2,3-Dihydrobenzofuran-2-ones: A New Class of Highly Potent Antiinflammatory Agents

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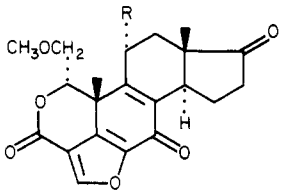
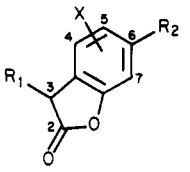
A series of 2,3-dihydrobenzofuran-2-one analogues of the mold metabolite wortmannin, which is a powerful antiinflammatory compound, was synthesized. Most of these compounds were tested for their ability to inhibit the carrageenin paw edema and the adjuvant-induced arthritis of the rat and for their ability to inhibit prostaglandin synthesis in vitro. Indomethacin and diclofenac were used as references. The results show that compounds bearing an alkyl or aryl group in position 6 and an additional substituent, preferably chlorine, in position 5 are very powerful antiinflammatory agents and inhibitors of prostaglandin synthesis. The most active among these compounds, 5-chloro-6-cyclohexyl-2,3-dihydrobenzofuran-2-one, was significantly more potent than diclofenac in all testing models, more powerful than indomethacin in inhibiting acute inflammation and prostaglandin synthesis, and somewhat less potent than the latter compound in the adjuvant arthritis model.

The mould metabolites wortmannin[1] (1) and deacetoxywortmannin[2] (2) are very powerful antiinflammatory agents, but they also show considerable toxicity.[3] Attempts to lower their toxic effects while retaining their antiinflammatory actions by chemical derivatization were unsuccessful.[4,5] That work, however, suggested that the intact furan ring was essential for the antiinflammatory activity and prompted the synthesis of a series of simpler compounds bearing the essential features of the natural product only.[6,7] This led us eventually to benzofuranone derivatives with the general formula 3, which are γ-lactones of *o*-hydroxyphenylacetic acids. Arylacetic acids have been extensively studied as potential antiinflammatory agents,[8] but their lactones are rarely mentioned in the literature. Kadin[9] has previously described antiinflammatory 2,3-dihydro-2-oxobenzofuran-3-carboxanilides without alkyl or aryl substituents in the phenyl ring. Some of the benzofuran-2-one derivatives described in the present paper have unusually strong antiinflammatory activities in the rat, particularly those bearing a cyclohexyl or phenyl substituent in position 6 (cf. 3).



1 (wortmannin), R = OAc
2 (deacetoxywortmannin), R = H



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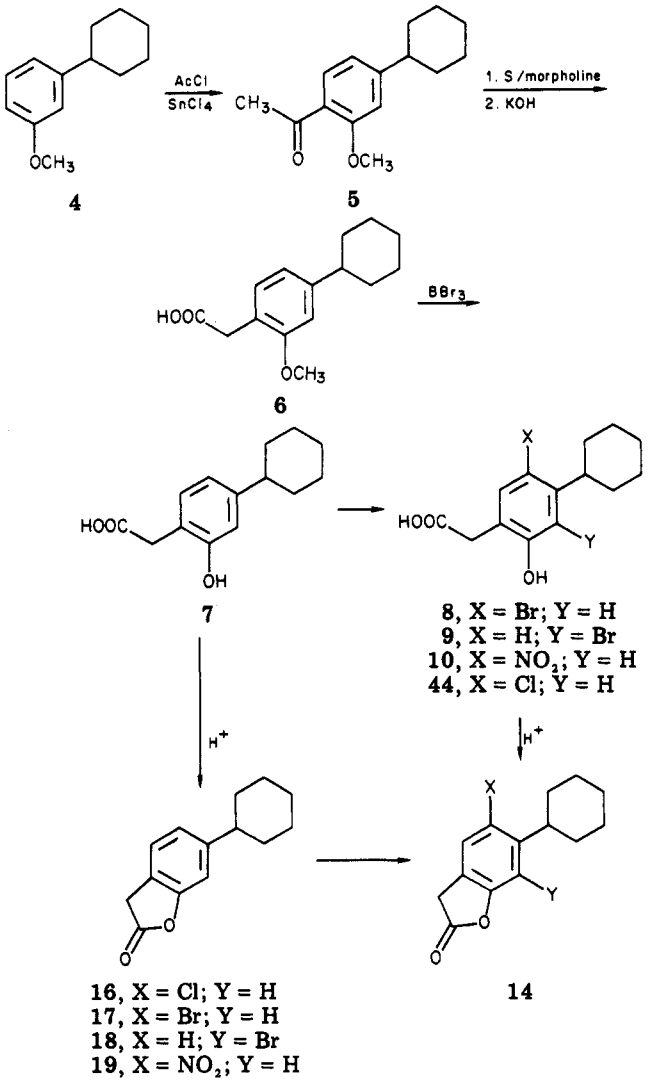
Chemistry. The synthesis of the lead compound, 6-cyclohexyl-2,3-dihydrobenzofuran-2-one (14), was accomplished in a classical manner as outlined in Scheme I (method A). 3-Bromoanisole was converted to the starting material 3-cyclohexylanisole (4) by a published[10] procedure. Friedel–Crafts acylation of 4 with acetyl chloride in the

Scheme I



(1) J. MacMillan, A. E. Vanstone, and S. K. Yeboah, *J. Chem. Soc., Perkin Trans. 1*, 2898 (1972).
(2) W. Haefliger and D. Hauser, *Helv. Chim. Acta*, **56**, 2901 (1973).
(3) D. Wiesinger, H. U. Gubler, W. Haefliger, and D. Hauser, *Experientia*, **30**, 135 (1974).
(4) W. Haefliger, Z. Kis, and D. Hauser, *Helv. Chim. Acta*, **58**, 1620 (1975).
(5) W. Haefliger and D. Hauser, *Helv. Chim. Acta*, **58**, 1629 (1975).
(6) W. Haefliger and D. Hauser, *Synthesis*, 236 (1980).
(7) W. Haefliger, A. Closse, and D. Hauser, in "Enzyme Inhibitors", U. Brodbeck, Ed., Verlag Chemie, Weinheim, 1980.
(8) P. F. Juby, in "Antiinflammatory Agents", Vol. 1, R. A. Scherrer and M. W. Whitehouse, Ed., Academic Press, New York, 1974, p 92.
(9) S. B. Kadin, *J. Med. Chem.*, **15**, 551 (1972).
(10) J. H. Gardner and J. R. Stevens, *J. Am. Chem. Soc.*, **69**, 3086 (1947).

