenzenealkanoic acids (3) were prepared by a modified Ullmann–Jourdan reaction involving the condensation of 2-chlorobenzoic acid (**1a**) with the appropriately substituted 4-aminophenylalkanoic acid (2), as shown in Scheme I. The reaction was carried out in *N,N*-dimethylformamide as solvent in the presence of anhydrous potassium carbonate and copper catalyst.[19] Compounds **3a**, **3b**, **3d**, and **3f** were prepared in this manner. In the case of **3c**, the reactant **2c** was the corresponding methyl ester of 2-(4-aminophenyl)propionic acid,[20] rather than the free acid, and a facile alkaline hydrolysis was carried out on the crude ester product to give **3c**. Compound **3e** was produced by a Type II Ullmann reaction between anthranilic acid (**1b**) and 4-chlorophenoxyacetic acid (**2e**).

Polyphosphoric acid cyclodehydration of the acids **3a**, **3b**, and **3c** provided the corresponding 9,10-dihydro-9-oxo-acridine-2-alkanoic acids **4a**, **4b**, and **4c** in good yields. However, **4d**, **4e**, and **4f**, which have a longer alkanoic acid side chain, could not be cleanly obtained by this cyclodehydration procedure on **3d**, **3e**, and **3f**, respectively, because of the byproducts formed from an intramolecular Friedel–Crafts-type acylation of the types 4′ and 4″ shown in Scheme II. This was particularly observed in the cyclodehydration of **3d**; shortening the reaction time or lowering reaction temperature did not resolve this problem as the conditions for the cyclodehydration to form the central ring of the 9-acridanone appear to be very similar to those for the side chain cyclodehydration reaction.

Alternative routes to prepare **4d**, **4e**, and **4f** were therefore



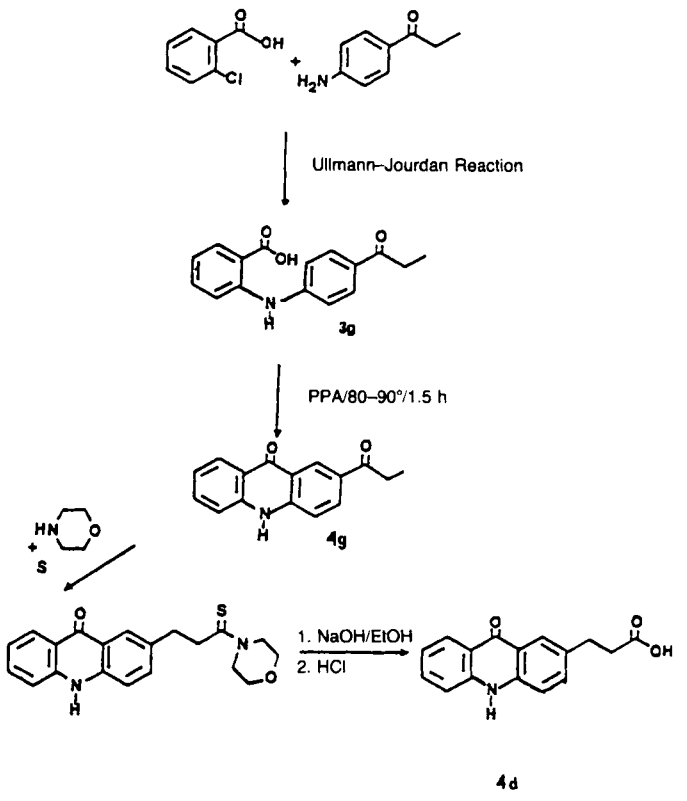
Scheme I



Scheme II

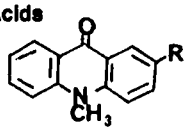
sought. Scheme III shows the route used to synthesize the 3-propionic acid derivative **4d**. Accordingly, 2-chlorobenzoic acid (**1a**) was condensed with 4′-aminopropiophenone in an Ullmann–Jourdan reaction, as described above, to give **3g**; polyphosphoric acid cyclodehydration of this intermediate provided 2-propionyl-9,10-dihydro-9-oxo-acridine (**4g**). This key compound was subjected to a Kindler–Willgerodt reaction with sulfur and morpholine, followed by in situ hydrolysis of the intermediate thiomorpholide, to provide the required 3-(9,10-dihydro-9-oxo-acridin-2-yl)propionic acid (**4d**). The carbonyl group in the 9,10-dihydro-9-oxo-acridine nucleus did not interfere with the reaction, being highly deactivated and presumably behaving more like a vinylogous amide.

The oxyacetic acid compound **4e** and the thioacetic acid **4f** were obtained via the common intermediate 2-hydroxy-9,10-dihydro-9-oxo-acridine (**4h**; Scheme IV); this was obtained from **3h** by a cyclodehydration effected by 98% sulfuric acid. An Ullmann–Jourdan reaction between 2-chlorobenzoic acid (**1a**) and 4-methoxyaniline provided **3h**. Treatment of **4h**

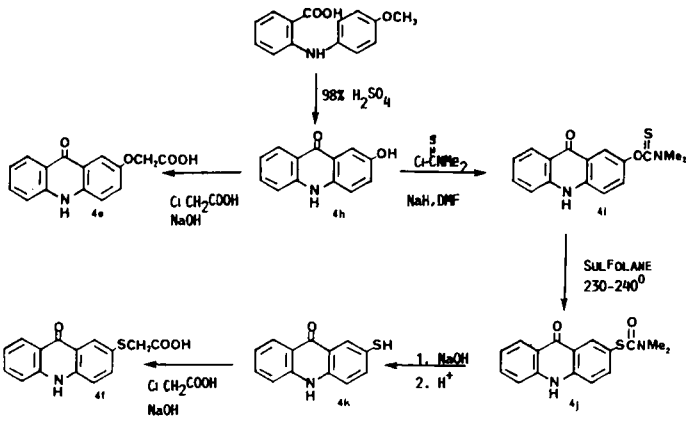


Scheme III

Table III—10-Methyl-9,10-dihydro-9-oxo-2-acridinealkanoic Acids



Compound	R	Solvent	Yield, %	mp, °C	Formula
5a	COOH	DMF	70	212–214	$C_{15}H_{11}NO_3 \cdot H_2O$
5b	CH_2COOH	DMSO	72	192–193	$C_{16}H_{13}NO_3$
5c	$CH(CH_3)COOH$	DMF	68	144–146	$C_{17}H_{15}NO_3$
5d	CH_2CH_2COOH	DMF	78	148–149	$C_{17}H_{15}NO_3$
5e	OCH_2COOH	DMSO	84	146–148	$C_{15}H_{11}NO_4 \cdot H_2O$
5f	SCH_2COOH	DMF	78	179–180	$C_{16}H_{13}NO_3S$



Scheme IV

with sodium hydroxide and chloroacetic acid, followed by acidification, provided **4e**. When the phenoxide of **4h** was treated with *N,N*-dimethylthiocarbamoyl chloride in *N,N*-dimethylformamide, 2-(*N,N*-dimethylaminothiocarbamoyloxy)-9,10-dihydro-9-oxo-acridine (**4i**) was obtained. This underwent thermal rearrangement to the thiolester 2-(*N,N*-dimethylaminocarbamoylthio)-9,10-dihydro-9-oxo-acridine (**4j**) by heating at 230 °C in tetramethylene sulfone, that is, a Newman–Karnes rearrangement.[21] A facile alkaline hydrolysis of **4j**, followed by acidification, provided 2-mercapto-9,10-dihydro-9-oxo-acridine (**4k**); treatment of this compound with sodium hydroxide and chloroacetic acid provided the required thioacetic acid (**4f**).

The 10-methyl-9-oxo-acridinealkanoic acids **5a–5f** were prepared by the *N*-methylation of the corresponding 9,10-dihydro-9-oxo-compounds **4a–4f** using sodium hydride and iodomethane in either *N,N*-dimethylformamide or dimethylsulfoxide. The methylation procedure actually provided the *N*-methyl methyl esters of carboxylic acids **4a–4f**, which were saponified without purification to the 10-methylated acids **5a–5f**.