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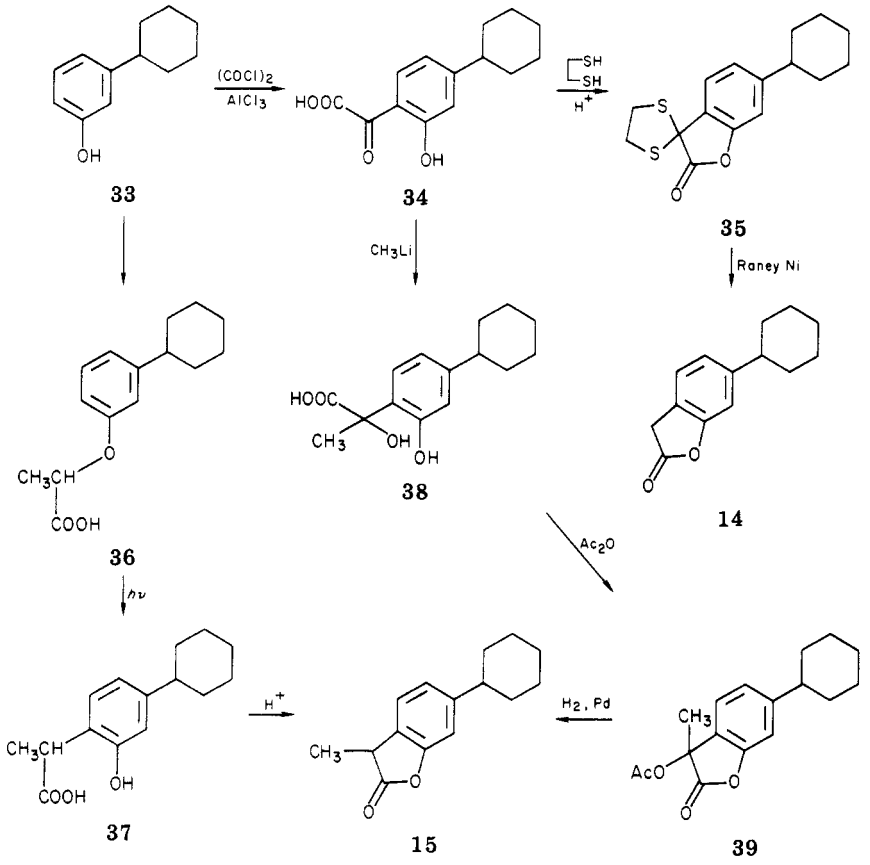
Scheme II



presence of $SnCl_4$ and subsequent Willgerodt–Kindler reaction of **5** gave the phenylacetic acid derivative **6**. Cleavage of the methoxy group in **6** with BBr_3 in CH_2Cl_2 yielded a mixture of 4-cyclohexyl-2-hydroxyphenylacetic acid (**7**) and the corresponding γ-lactone **14**. Unreacted hydroxy acid was smoothly cyclized to the lactone by treatment in a water separator with *p*-toluenesulfonic acid in benzene or toluene.

A halogen or a nitro group was introduced in position 5 of the benzofuranone system by treating the phenylacetic acid derivative **7** with NCS in DMF, NBS in CH_2Cl_2, or HNO_3 in CH_2Cl_2, respectively (Scheme I), and converting the hydroxy acids to the corresponding γ-lactones. In the case of the bromination, the 7-bromo compound **9** was formed in comparable yield to the 5-bromo isomer **8**. The position of the bromo substituent was deduced from the NMR spectra. Alternative syntheses for the construction of the benzofuran-2-one ring system (methods B and C) are outlined in Scheme II.

Method B is based on the reaction of the phenol **33** with oxalylchloride and subsequent hydrolysis to the keto acid **34**. This reaction appears to be highly regioselective, since in this and other cases no isomeric products were detected. Reduction of the keto function was accomplished by conversion of **34** into the dithiolane derivative **35** and subsequent desulfurization with Raney nickel. Keto acid **34** was also used as the starting material for the benzofuranone derivative **15**. In the latter case, **34** was reacted with 3 mol of CH_3Li, and the resulting intermediate, **38**, was cyclized to **39** in the presence of Ac_2O. Hydrogenolytic cleavage of **39** yielded **15**.

The key step of *method C* consists of the photochemical rearrangement[11,12] of the α-phenoxypropionic acid **36** to the phenylpropionic acid **37**. Under the conditions employed, the yield of pure material was only 12%, the main byproduct being the starting 3-cyclohexylphenol (**33**).

Compounds with substituents in position 6 other than cyclohexyl (i.e., isobutyl, cyclopentyl, and phenyl; see **3**) were prepared in analogous fashion, according to one of the methods described.

An alternative synthesis of 5-chloro derivatives started from compounds with protected ester and phenol functions (Scheme III). Best results in the chlorination step were obtained with 1 equiv of SO_2Cl_2 in CH_2Cl_2.[13] The same procedure was successful in the chlorination of γ-lactone derivatives (cf. **14** → **16**). Treatment of **40** with an excess of SO_2Cl_2 led to the dichloro derivative **21**. Ester derivatives were also convenient starting materials for the alkylation of the acetic acid side chain (**40** → **41**). Alternative possibilities for the introduction of a methyl group in the γ-lactone ring have been mentioned in the discussion of Scheme II. Attempts to prepare the enantiomers of the 3-methyl derivative **15** in pure form were so far unsuccessful, since the chiral center in the lactone is very susceptible to racemization.

The 5-methyl analogue **20** was prepared via the formyl compound **46**, which was reduced in two steps (**46** → **47** → **48**) to 4-cyclohexyl-2-methoxy-5-methylphenylacetic acid (**48**). 5-Hydroxy-2-methoxybiphenyl (**50**), synthesized from 3-hydroxybiphenyl[14] via 2-methoxy-5-phenylazobiphenyl (**49**), was converted to **29** by method B. Cleavage of **29** with BBr_3 gave **30** (Scheme IV). The lactones **31** and **32** (Table I), bearing the cyclohexyl ring in positions

(11) D. P. Kelly, J. T. Pinhey, and R. D. G. Rigby, *Aust. J. Chem.*, **22**, 977 (1969).

(12) J. R. Collier, M. K. M. Diramia, and J. Hill, *J. Chem. Soc. C*, 155 (1970).

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(14) A. S. Hay, U.S. Patent 3 363 008 (1968); *Chem. Abstr.*, **69**, 2702 (1968).

Scheme III

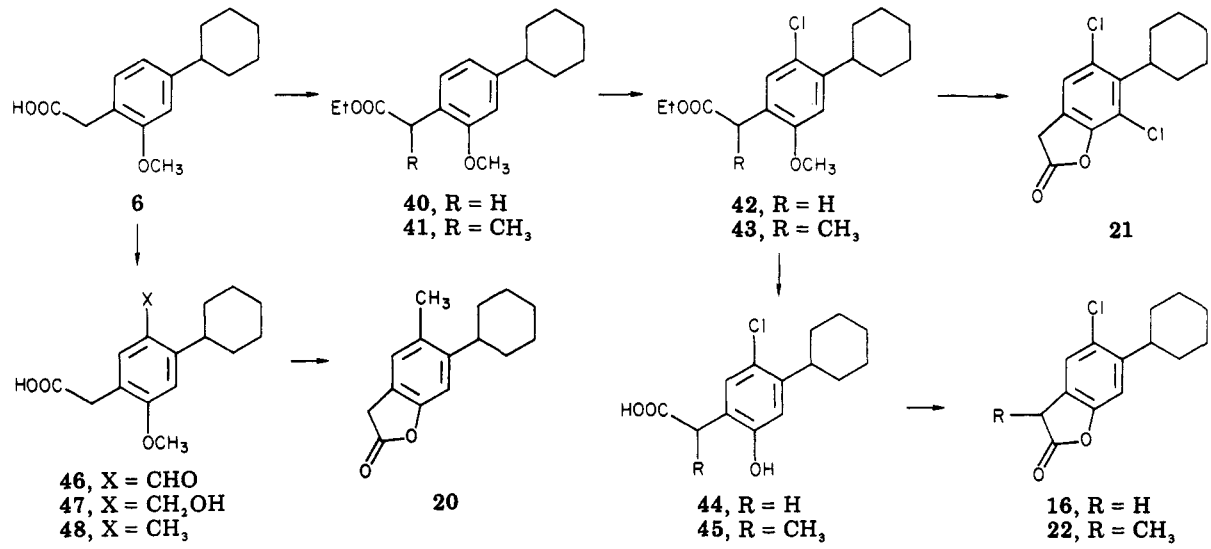
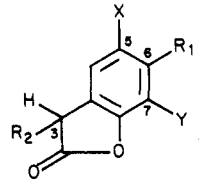


Table I. 2,3-Dihydrobenzofuranone derivatives



compd	R_1	R_2	X	Y	mp or bp (mm), °C	emp formula	ED_{50}, mg/kg po CPE[a]	ED_{50}, mg/kg po AA[b]	PG[c] IC_{50}, μM
11	H	H	H	H	43-45	$C_8H_6O_2$	>60		>100
12	isobutyl	H	H	H	24-25	$C_{12}H_{14}O_2$	8	>40	3
13	cyclopentyl	H	H	H	42-43	$C_{13}H_{14}O_2$	30	60	10
14	cyclohexyl	H	H	H	80-81	$C_{14}H_{16}O_2$	1	20	0.8
15	cyclohexyl	CH_3	H	H	87-89	$C_{15}H_{18}O_2$	0.8	2.5	0.4
16	cyclohexyl	H	Cl	H	100-102	$C_{14}H_{15}ClO_2$	0.5	1	0.05
17	cyclohexyl	H	Br	H	124-126	$C_{14}H_{15}BrO_2$	2	7	0.03
18	cyclohexyl	H	H	Br	108-109	$C_{14}H_{15}BrO_2$	36	>30	3
19	cyclohexyl	H	NO_2	H	132-133	$C_{14}H_{15}NO_4$	6	4	0.5
20	cyclohexyl	H	CH_3	H	157-158	$C_{15}H_{18}O_2$	3	1.5	0.1
21	cyclohexyl	H	Cl	Cl	131-132	$C_{14}H_{14}Cl_2O_2$	2	30	1
22	cyclohexyl	CH_3	Cl	H	92-93	$C_{15}H_{17}ClO_2$	0.3	1	0.07
23	cyclohexyl	CH_3	CH_3	H	67-68	$C_{16}H_{20}O_2$	6	<3	0.1
24	phenyl	H	H	H	109-110	$C_{14}H_{10}O_2$	6	20	0.7
25	phenyl	CH_3	H	H	100-102	$C_{15}H_{12}O_2$	2.5	14	0.5
26	phenyl	CH_2CH_3	H	H	75-76	$C_{16}H_{14}O_2$	25	>30	50
27	phenyl	H	Cl	H	111-112	$C_{14}H_9ClO_2$	1	3	0.08
28	phenyl	CH_3	Cl	H	88-89	$C_{15}H_{11}ClO_2$	0.3	2	0.08
29	phenyl	H	OCH_3	H	132-133	$C_{15}H_{12}O_3$	30	30	10
30	phenyl	H	OH	H	149-150	$C_{14}H_{10}O_3$	>30		50
31	H	H	H	cyclohexyl	86-87	$C_{14}H_{16}O_2$	>60	>30	100
32	H	H	cyclohexyl	H	100 (2)	$C_{14}H_{16}O_2$	>60		10
diclofenac							3	2	0.5
indomethacin							2	0.4	0.2

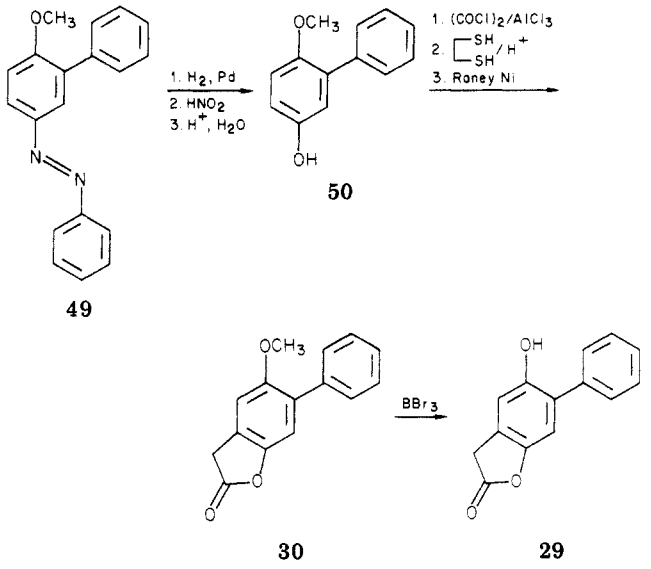
[a] CPE = carrageenin-induced paw edema. [b] AA = adjuvant-induced polyarthritis (treatment, days 1 to 19). [c] PG = inhibition of prostaglandin synthesis in vitro.

other than all the previous compounds, were prepared from 2- and 4-cyclohexylphenol[15] by method A or B.

Pharmacology. Structure–Activity Discussion. Most of the compounds described were tested for antiinflammatory activity in the rat (carrageenin-induced paw edema and adjuvant-induced polyarthritis) and for inhibition of prostaglandin synthesis in vitro. The results are summarized in Table I. In general, there is a good correlation between the inhibitory activities found in the two in vivo models and in the PG synthesis test. A notable exception is compound 17, which is the most potent compound of the series in inhibiting PG synthesis, but is moderately active in the in vivo tests. From the data presented in Table I, the following structure–activity relationships can be recognized. In general, compounds with a cyclohexyl ring in position 6 (compounds 14–23) were more active than the corresponding phenyl or alkyl derivatives. Compounds with the cyclohexyl ring in position 5 or 7 instead of 6 (see 31 and 32 in Table I) were completely inactive. The antiphlogistic activity of compounds bearing a cyclohexyl or phenyl residue in position 6 was

(15) R. R. Burtner and J. M. Brown, *J. Am. Chem. Soc.*, 75, 2334 (1953).