Biological Testing—The anti-inflammatory activity of the test compounds was evaluated using the model of inhibition of rat carrageenan-induced paw edema as a criterion, using a slightly modified version of the procedure of Winter et al.[22] The results of this screen are provided in Table IV. Naproxen was used as the control drug; the percent edema inhibition produced by this drug at both 3 and 5 h in this study compares well with that reported by Lombardino et al.[23]

In addition, some selected compounds were evaluated for inhibition of soybean lipoxygenase by the UV absorbance-based enzyme assay of Sircar et al.[24] While one may not extrapolate the quantitative results of this assay to the inhibition of mammalian 5-lipoxygenase, it has been shown that inhibition of plant lipoxygenase activity by NSAIDs is

Table IV—Inhibition of Carrageenan-Induced Paw Edema and Soybean Lipoxygenase[a]

Compound	Carrageenan Paw Edema Inhibition %		Inhibition of Soybean Lipoxygenase
	3 h	5 h	IC_{50}, μM
Naproxen	56	39	24
3a	2	6	249
3b	17	8	>300
3c	48	35	>300
3d	0	0	N.A.[b]
3e	0	0	N.A.
3f	0	4	N.A.
4a	2	6	17.2
4b	24	22	>300
4c	10	16	133
4d	0	8	>300
4e	0	0	N.A.
4f	0	0	N.A.
5a	10	2	8.4
5b	37	24	>300
5c	—[c]	—[c]	76
5d	0	20	>300
5e	—[c]	—[c]	>300
5f	0	7	>300

[a] All compounds tested as sodium salts po at dose equivalent of 50 mg/kg of free acid; % inhibitions reported are calculated from mean paw volumes of eight animals per experimental group; *t* test of significance was applied to mean ± SEM paw volumes in each series at $p < 0.05$ for calculating percent inhibition. [b] Data not available. [c] Data not reported; solutions of sodium salts of these compounds darkened rapidly with loss of fluorescence intensity upon exposure to light, suggesting possible photodecomposition.

qualitatively similar to their inhibition of the rat mast cell lipoxygenase, and may be used as a simple qualitative screen for such activity.[24] Recently it has been shown[25] for a series of hydroxythiazole derivatives that NSAIDs inhibit mammalian 5-lipoxygenase with lower IC_{50} values than for their inhibition of plant-derived 12-lipoxygenase.

Discussion

The results in Table IV indicate that the test compounds containing the acetic acid side chain (**3b**, **4b**, and **5b**) and those containing the 2-propionic acid side chain (**3c** and **4c**) show edema inhibitory activity at 50 mg/kg po; the remaining compounds essentially show no activity at this dose level. This indicates that while certain arylalkanoic acids and heteroarylalkanoic acids with 3-propionic, oxyacetic acid, and thioacetic acid side chains have been reported[1,14] to have anti-inflammatory properties, the tricyclic 9,10-dihydro-9-oxo-2-acridinealkanoic acids and the structurally related 4-(2-carboxyphenyl)aminobenzenealkanoic acids containing such side chains show inactivity in the carrageenan paw

edema model. The most active compound in the present series was 3c, which, as the racemate, showed activity comparable to that of (+)-naproxen, both at 3 and 5 h following the carrageenan challenge. Compound 3c has been reported by Picciola[26] as an intermediate in the synthesis of an indazolylpropionic acid anti-inflammatory agent, but was apparently not itself evaluated for such activity during that study.

At the present time, there is still no consensus on the mode of action of NSAIDs at the cycloxygenase active site. Various receptor models have been proposed, the foremost among them being those of Appleton and Brown[16,17] and that of Gund and Shen.[18] The latter model equates the binding site of the carboxyl group of NSAIDs with the carboxyl binding site of the substrate arachidonic acid at the active site of the enzyme, and thus allows a greater structural diversity around the alkanoic acid side chain moiety for receptor fit. The Appleton and Brown model is based on the NSAIDs having structural analogy not with arachidonic acid itself, but with a hydroperoxy intermediate formed from arachidonic acid during cyclo-oxygenation, in its low energy preferred conformation, just prior to its cyclization to prostaglandin H_2. As can be seen from Figure 1, 3c (the most potent compound in the present series) and also 4b and 5b (which have moderately good activity) fit in well with this template model. The Appleton and Brown model would predict activity of only the 2-propionic acid series and acetic acid side chain compounds in the present series; those with shorter or longer side chains would be less active or inactive as they would not fit the receptor template. Thus, the results of the present study appear to favor the Appleton and Brown model over that of Gund and Shen.