Scheme IV



49 50 30 29

strongly enhanced by substitutions in position 5 other than hydroxy or methoxy. In the adjuvant polyarthritis test, the strongest effect was obtained with Cl and the weakest with Br; NO_2 and CH_3 were intermediate. Halogen substitution was more effective in position 5 than in position 7, as shown in the case of Br (**17** and **18**). Methoxy and hydroxy groups in position 5 (**29** and **30**) exerted a negative effect on the activities tested. A methyl group in position 3 (**15**) improved activity, but a corresponding ethyl group strongly lowered it (**26**).

The most active compound of this series was 5-chloro-6-cyclohexyl-2,3-dihydrobenzofuran-2-one (**16**). It was more active than the reference compounds, indomethacin and diclofenac, in inhibiting the acute inflammation induced by carrageenin, it was half as active as indomethacin and 4 times more active than diclofenac as an inhibitor of the adjuvant-induced arthritis, and it was about 10-fold more potent than the above reference compounds in inhibiting PGE_2 and $PGF_{2\alpha}$ synthesis in vitro. Compound **16** was strongly ulcerogenic in the rat, its activity being similar to that of indomethacin.

The phenylacetic acid derivatives of the type **7** or **44** (Scheme I) are formed from the corresponding lactones (**14** and **16**) when exposed to alkaline media. They are *o*-hydroxy analogues of the very potent but ulcerogenic 4-cyclohexylphenylacetic acids[7] and exhibit antiinflammatory activities comparable to the parent benzofuran-2-ones. It remains to be determined whether the γ-lactones described in the present publication are acting per se or as prodrugs for the corresponding phenylacetic acids.

Experimental Section

Chemistry. Microanalyses were carried out in the Microanalytical Department, Sandoz Ltd., Basle, Switzerland. Where analyses are indicated by the symbols of the elements, the results obtained were within 0.4% of the theoretical values. The melting points are uncorrected. The IR and NMR spectra of all new compounds were consistent with their structures. Chemical shifts (δ) were measured downfield from Me_4Si (δ 0).

Pharmacology. Carrageenin Paw Edema. The method of Winter et al.[16] was employed for the paw edema test. The compounds were administered orally as a suspension in physiological saline containing 0.5% of tragacanth, 1 h prior to the subplantar injection of 0.1 mL of 1% carrageenin in physiological saline. Control reading was taken immediately after the carrageenin injection (0-h value) and the swelling was measured after 3 and 5 h using the antiphlogmeter of Kemper and Ameln.[17] Five rats (Sprague–Dawley, male, 150–170 g) per dose were used in each experiment. The mean values of the 3- and 5-h readings after deduction of the corresponding control reading (0-h value) were calculated. The values obtained in the treated animals were expressed in percent of the values obtained in control animals. ED_{50} is the dose causing a 50% inhibition of carrageenin-induced swelling after 3 h.

Adjuvant Arthritis. The method described by Pearson and Wood[18] was used for the adjuvant polyarthritis. Complete Freund adjuvant, i.e., 0.1 mL of an homogenate of 6 mg of *Mycobacterium smegmatis* S 1043 per milliliter of mineral oil, was injected intracutaneously into the plantar region of one hind paw (day 1). Under these conditions, a primary swelling developed at the injection site, which was followed on days 12 to 15 by a secondary swelling in the joints of the other feet. The test compounds were given orally as a suspension in physiological saline containing 0.5% of tragacanth during development of the adjuvant disease (i.e., from day 1 to 19). Paw diameters were measured at the beginning and at the end of the treatment under light either anesthesia by means of a caliper. The body weights were determined every 2 days. Five to ten rats (strain OFA, male Sandoz Ltd., Switzerland) with initial weights of about 150 g were used for each dosage. The values obtained in the treated animals were expressed in percent of the control values obtained in untreated animals. ED_{50} is the dose causing a 50% inhibition of the secondary swelling.

Ulcerogenic Activity. Rats which had been fasted overnight were given the test compound orally, and after 6 h their stomachs were examined for mucosal damage. Test compounds were compared on the basis of their UD_{50}, which is the dose inducing petechial bleeding or hemorrhages of the gastric mucosa in half of the animals.

Prostaglandin Synthesis Inhibition. Inhibition of prostaglandin (PG) synthesis was tested in vitro using a microsomal fraction from bull seminal vesicles as the enzyme source. The tissue was homogenized with a varying blender, and the microsomes were obtained as a pellet after centrifugation at 40000*g* for 30 min.[19] This preparation was lyophilized and dissolved in saline prior to use. The compounds to be tested were incubated for 30 min at 37 °C and pH 7.4 in the presence of 0.033 mM [^{3}H]arachidonic acid (ca. 100 000 cpm) and 2–3 mg of the lyophilized enzyme preparation. The incubation was stopped by acidification, and the products were immediately extracted into ethyl acetate and separated on thin-layer chromatography.[20] PGE_2 and $PGF_{2\alpha}$ were then eluted from the plates and measured radiometrically.

4-Cyclohexyl-2-methoxyacetophenone (5). 3-Cyclohexylanisole[10] (**4**; 84.7 g, 0.45 mol) was dissolved in CH_2Cl_2 (800 mL), and then acetyl chloride (35 g, 0.45 mol) and subsequently $SnCl_4$ (116 g, 0.45 mol) were added dropwise at 5–10 °C while stirring. After stirring at room temperature for another 20 h, the reaction mixture was poured on ice and extracted with CH_2Cl_2 (thrice). The organic phase was successively washed with 2 N HCl, 2 N Na_2CO_3, and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from acetone–petroleum ether to give **5** (69 g, 69%), mp 99–100 °C. Anal. ($C_{15}H_{20}O_2$) C, H, O.

4-Cyclohexyl-2-methoxyphenylacetic Acid (6). 4-Cyclohexyl-2-methoxyacetophenone (**5**; 16.3 g, 0.07 mol), morpholine (12.2 g, 0.14 mol), and sulfur (4.5 g, 0.14 mol) were refluxed for 6 h. The hot reaction mixture was poured on a mixture of ice, H_2O, and CH_2Cl_2 and stirred until all material was in solution. The H_2O phase was extracted with CH_2Cl_2 (thrice). The combined extracts were washed with H_2O (twice) and dried (Na_2SO_4), and the solvent was removed in vacuo. Recrystallization from EtOH gave 4-cyclohexyl-2-(methoxythiono)phenylacetomorpholide (21.3 g, 91%), mp 118–122 °C. Anal. ($C_{19}H_{27}NO_2S$) C, H, N, O.

(16) C. A. Winter, E. A. Riley, and G. W. Nuss, *Proc. Soc. Exp. Biol.*, 111, 544 (1962).
(17) F. Kemper and G. Ameln, *Z. Gesamte Exp. Med.*, **131**, 407 (1959).
(18) C. M. Pearson and F. P. Wood, *Arthritis Rheum.*, **2**, 440 (1959).
(19) C. Takeguchi, E. Kohno, and C. J. Sih, *Biochemistry*, **10**, 2372 (1971).
(20) E. Anggard and B. Samuelson, *J. Biol. Chem.*, **240**, 3518 (1965).

The morpholide (10.7 g, 0.03 mol), KOH (9.0 g, 0.16 mol), EtOH (50 mL), and H_2O (2.6 mL) were refluxed for 20 h. The reaction solution was concentrated to about half of its volume, carefully acidified with concentrated HCl, and extracted with CH_2Cl_2 (thrice). The organic phases were washed with H_2O (twice), treated with C, dried (Na_2SO_4), and evaporated to dryness. The product was crystallized from CH_2Cl_2–petroleum ether to give **6** (11.9 g, 75%), mp 137–139 °C. Anal. ($C_{15}H_{20}O_3$) C, H, O.

4-Cyclohexyl-2-hydroxyphenylacetic Acid (7). 4-Cyclohexyl-2-methoxyphenylacetic acid (**6**; 6.0 g, 0.024 mol) was dissolved in dry CH_2Cl_2 (100 mL), and BBr_3 (12 mL, 0.13 mol) was added dropwise at 0 °C while stirring. After 1 h the reaction mixture was added dropwise to 2 N NaOH (200 mL) while stirring and cooling. The solution was acidified to pH 1 with 2 N HCl and extracted with CH_2Cl_2 (thrice). The organic phase was washed with H_2O (twice) and dried (Na_2SO_4), and the solvent was evaporated. The residue was recrystallized from CH_2Cl_2–petroleum ether to yield **7** (3.5 g, 62%), mp 109–110 °C. Anal. ($C_{14}H_{18}O_3$) C, H, O.

3-Bromo- (9) and 5-Bromo-4-cyclohexyl-2-hydroxyphenylacetic Acid (8). 4-Cyclohexyl-2-hydroxyphenylacetic acid (**7**; 7 g, 0.03 mol), *N*-bromosuccinimide (5.3 g, 0.03 mol), and succinimide (6 g, 0.06 mol) in dry CH_2Cl_2 (500 mL) were stirred for 5 days at room temperature. The mixture was washed with water (thrice), dried (Na_2SO_4), and evaporated to dryness. The residue was purified by crystallization from ether–petroleum ether. The first crop contained 3-bromo-4-cyclohexyl-2-hydroxyphenylacetic acid (**9**, 2.6 g, 28%) and the third crop its 5-bromo isomer (**8**; 3.8 g, 40%). Both products were used for the next step without purification.

4-Cyclohexyl-2-hydroxy-5-nitrophenylacetic Acid (10). HNO_3 (100%, 3.9 mL) was added slowly to a solution of 4-cyclohexyl-2-hydroxyphenylacetic acid (18.7 g, 0.08 mol) in dry CH_2Cl_2 (300 mL) while stirring at −10 °C. The solution was allowed to come to room temperature and stirred for another 2 h. The solution was washed with H_2O (thrice), dried (Na_2SO_4), and evaporated to dryness. The crystalline residue consisted of **10** (7.1 g, 31%), which was employed without further purification. The mother liquor contained mainly the 3-nitro isomer.

6-Cyclohexyl-2,3-dihydrobenzofuran-2-one (14). (i) 4-Cyclohexyl-2-methoxyphenylacetic acid (**6**; 35 g, 0.14 mol) in CH_2Cl_2 (600 mL) was treated dropwise with BBr_3 (30 mL, 0.32 mol) at 0 °C while stirring. After stirring for 1 h at 22 °C, the reaction mixture was added dropwise to ice–H_2O while stirring and cooling. The solution was extracted with CH_2Cl_2 (thrice). The extract was washed with water (thrice), dried (Na_2SO_4), and evaporated to dryness. The residue and *p*-toluenesulfonic acid (100 mg) were dissolved in toluene (300 mL) and refluxed for 3 h with removal of H_2O via a Dean–Stark trap. The solvent was removed in vacuo. Chromatography on 200 g of silica gel (elution with CH_2Cl_2) and recrystallization of the eluate from ether–petroleum ether gave 24.7 g of pure **14**. Crystallization of the mother liquor gave an additional 3.9 g (total yield 94%) of the lactone **14**: mp 80–81 °C; IR (CH_2Cl_2) 1805, 1630, 1590 cm^{-1}; NMR ($CDCl_3$) 2.47 (br s, 1 H), 3.57 (s, 2 H), 6.87 (s, 1 H), 6.92 (d, 1 H, J = 7 Hz), 7.11 (d, 1 H, J = 7 Hz). Anal. ($C_{14}H_{16}O_2$) C, H, O.

(ii) Treatment of pure 4-cyclohexyl-2-hydroxyphenylacetic acid (**7**) with *p*-toluenesulfonic acid in boiling toluene as described in i gave **14**, which was identical with the previous sample.

(iii) Dithiolane **35** (306 mg, 0.001 mol) was dissolved in ethyl acetate (20 mL) and treated with moist Raney nickel (1 g). The reaction mixture was stirred at room temperature for 16 h, filtered, dried (Na_2SO_4), and evaporated in vacuo. Recrystallization from $CHCl_3$–hexane furnished **14** (100 mg, 46%), which was identical with the previous sample.

6-Cyclohexyl-2,3-dihydro-3-methylbenzofuran-2-one (15). (i) Hydroxy acid **37** was cyclized with *p*-toluenesulfonic acid in boiling toluene under the conditions described for **14**. Crystallization from petroleum ether gave **15** (95% yield): mp 87–89 °C; IR (CH_2Cl_2) 1800, 1630, 1595 cm^{-1}; NMR ($CDCl_3$) δ 1.52 (d, 3 H, J = 8 Hz), 3.66 (m, 1 H), 6.9–7.3 (m, 3 H). Anal. ($C_{15}H_{18}O_2$) C, H, O.

(ii) Compound **39** (288 mg, 0.001 mol) was hydrogenated in ethyl acetate at 1 atm using 30 mg of 10% Pd/C. Filtration, followed by evaporation of the solvent and crystallization of the crude material from $CHCl_3$–hexane, gave **15**, which was identical with the sample described above. The hydrogenation gave better yields (90%) when performed in a mixture of dioxane and phosphate buffer (pH 7).

5-Chloro-6-cyclohexyl-2,3-dihydrobenzofuran-2-one (16). Crude hydroxy acid **44** was converted to the γ-lactone in analogy to the procedure described for **14**. The product (**16**) was crystallized from ether–hexane: mp 100–102 °C; NMR ($CDCl_3$) δ 3.02 (m, 1 H), 3.68 (s, 2 H), 6.99 (s, 1 H), 7.22 (s, 1 H). Anal. ($C_{14}H_{15}ClO_2$) C, H, Cl, O.

5-Bromo-6-cyclohexyl-2,3-dihydrobenzofuran-2-one (17). Compound **8** was cyclized to the corresponding γ-lactone as described for **14**: mp 124–126 °C; NMR (Me_2SO-d_6) δ 2.9 (m, 1 H), 3.95 (s, 2 H), 7.27 (s, 1 H), 7.66 (s, 1 H). Anal. ($C_{14}H_{15}BrO_2$) C, H, Br, O.