Support for this template model has also recently been provided by Walsh et al. who investigated a different chemical class of anti-inflammatory agents.[27] Moreover, noncarboxylic acidic NSAIDs such as piroxicam are accommodated by the Appleton and Brown template model also.[28] Since 4a and 5a have previously been shown to have antiallergy and bronchodilatory activity,[12] it appears that the shorter carboxylic acid chain may impart that activity, while acetic and 2-propionic acid side chains result in anti-inflammatory activity in the present class of compounds. One apparent discrepancy in the present report is 5d which shows edema inhibition at 5 h but not at 3 h. This could be due to the fact that anti-inflammatory activity resides not in the 5d molecule itself but in that of a metabolite which manifests at 5 h, since the maximal anti-inflammatory response of an NSAID is typically achieved at ~3 h following dosing in the paw edema bioassay.[23]

In the soybean lipoxygenase assay, only compounds with the carboxylic acid or 2-propionic acid side chains showed IC_{50} values of <300 μM, suggesting a possible structural constraint at the active site of the enzyme for the binding of inhibitor molecules. While one should exercise caution in extrapolating results for mammalian 5-lipoxygenase inhibition from the data for soybean 12-lipoxygenase, it is interesting to note that the compounds reported in literature to have bronchodilatory and antiallergic activity, 4a and 5a, showed the greatest inhibition in the present series. Further studies with these compounds, using the recently reported rat basophil-derived 5-lipoxygenase preparations,[25] are needed to determine if they are also good inhibitors of that enzyme. If they prove to be so, their antiallergic and bronchodilatory activities may be derived from their ability to block leukotriene biosynthesis.

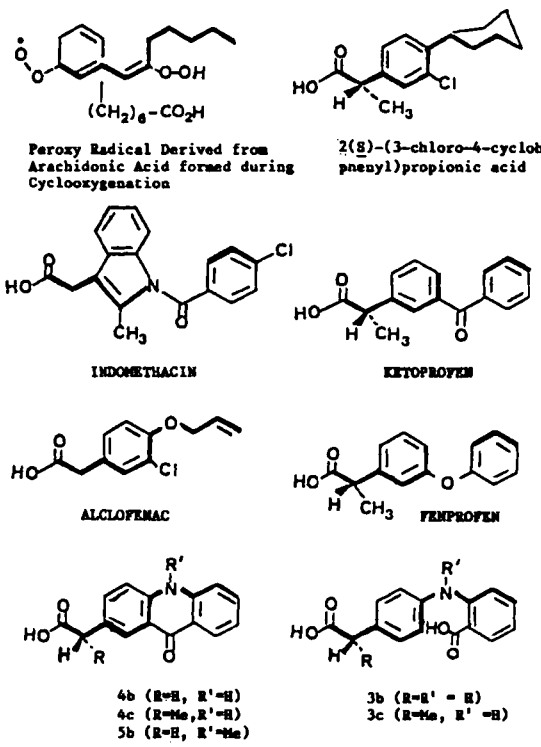


Figure 1—Structural analogy between the peroxy radical derived from arachidonic acid at the cyclooxygenase active site, known NSAIDs, and 2-propionic and acetic acid side chain compounds in the reported series. After Appleton and Brown (ref 16).

Experimental Section

Melting points were determined in capillary tubes in a Thomas Hoover Unimelt apparatus and are uncorrected. The IR spectra were obtained as KBr discs or nujol mulls, recorded on a Beckman 4230 prism spectrophotometer or a Perkin Elmer 700 grating spectrophotometer. The ^1H NMR spectra were obtained on a Perkin Elmer R12 60 MHz instrument, and the chemical shifts are reported in ppm (δ) downfield from tetramethylsilane as the internal standard. The IR and NMR spectra were in agreement with the assigned structure in each case. Homogeneity of samples was determined by thin-layer chromatography using MK6F silica gel plates with fluorescent indicator or on Eastman Chromagram 13181 sheets; spots were visualized under UV light or by exposure of the plates to iodine vapor. Neutralization equivalents were determined by potentiometric titration, using 0.1 M NaOH as the titrant, and the values found were within ±2% of the theoretical for all test compounds. Column chromatographic separations were carried out on silica gel, Davison grade 62. Microanalyses were performed by Microanalysis, Wilmington, DE. Organic reagents were supplied by Aldrich Chemical or by Pfaltz and Bauer. The copper catalyst used to synthesize the 4(2-carboxyphenyl)aminobenzenealkanoic acids was a finely powdered metal obtained from Alcan Metal Powders (Code No. MD 650B). This was activated by washing with a 1:1 mixture of acetone and concentrated HCl and then with aqueous acetone, and drying in a vacuum oven. Naproxen reference standard was a gift of the Quality Control Department, Syntex, Palo Alto, CA. Lyophilized soybean lipoxygenase (EC 1.13.11.12) and its substrate, sodium linoleate, were obtained from Sigma Chemical. Elemental analysis data are shown in Table V.