7-Bromo-6-cyclohexyl-2,3-dihydrobenzofuran-2-one (18). The hydroxy acid **9** was converted to the respective γ-lactone **18**: mp 108–109 °C; NMR (Me_2SO-d_6) δ 2.9 (m, 1 H), 3.97 (s, 2 H), 7.06 (d, 2 H, J = 7 Hz), 7.25 (d, 2 H, J = 7 Hz). Anal. ($C_{14}H_{15}BrO_2$) C, H, Br, O.

4-Cyclohexyl-2,3-dihydro-5-nitrobenzofuran-2-one (19). Compound **10** was cyclized to **19** as described for **14**: mp 132–133 °C; NMR ($CDCl_3$) δ 3.1 (m, 1 H), 3.8 (s, 2 H), 7.16 (s, 1 H), 7.73 (s, 1 H). Anal. ($C_{14}H_{15}NO_4$) C, H, N, O.

6-Cyclohexyl-2,3-dihydro-5-methylbenzofuran-2-one (20). Compound **48** (2.9 g, 0.011 mol) was cleaved with BBr_3 and cyclized to the γ-lactone as described for **14**. Crystallization from ether–hexane gave pure **20** (2.2 g, 87%): mp 157–158 °C; NMR ($CDCl_3$) δ 2.28 (s, 3 H), 2.7 (br, 1 H), 3.61 (s, 2 H), 6.93 (s, 1 H), 7.00 (s, 1 H). Anal. ($C_{15}H_{18}O_2$) C, H, O.

5-Chloro-6-cyclohexyl-2,3-dihydro-3-methylbenzofuran-2-one (22). The chloro derivative **43** was cleaved with BBr_3 (cf. preparation of **44**), yielding the acid **45**. The latter was cyclized to the desired γ-lactone **22** (cf. preparation of **14**), mp 92–93 °C. Anal. ($C_{15}H_{17}ClO_2$) C, H, O.

2,3-Dihydro-5-methoxy-6-phenylbenzofuran-2-one (29). 5-Hydroxy-2-methoxybiphenyl (**50**) was converted to the benzofuranone derivative **29** according to method B (cf. Scheme II, **33** → **34** → **35** → **14**): mp 132–133 °C; NMR ($CDCl_3$) δ 3.76 (s, 5 H), 6.92 (s, 1 H), 7.05 (s, 1 H), 7.4 (m, 5 H). Anal. ($C_{15}H_{12}O_3$) C, H, O.

2,3-Dihydro-5-hydroxy-6-phenylbenzofuran-2-one (30). The 5-methoxy derivative **29** was treated with BBr_3 in CH_2Cl_2 as described for other ether cleavages (cf. **6** → **7**): mp 149–150 °C; NMR ($CDCl_3$) δ 3.71 (s, 2 H), 5.28 (s, 1 H), 6.8–7.6 (m, 7 H). Anal. ($C_{14}H_{10}O_3$) C, H, O.

3-Cyclohexylphenol (33). 3-Cyclohexylanisol (**4**) was cleaved with BBr_3 in CH_2Cl_2 instead of HBr in AcOH (cf. ref 10).

4-Cyclohexyl-2-hydroxy-α-oxophenylacetic Acid (34). 3-Cyclohexylphenol (**33**; 3.4 g, 0.019 mol) was dissolved in CH_2Cl_2 (100 mL) and cooled in an ice bath. $AlCl_3$ (5.3 g, 0.04 mol) was added, and the mixture was stirred for 30 min. A solution of oxalyl chloride (3.54 g, 0.028 mol) in CH_2Cl_2 (20 mL) was added slowly dropwise. After stirring at 0 °C for 2 h, the solution was poured into ice and left at room temperature overnight. The mixture was extracted with CH_2Cl_2 (thrice). The organic phase was washed with H_2O (twice), followed by extraction with 3% NaOH (twice). The basic extract was acidified, and the product was isolated by CH_2Cl_2 extraction (twice). The CH_2Cl_2 layers were combined, dried (Na_2SO_4), and concentrated. Crystallization of the residue from ether–hexane gave **34** (2.7 g, 56%), mp 91–93 °C. Anal. ($C_{14}H_{16}O_4$) C, H, O.

6-Cyclohexyl-2,3-dihydro-3-(1,3-dithiolan-2-yl)benzofuran-2-one (35). A solution of **34** (2.5 g, 0.01 mol), ethane–dithiol (2 mL), and *p*-toluenesulfonic acid (100 mg) was refluxed for 18 h with removal of H_2O via a Dean–Stark trap. After cooling, the reaction mixture was washed with saturated NaCl solution and dried (Na_2SO_4), and the solvent was removed in vacuo. Crystallization of the crude product from $CHCl_3$–hexane gave **35** (1.68 g, 55%): mp 156–158 °C. Anal. ($C_{16}H_{18}O_2S_2$) C, H, O.

2-(3-Cyclohexylphenoxy)propionic Acid (36). NaH (11 g of a 55% oil dispersion, 0.25 mol) was washed with petroleum ether under an atmosphere of N_2, and THF (500 mL) was added. 3-Cyclohexylphenol (**33**; 44 g, 0.25 mol) in dry THF (500 mL) was added dropwise within ca. 0.5 h while stirring. The reaction mixture was then treated dropwise with ethyl 2-bromopropionate (33 mL, 0.25 mol) while stirring. The solution was kept at 60 °C

for 1 h and then at room temperature for 60 h. The solvent was evaporated in vacuo, and the residue was taken up in H_2O and extracted with CH_2Cl_2 (thrice). The organic phase was washed with (H_2O), dried (Na_2SO_4), and evaporated to dryness. The residue (70 g) without purification was refluxed in 5% methanolic KOH (1 L) for 2 h. The solvent was removed in vacuo. The residue was dissolved in H_2O, acidified (pH 2) with 2 N HCl while cooling, and extracted with CH_2Cl_2 (thrice). The extracts were washed with H_2O (twice) and dried (Na_2SO_4), and the solvent was removed in vacuo. Crystallization from petroleum ether gave 38 g of **36**. Concentration of the mother liquor yielded another 15 g (total yield 85%), mp 80–81 °C. Anal. ($C_{15}H_{20}O_3$) C, H, O.

2-(4-Cyclohexyl-2-hydroxyphenyl)propionic Acid (37). (i) **36** (2 g, 0.0081 mol) in 95% EtOH (180 mL) while cooling with water was irradiated with a 150-W mercury high-pressure lamp for 2 h in an atmosphere of argon. The solvent was removed in vacuo, and the residue was chromatographed on 150 g of silica gel. With ethyl acetate–hexane (1:1), 3-cyclohexylphenol (**33**; 550 mg) was eluted. Further elution with ethyl acetate afforded **37** (250 mg, 12%), mp 128–130 °C. Anal. ($C_{15}H_{20}O_3$) C, H, O.

(ii) Better yields of **37** were obtained by cleavage of **41** with BBr_3 (cf. **6** → **7**).