4-(2-Carboxyphenyl)aminobenzenealkanoic Acids (3a, 3b, 3d, and 3f)—A mixture of 75 mL of *N,N*-dimethylformamide, 2-chlorobenzoic acid (1a, 0.033 mol, 5.2 g), the appropriate 4-aminophenylalkanoic acid (2; 0.036 mol), anhydrous potassium carbonate (0.18 mol, 15 g), and copper powder (0.4 g) was heated, while being stirred, at 140–145 °C, for 3.5 h under a stream of nitrogen gas. The mixture was then cooled to 60 °C and quenched in 200 mL of ice water. The dark solution was decolorized using 1.5 g of activated carbon (Norit A) at 100 °C for 5 min, filtered, and neutralized with 6 M HCl while cooling in an ice bath. The crude solid product was recrystallized from 95% ethanol or from aqueous *N,N*-dimethylformamide.

In the case of 3c, the above procedure was followed starting with 2c.[20] The crude product obtained after acidification of the decolorized

Table V—Elemental Analysis Data

Compound	% Carbon		% Hydrogen	
	Theor.	Found	Theor.	Found
3a	65.37	65.32	4.31	4.40
3b	63.69	63.78	4.83	4.84
3c	67.36	67.53	5.30	5.32
3d	67.37	66.91	5.23	4.84
3e	62.72	62.95	4.56	4.40
3f	59.41	59.32	4.32	4.17
3g	71.91	71.98	4.87	4.63
4a	65.34	65.28	4.28	4.12
4b	71.12	70.86	4.38	4.41
4c	71.90	72.15	4.90	4.84
4d·$2H_2O$	63.31	63.31	5.60	5.54
4e	62.72	63.17	4.56	4.62
4f	63.16	63.23	3.89	3.87
5a	66.42	66.12	4.79	4.95
5b	71.90	72.05	4.90	4.83
5c	72.58	73.14	5.37	5.53
5d	72.58	73.01	5.37	5.35
5e	63.79	63.77	4.98	5.06
5f	64.21	63.96	4.38	4.38

filtrate was suspended in a mixture of 9.0 g of NaOH in 40 mL of water and 100 mL of 95% ethanol, heated at reflux for 30 min, filtered, and acidified with 2 M HCl; the precipitate so obtained was filtered under suction and recrystallized from 95% ethanol.

The remaining 4-(2-carboxyphenyl)aminobenzenes (**3g**, **3h**, and **3j**) were also obtained by this modified Ullmann–Jourdan reaction, except that in these cases, 0.09 mol (7.5 g) of anhydrous potassium carbonate was used in the reaction.

9,10-Dihydro-9-oxo-acridine-2-alkanoic Acids (4a, 4b, and 4c) and 2-Substituted 9,10-Dihydro-9-oxo-acridine (4g and 4l)—Polyphosphoric acid (15 mL) was heated to 80–90 °C in a water bath while being stirred mechanically; the appropriate 4-(2-carboxyphenyl)aminobenzene (**3**) was added over a period of 15 min in a finely powdered form. After an additional 1–1.5 h at 90–100 °C (monitored by a highly fluorescent product spot on TLC, silica gel, $CHCl_3$:MeOH, 8:2), the reaction mixture was cooled to 40 °C and poured slowly with stirring into 100 mL of ice water (exotherm). The resulting suspension was boiled for 2 min, cooled to 20 °C, and filtered to yield the crude product, which was then recrystallized from a 2:1 acetone:*N,N*-dimethylformamide mixture or from glacial acetic acid.

3-(9,10-Dihydro-9-oxo-acridin-2-yl)propionic Acid (4d)—A mixture of 2-propionyl-9,10-dihydro-9-oxo-acridine (**4g**, 0.02 mol, 5.1 g), sulfur powder (0.031 mol, 1.0 g), and freshly distilled morpholine (0.033 mol, 3 mL) was heated at reflux for a period of 10 h. The dark reaction mixture was cooled to 70 °C, poured into a flask containing 40 mL of a 10% ethanolic solution of NaOH, and heated at reflux for an additional 6 h, during which time the thiomorpholide was hydrolyzed. The mixture was poured into 200 mL of water, activated carbon (Norit A, 0.5 g) was added, and the solution was boiled for 5 min and then filtered through a pad of Celite filter aid. The clear pale brown filtrate was cooled to 15 °C and acidified with 6 M HCl, and the precipitated pale yellow solid was filtered, washed well with water, and recrystallized from methanol:*N,N*-dimethylformamide (2:1) to give dull yellow flakes. The yield was 2.37 g (41%) of the dihydrate, mp (dihydrate) 192–195 °C; IR (KBr):3300 (NH), 1720 (COOH), and 1640 (C=O) cm^{-1}; ^1H NMR (12% in $(CH_3)_2SO$-d_6): 2.40–3.10 (*m*, 4H, two fused triplets of $-CH_2-CH_2-$), 6.85–8.35 (*m*, 7H, arom. H), and 12.45 (broad *s*, exchangeable, 2H, COOH, NH) ppm. The anhydrous form was obtained on heating overnight (vacuum oven, 110 °C); mp 246–248 °C).

9,10-Dihydro-9-oxo-acridin-2-yloxy)acetic Acid (4e)—In a 400-mL beaker were placed 2-hydroxy-9,10-dihydro-9-oxo-acridine (**4h**, 0.025 mol, 5.28 g) and chloroacetic acid (0.025 mol, 2.40 g). A solution of NaOH pellets (0.056 mol, 2.25 g) in 15 mL of distilled water was added slowly, with stirring, until all the solid dissolved. The dark brown mixture so obtained was heated with a medium flame until most of the liquid had evaporated. The residue was treated with 15 mL of water and with activated carbon (Norit A, 0.5 g) at 60 °C for 5 min and then cooled to room temperature and filtered. The clear amber filtrate so obtained was acidified with 6 M HCl. The solid that precipitated was filtered and recrystallized from 75% aqueous ethanol. The yield was 4.3 g (64%), mp 202–205 °C, IR (KBr):3320 (NH), 1740 (COOH), 1635 (C=O), and 1595 (NH) cm^{-1}; ^1H NMR (15% in $(CD_3)_2SO$): 4.61 (*s*, 2H, $-CH_2-OAr$), 7.05–8.22 (*m*, 7H, arom. H), and 12.15 (diffuse *s*, 2H, NH, COOH) ppm.

2-(*N,N*-Dimethylaminothiocarbamoyloxy)-9,10-dihydro-9-oxo-acridine (4i)—To a solution of 2-hydroxy-9,10-dihydro-9-oxo-acridine (**4h**, 0.02 mol, 5.68 g) in 70 mL of dry *N,N*-dimethylformamide was added sodium hydride (0.04 mol, 1.4 g of a 60% mineral oil dispersion). After 10 min of stirring under a nitrogen atmosphere, *N,N*-dimethylthiocarbamoyl chloride (0.022 mol, 2.7 g) was added in one portion. The mixture was stirred at 70 °C for 5 h and then at room temperature for 16 h. The reaction mixture was then poured into 200 mL of cold water containing 1 mL of glacial acetic acid. The resulting orange-yellow precipitate was filtered, washed with water, drained, dried in a vacuum oven (80 °C, 2.5 h), and then recrystallized from *N,N*-dimethylformamide:water in 72% yield (2.04 g), mp 219–222 °C; TLC: R_f = 0.42 (benzene:tetrahydrofuran:acetic acid, 30:3:1); IR (Nujol):3300 (NH), 1630 (C=O, 9,10-dihydro-*9-oxo*-acridine nucleus), and 1150 (C—O) cm^{-1}.

Anal.—Calc. for $C_{16}H_{14}N_2O_2S$: C, 64.42; H, 4.69%. Found: C, 64.63; H, 4.71%.