3-Acetoxy-6-cyclohexyl-2,3-dihydro-3-methylbenzofuran-2-one (39). Methyllithium (6 mL, 2 N in ether) was added to a solution of **34** (992 mg, 0.004 mol) in THF (100 mL) at −78 °C under an atmosphere of argon. After 3 h at room temperature, the solution was poured into buffer solution (pH 7), extracted with ethyl acetate, dried (Na_2SO_4), and evaporated to dryness. The intermediate product (**38**) without purification was dissolved in acetic anhydride (5 mL) and refluxed for 20 min. Evaporation in vacuo and filtration through silica gel (6 g) with benzene gave pure **39** (206 mg, 18%), mp 137–139 °C.

Ethyl 4-Cyclohexyl-2-methoxyphenylacetate (40). A stream of HCl was passed through a solution of 4-cyclohexyl-2-methoxyphenylacetic acid (**6**; 10 g) in EtOH (100 mL) for 30 min. The solution was evaporated to dryness, and the residue was chromatographed on silica gel (200 g). With $CHCl_3$, pure ester (**40**) was eluted, which was used for the subsequent reaction without further purification.

Ethyl 4-Cyclohexyl-2-methoxy-α-methylphenylacetate (41). *n*-Butyllithium (2.2 N in hexane, 16.2 mL, 0.036 mol) was added dropwise at −78 °C while stirring under an atmosphere of N_2 to a solution of *N*-cyclohexylisopropylamine (5.9 mL, 0.036 mol) in dry THF (350 mL). Stirring was continued for 15 min at −78 °C. Ethyl 4-cyclohexyl-2-methoxyphenylacetate (**40**; 8.3 g, 0.030 mol) dissolved in THF (150 mL) was added slowly, and stirring was continued for another 20 min at −78 °C. A solution of methyl iodide (2.24 mL, 0.036 mol) in a mixture of HMPT (6.45 g, 0.036 mol) and THF (50 mL) was added slowly. The reaction was allowed to come to room temperature, and stirring was continued for 18 h. The solution was poured onto a mixture of 1 N HCl (100 mL) and ice (100 g) and extracted with CH_2Cl_2 (thrice). The extracts were washed with H_2O, combined, dried (Na_2SO_4), and evaporated to dryness. The residual oil (11.9 g) was chromatographed on silica gel (1.2 kg). Elution with hexane–ethyl acetate (7:1) gave pure **41**, as an oil (8.5 g, 98%), which was used for the next step without further purification.

Ethyl 5-Chloro-4-cyclohexyl-2-methoxyphenylacetate (42). SO_2Cl_2 (63 mL, 0.79 mol) was added slowly at room temperature to a well-stirred solution of ethyl 4-cyclohexyl-2-methylphenylacetate (**40**; 218 g, 0.79 mol) in dry CH_2Cl_2 (1 L), and stirring was continued overnight. Air was blown through the solution for 30 min, and the solvent was removed in vacuo. The residual oil consisted of almost pure **42** (225 g, 92%) and was used without further purification.

Ethyl 5-Chloro-4-cyclohexyl-2-methoxy-α-methylphenylacetate (43). Ethyl 4-cyclohexyl-2-methoxy-α-methylphenylacetate (**41**) was reacted with SO_2Cl_2 as described for the preparation of **42**. The crude product (**43**) was used without further purification.

5-Chloro-4-cyclohexyl-2-hydroxyphenylacetic Acid (44). BBr_3 (400 mL) was added slowly to a solution of ethyl 5-chloro-4-cyclohexyl-2-methoxyphenylacetate (**42**; 225 g, 0.72 mol) in CH_2Cl_2 (1 L) while stirring at 0 °C. Stirring was continued for 1 h at room temperature. The reaction mixture was poured onto ice (500 g). The organic layer was washed with water (twice), dried (Na_2SO_4), and evaporated to dryness. The residue was treated with 2 N ethanolic NaOH (500 mL) at room temperature for 4 h. The solution was acidified, concentrated, diluted with H_2O, and extracted with $CHCl_3$ (twice). The extracts were washed with H_2O (twice), dried (Na_2SO_4), and evaporated to dryness. The crystalline residue (120 g) consisted of crude **44** and was used without purification.

4-Cyclohexyl-5-formyl-2-methoxyphenylacetic Acid (46). $TiCl_4$ (16 mL) was added at 0 °C to a solution of 4-cyclohexyl-2-methoxyphenylacetic acid (**6**; 18.1 g, 0.073 mol) in CH_2Cl_2 (350 mL). Dichloromethyl methyl ether (6.3 mL, 0.073 mol) was added dropwise while stirring. The reaction mixture was allowed to come to room temperature, and stirring was continued for 1 h. The solution was poured onto ice and extracted with ethyl acetate (twice). The extract was washed with H_2O (twice) and dried (Na_2SO_4). The solvent was removed in vacuo, and the residue was crystallized from CH_2Cl_2–hexane to give **46** (14.5 g, 72%): mp 128–129 °C; NMR ($CDCl_3$) δ 3.63 (s, 2 H), 3.87 (s, 3 H), 6.82 (s, 1 H), 7.62 (s, 1 H), 10.18 (s, 1 H). Anal. ($C_{16}H_{20}O_4$) C, H, O.

4-Cyclohexyl-2-methoxy-5-(hydroxymethyl)phenylacetic Acid (47). A solution of 4-cyclohexyl-5-formyl-2-methoxyphenylacetic acid (**46**; 7 g, 0.025 mol) in THF (450 mL) was treated with several portions of $NaBH_4$ (4 g, 0.110 mol). The mixture was refluxed for 40 min, cooled, and poured on crushed ice (200 g), acidified, and extracted with ethyl acetate (thrice). The extracts were washed with saturated NaCl solution (thrice), combined, dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from ethyl acetate–petroleum ether to give **47** (6.7 g, 95%): mp 169–171 °C; NMR (Me_2SO-d_6) δ 3.40 (s, 2 H), 3.70 (s, 3 H), 4.41 (s, 2 H), 6.77 (s, 1 H), 7.05 (s, 1 H).

4-Cyclohexyl-2-methoxy-5-methylphenylacetic Acid (48). The hydroxymethyl derivative **47** (6.5 g, 0.023 mol) in ethyl acetate (150 mL) was hydrogenated at 2 atm and room temperature for 14 h using 10% Pd/C. Filtration, followed by evaporation of the solvent and crystallization from petroleum ether, gave **48** (5.5 g, 91%), mp 124–126 °C. Anal. ($C_{16}H_{22}O_3$) C, H, O.