2-(*N,N*-Dimethylaminocarbamoylthio)-9,10-dihydro-9-oxo-acridine (4j)—A magnetically stirred mixture of 2-(*N,N*-dimethylaminothiocarbamoyloxy)-9,10-dihydro-9-oxo-acridine (**4i**; 0.01 mol, 2.84 g) and tetramethylene sulfone (20 mL) was heated at 230–240 °C under a nitrogen atmosphere for 3 h. The reaction was monitored by TLC (silica gel, benzene:tetrahydrofuran:acetic acid, 30:3:1); the product spot had a higher R_f value (0.67) than the starting material (0.43). The mixture was poured into 200 mL of cold water and then 1 g of decolorizing carbon (Norit A) was added and the solution was diluted with 100 mL of 95% ethanol. The mixture was heated to 70 °C and filtered hot, and then the filtrate was concentrated on a rotary evaporator to a third of its original volume. This solution was placed in the refrigerator overnight and the orange-brown solid that precipitated was collected by filtration and dried. The material was used for the subsequent step without further purification, being homogeneous on TLC, but not very stable on storage. The yield was 1.93 g (68%), mp 264–268 °C dec.; IR (KBr):3260 (NH), 1640 (C=O, 9-oxo group), and 1655 (O=C—N) cm^{-1}.

2-Mercapto-9,10-dihydro-9-oxo-acridine (4k)—A mixture of 2-(*N,N*-dimethylaminocarbamoylthio)-9,10-dihydro-9-oxo-acridine (**4j**, 0.079 mol, 2.34 g) and NaOH (0.20 mol, 8.0 g) in 35 mL of distilled water and 80 mL of 95% ethanol was heated at reflux for 3 h under a slow stream of nitrogen, cooled to room temperature, and poured into 200 mL of distilled water. The solution was treated with activated carbon (Norit A) for 5 min and suction filtered over Celite filter aid. The filtrate was acidified with 2 M HCl to precipitate an orange-yellow solid that was collected by suction filtration. The filter cake was washed well with water, drained dry, and recrystallized from ethanol to yield **4k** as orange flakes which were dried in a vacuum oven. The yield was 1.57 g (88%), mp 289–291 °C dec. (changes crystalline appearance at ~210 °C); IR (Nujol):3300 (NH), 2520 (SH), 1635 (C=O), and 1565 (NH) cm^{-1}; ^1H NMR (15% in $(CD_3)_2SO$): 3.45 (*s*, 1H, exchangeable, SH), and 7.05–8.25 (*m*, 7H, arom. H) ppm (NH proton not observed below 10 ppm).

Anal.—Calc. for $C_{13}H_9NOS$: C, 68.72; H, 3.96%. Found: C, 69.17; H, 4.12%.

9,10-Dihydro-9-oxo-acridin-2-ylthio)acetic Acid (4f)—To a solution of NaOH pellets (0.028 mol, 1.12 g) dissolved in distilled water (50 mL) and *N,N*-dimethylformamide (50 mL) was added 2-mercapto-9,10-dihydro-9-oxo-acridine (**4k**, 0.01 mol, 3.20 g), and the mixture was stirred at room temperature under a nitrogen atmosphere for 10 min. This was followed by the addition of chloroacetic acid (0.014 mol, 1.32 g); the mixture (color change to deep brown) was stirred at room temperature for 10 min and then heated at 60–70 °C for 3 h. The reaction mixture was then poured into 100 mL of ice water. The dark solution so obtained was treated with Norit A and filtered over Celite filter aid, and the clear amber-brown filtrate was acidified with 1 M HCl until a pale green-gray precipitate formed. This was collected by filtration, washed with water, recrystallized from water:*N,N*-dimethylformamide (60:40) to give crystals of **4f** in a yield of 2.59 g (65%), mp 288–292 °C dec.; IR (KBr):3360 (NH), 1730 (COOH), 1645 (C=O), and 1585 (NH) cm^{-1}; ^1H NMR (10% in $(CD_3)_2SO$): 3.87 (*s*, 2H, $-S-CH_2-$), 7.10–8.25 (*m*, 7H, arom. H), and 12.55 (*bs*, 2H, NH, COOH) ppm.