2-Methoxy-5-(phenylazo)biphenyl (49). Aniline (3.1 g, 0.033 mol) was dissolved in a mixture of concentrated HCl (8.2 mL, 0.096 mol) and ice–H_2O (60 mL), and treated dropwise with $NaNO_2$ (2.3 g, 0.033 mol) in H_2O (5 mL) while stirring at 0 °C. In a separate reaction flask, 2-hydroxybiphenyl (5.1 g, 0.03 mol) was dissolved in a solution of NaOH (4.4 g, 0.11 mol) in H_2O (60 mL). To this solution was added slowly the cold solution of phenyldiazonium chloride at 0–2 °C while stirring. The reaction mixture was filtered, and the filtrate was neutralized by treating it with dry ice (ca. 5 g). The mixture was extracted with toluene (twice). The organic phase was treated with 2 N NaOH (thrice). The basic extract was extracted with toleuen (twice) and acidified (pH 3). The product was filtered off and recrystallized from ether–petroleum ether to yield 2-hydroxy-5-(phenylazo)biphenyl (5.1 g, 62%), mp 91–92 °C.

NaH (290 mg of a 55% oil dispersion, 0.0067 mol) was washed with petroleum ether under an atmosphere of N_2, and DMF (30 mL) was added. 2-Hydroxy-5-(phenylazo)biphenyl (1.37 g, 0.005 mol) was added, and the solution was warmed to 70 °C.

The reaction mixture was treated dropwise with CH_3I (2.82 g, 1.65 mL, 0.01 mol) and kept at 70 °C for 2 h. Methanol (2 mL) was added, and the solvent was evaporated in vacuo. The residue was taken up in H_2O and extracted with ether (thrice). The organic phase was washed with 2 N NaOH (once) and H_2O (twice), dried (Na_2SO_4), and concentrated in vacuo. Crystallization from ether–petroleum ether gave, in two crops, 1.15 g of **49** (80% from 2-hydroxy-5-(phenylazo)biphenyl): mp 87–88 °C; NMR ($CDCl_3$) δ 3.85 (s, 3 H), 7.0–7.95 (m, 13 H). Anal. ($C_{19}H_{16}N_2O$) C, H, N.

5-Hydroxy-2-methoxybiphenyl (50). 2-Methoxy-5-(phenylazo)biphenyl (**49**; 3.4 g, 0.012 mol) was hydrogenated in ethanol (50 mL) at 1 atm using 450 mg of 10% Pd/C. The reaction mixture was filtered and evaporated in vacuo. The residue was distilled in vacuo, yielding 5-amino-2-methoxybiphenyl (2.3 g): bp 138–143 °C (0.1 mm); NMR ($CDCl_3$) δ 3.35 (d, 2 H), 3.64 (s, 3 H), 6.5–7.5 (m, 8 H).

5-Amino-2-methoxybiphenyl (1.7 g, 0.0085 mol) was dissolved in AcOH (3 mL) and added to a mixture of concentrated H_2SO_4 (0.7 mL) and ice–H_2O (15 mL). $NaNO_2$ (620 mg) in H_2O (4.5 mL) was added dropwise while stirring at 0 °C. Stirring was continued for 30 min. Urea was added until the KI–starch paper test was

negative. The cold solution was slowly added under the surface of boiling 2 N H_2SO_4 (75 mL). Boiling was continued for 1 h. The solution was allowed to come to room temperature and extracted with CH_2Cl_2 (thrice). The organic phase was washed with H_2O (twice), dried (Na_2SO_4), and evaporated to dryness. Chromatography on 30 g of silica gel (elution with CH_2Cl_2) gave pure **50** (990 mg, 57% from 5-amino-2-methoxybiphenyl) as an oil: IR (CH_2Cl_2) 3600 cm^{-1}; NMR ($CDCl_3$) δ 3.74 (s, 3 H), 5.35 (s, 1 H), 6.87 (s, 3 H), 7.3–7.7 (m, 5 H).

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Synthesis of Some Novel Amodiaquine Analogues as Potential Antimalarial and Antifilarial Compounds

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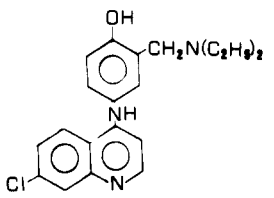
Ten amodiaquine analogues, which are hybridized molecules of amodiaquine and diethylcarbamazine, were designed and synthesized. Six analogues, all bearing a basic tertiary amino function at their side chain, were active against *Plasmodium berghei* in mice and inhibited the mobility of adult worms and microfilariae of *Breinlia booliati* in vitro. They were inactive against *Litomosoides carinii* in *Mastomys natalensis*. The most active antimalarial compound, 7-chloro-4-[α-[[*N*-(4-methyl-1-piperazinyl)carbonyl]amino]-4-hydroxy-*m*-toluidino]quinoline, had twice the activity of amodiaquine. O-Methylation and N-ethylation generally reduced antimalarial activity. Analogues which lack a basic tertiary amino function at their side chain were also lacking in both antimalarial and antifilarial activities.

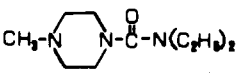
Malaria and filariasis are notable for their overlapping distribution in many parts of Asia and Africa. The development of an active agent against both malaria and filariasis would clearly have the advantages of convenience and economy in its usage, especially for mass chemotherapy and prophylaxis in endemic areas. A rational approach to the design of such a dual-acting agent is to start from a parent molecule which has both antimalarial and antifilarial properties. A satisfactory candidate is amodiaquine[1] (**1**).



1

The 4-aminoquinolines amodiaquine and chloroquine[2] are among the most widely used drugs for the treatment of malaria. Thompson and co-workers[3] reported that amodiaquine when given orally to Mongolian gerbils infected with *Litomosoides carinii* at doses of 25–100 (mg/kg)/day × 5 elicited strong macrofilaricidal action. However, it had no direct action on the circulating microfilariae. Subsequent clinical trials in man revealed that amodiaquine in a total dose of 40 mg/kg was also macrofilaricidal in bancroftian filariasis.[4] Unfortunately, because of the unpleasant side effects at this dose level (dizziness, nausea, and vomiting) and the possibility of inducing blood dyscrasias,[5] mass chemotherapy of filariasis with amodiaquine could not be recommended.[4]

In this work, we have designed five series of potential dual-acting amodiaquine analogues based on the principles of drug hybridization.[6] In this approach, amodiaquine is condensed with a potent microfilaricidal agent, diethylcarbamazine (DEC, **2**), in such a way that the important functional groups and ideal conformations of both drugs are retained.[7,8]



2

Structure–activity studies have shown that wide liberties can be taken in the modification of the side chain of amodiaquine without loss of antimalarial activity.[7] Through drug hybridization, we hope to obtain dual-acting amodiaquine analogues which may still retain the main therapeutic activities of the parent molecule. Since DEC is essentially a microfilaricidal agent,[9] the hybridized molecules may possess the antimalarial and macrofilaricidal activities of amodiaquine, with additional microfilaricidal activity imparted by the DEC moiety.

The design of these hybridized amodiaquine–DEC molecules has been accomplished by replacing the diethylamino function of amodiaquine with the N_1, N_2, and N_3 nitrogen of DEC (Scheme I). In this way, compounds **12a,b** (series A), **13a,b** (series B), **14a,b** (series C), **15a,b** (series D), and **16a,b** (series E) were designed and subsequently synthesized.

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