9,10-Dihydro-10-methyl-9-oxo-acridine-2-alkanoic Acids (5a–5f)—To a stirred solution of sodium hydride (0.025 mol, 60% dispersion in mineral oil) in *N,N*-dimethylformamide (dried over molecular sieve 4A, 40 mL) or dimethylsulfoxide (40 mL) was added the 9,10-dihydro-9-oxo-acridine-2-alkanoic acid 4 (0.01 mol) in portions. The mixture was stirred under a nitrogen atmosphere for 30 min at 40 °C; and then idomethane (0.025 mol, 3.7 g) was added in a dropwise manner through a dropping funnel. At this stage, the yellow slurry became a clear brown solution. The mixture was heated at 65–70 °C for 1 h, and the reaction mixture was then poured into 200 mL of ice water while stirring. The solution so obtained was acidified with 6 M HCl, added in a dropwise manner until a yellow or green precipitate was obtained. This was collected by filtration and washed well with cold water, and the crude methyl ester so obtained was hydrolyzed by heating at reflux for 30 min with a mixture of 10 mL of 6 M NaOH and 30 mL of 95% ethanol. The hydrolysate was diluted with 100 mL of water and then acidified with 6 M HCl to yield a pale yellow-green solid. The crude acids (5) so obtained were purified by recrystallization from ethyl acetate or by column chromatography on silica gel, eluting with chloroform:petroleum ether:methanol (60:30:10). The *N*-methyl group of these compounds appears as a singlet at 3.85–3.95 ppm in $(CD_3)_2SO$.

Biological Testing—*Carrageenan-Induced Rat Paw Edema Bioassay*—The anti-inflammatory activity of 16 of the test compounds was evaluated in vivo by the rat carrageenan-induced hindpaw edema test (by a variation of the procedure of Winter et al.,[22] a standard test for evaluating the activity of NSAIDs. Male albino rats, Charles River strain, weighing 200–300 g were used. The animals were fasted overnight, grouped (8 animals per group), weighed, and marked. The test compounds (all carboxylic acids) were administered as aqueous solutions of their sodium salts in normal saline. The dose level for each of the compounds was equivalent to 50 mg/kg of the free acid. The compounds were administered orally, by gastric gavage. Naproxen (50 mg/kg) was used as a positive control and a 0.9% NaCl solution was used as the negative control. The left and right hindpaw volumes of the animals were determined immediately after oral dosing using a mercury well pressure transducer connected to a Grass polygraph recorder; the paws were dipped to the ankle hairline level into the mercury well for two 5-s intervals, producing a spike response on the polygraph chart. Calibration of the chart response to the volume measurements was effected by the immersion of a known 2.0-mL volume into the mercury well. One hour following dosing, all rats were given an intrapedal injection, in the plantar region of the left hindpaw, of 0.1 mL of carrageenan solution (1% sterile solution in 0.9% NaCl) using a 26-gauge needle. The left and right hindpaw volumes were measured on each of the animals at 3 and 5 h following the carrageenan injection.

Calculations—The percent inhibition of inflammatory response was calculated from the paw volume measurements taken at 3 and 5 h following injection. The following parameters were determined: d = (left hindpaw volume − right hindpaw volume), calculated for each rat; and D = arithmetic mean of 'd' values, calculated for each experimental group of eight animals (those receiving test compounds and positive control, naproxen). The percent inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{D_{\text{cont}} - D_{\text{expt}}}{D_{\text{cont}}} \times 100 \qquad (1)$$

where D_{cont} is the D value for the group receiving the normal saline negative control and D_{expt} is that for the group receiving test compounds. All 'd' values used in the calculation of $D \pm$ SEM were subjected to a t test at a confidence limit of $p < 0.05$.

Soybean Lipoxygenase Inhibition Study—The UV absorbance-based assay of Sircar et al.[24] was used with only slight modification; the conversion of sodium linoleate to 13-hydroperoxylinoleic acid was followed by the appearance of a conjugated diene absorption at 234 nm. The reaction was monitored using a Beckman 5260 UV spectrophotometer. The enzymatic reaction was carried out at room temperature (28 ± 2 °C). Each sample had a total volume of 2 mL, containing sodium linoleate (100 μM), 150 μL of enzyme solution [1:10^4 dilution of lyophilized enzyme (w/v) in 0.9% NaCl or the dilution to give satisfactory range of initial absorbance] and 2% ethanol. The pH was adjusted to 9.0 (Tris buffer). Test compounds were added as aliquots of freshly prepared stock solutions in 60% aqueous ethanol to give final concentrations of 5 to 300 μM. The enzymatic reaction mixture with inhibitors added was compared in each case with the appropriate standard lacking the compound. The reported IC_{50} values were determined graphically from four concentrations of each compound as the mean of duplicate determinations. The standard deviation (SD) of the mean was ±12%. The IC_{50} value obtained for the control drug (naproxen) in this study was 24 μM; a value of 16 μM was obtained by Sircar et al.[24]

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