Synthesis and Quantitative Structure-Activity Relationships of Diclofenac Analogues

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The synthesis of a series of 2-anilinophenylacetic acids, close analogues of diclofenac, is described. These compounds were tested in two models used for evaluating the activity of nonsteroidal antiinflammatory drugs (NSAID's), inhibition of cyclooxygenase enzyme activity in vitro, and adjuvant-induced arthritis (AdA) in rats. Statistically significant correlations were found between the inhibitory activities of the compounds in these two models, indicating that cyclooxygenase inhibition seems to be the underlying mechanism for the antiinflammatory activity of these compounds. Quantitative structure-activity relationship (QSAR) analysis revealed that the crucial parameters for activity in both models were the lipophilicity and the angle of twist between the two phenyl rings. Optimal activities were associated with halogen or alkyl substituents in both ortho positions of the anilino ring. Compounds with OH groups in addition to two ortho substituents or compounds with only one or no ortho substituents were less active.

Diclofenac (sodium [2-[(2,6-dichlorophenyl)amino]-phenyl]acetate, Figure 1) is a potent NSAID (nonsteroidal antiinflammatory drug) therapeutically used in inflammatory and painful diseases of rheumatic and nonrheumatic origin.[1]

Like other NSAID's, diclofenac interacts with the arachidonic acid cascade at the level of cyclooxygenase. This fundamental mechanism for the action of NSAID's was first described by Vane.[2] Diclofenac inhibits cyclooxygenase at micromolar concentrations, and as a consequence, the formation of thromboxanes, prostaglandins, and prostacyclin is inhibited under various experimental and clinical conditions. The competitive character of the inhibition of the cyclooxygenase-arachidonic acid interaction and the structural similarity of most NSAID's with certain conformers of arachidonic acid indicates that at least one binding site is common to NSAID's and arachidonic acid.

Various receptor models have been proposed. However, since no X-ray structure determination of the enzyme exists, all models of the active site have been derived by complementary cavity mapping. Scherrer et al.[3] have designed a hypothetical receptor area to accommodate competitive inhibitors of UV-erythema and bradykinin induced bronchoconstriction (e.g. mefenamic acid and indomethacin). Using the results of conformational studies on indomethacin, Shen[4] proposed an antiinflammatory receptor site for this molecule, a feature of which was the presence of a cavity to accommodate the *p*-chlorobenzoyl substituent, which has been shown to be twisted out of plane and out of conjugation with the indole nucleus.

The proposals of Gund et al.[5] and Salvetti[7] were based upon the assumption that the carboxyl groups of the NSAID's, which inhibit PG synthetase, competitively bind to the enzyme at the same site as the carboxyl group of the fatty acid substrate. Appleton and Brown[6] and Nicholson et al.[8] assumed that the NSAID's carboxyl groups should rather be located at that part of the enzyme normally occupied by the 11-peroxy radical moiety of arachidonic acid. Peterson et al.[9] postulated a heme-arachidonic acid interaction in PG synthesis, whereby oxygen bound to Fe^{2+} of the heme group adds to C11 of arachidonic acid.

In the early 1960s, it was decided to develop a new class of highly potent antiinflammatory drug. The chemical structure of the target compound was rationally designed based on information on structure-activity relationships of antiinflammatory drugs available at that time: acetylsalicylic acid, phenylbutazone, mefenamic acid, ibuprofen, and indomethacin. By comparison of these drugs, important physicochemical and structural properties associated with antiinflammatory activity were discerned.

Structurally, all, except acetylsalicylic acid and ibuprofen, have two aromatic rings which are capable of forming a twisted conformation relative to each other. This was regarded as conducive to a proper fit to the receptor site of an enzyme, unknown at that time, later identified as arachidonic acid cyclooxygenase.[2] However, Scherrer and Shen's relatively crude hypothetical receptor outlines were already available. Using these, space-filling stereomodels of the NSAID's mentioned were fitted to derive the spacial requirements for the new molecules to be synthesized. The main conclusion reached was that the two rings should be twisted as much as possible.

Regarding the physicochemical properties of these drugs, their acidities (pK values between 4 and 5) are such that at physiological pH of 7.4 the molecules are dissociated to more than 99% and thus are essentially confined to plasma and intra- and extracellular water.

Their octanol/water partition coefficients at physiological pH, log P (or log D, the distribution coefficient, as the partition coefficient is often called in the case of partly ionized compounds), are in the range of log P 0.7-2.0, which is considered optimal for the molecules to readily cross biological membranes. It is the partition coefficient which largely determines the pharmacodynamic behavior of any drug, including its absorption, binding to proteins, and excretion.[10] It has, for instance, recently been shown, that for 12 clinically active NSAID's the rate of transport from an aqueous to a lipophilic medium (*n*-octanol) has

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(1) Skoutakis, V. A.; Carter, Ch. A.; Mickle, T. R.; Smith, V. H.; Arkin, Ch. R.; Alissantros, J.; Petty, D. A. *Drug Intelligence Clin. Pharm.* **1988**, *22*, 850.
(2) Vane, J. R. *Nature (London), New Biol.* **1971**, *231*, 232.
(3) Scherrer, R. A.; Winder, C. V.; Short, F. W. In Abstracts of the 9th National Medicinal Chemistry Symposium; American Chemical Society: Washington, DC, 1964; Abstract 11G.
(4) Shen, Z. Y. In International Congress Series No. 82; Garattini S., Dukes M. N. G. Eds.; Excerpta Medica Foundation: Amsterdam, 1965; p 13.
(5) Gund, P.; Shen, T. Y. *J. Med. Chem.* **1977**, *20*, 1146.
(6) Appleton, R. A.; Brown, K. *Prostaglandins* **1979**, *18*, 29.
(7) Salvetti, F.; Buttinoni, A.; Cesarini, R. *Eur. J. Med. Chem.* **1981**, *16*, 81.
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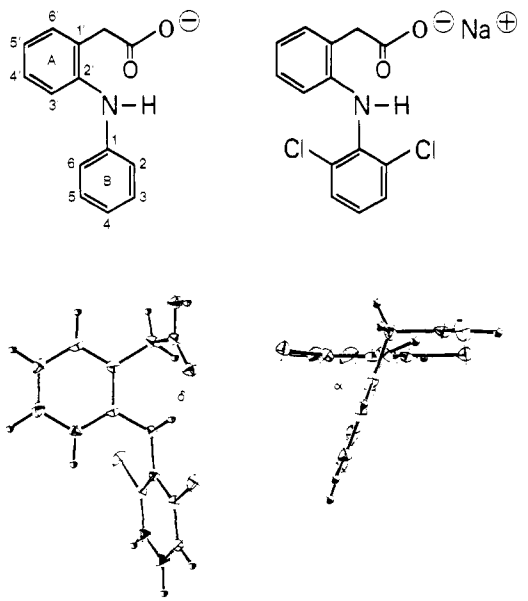
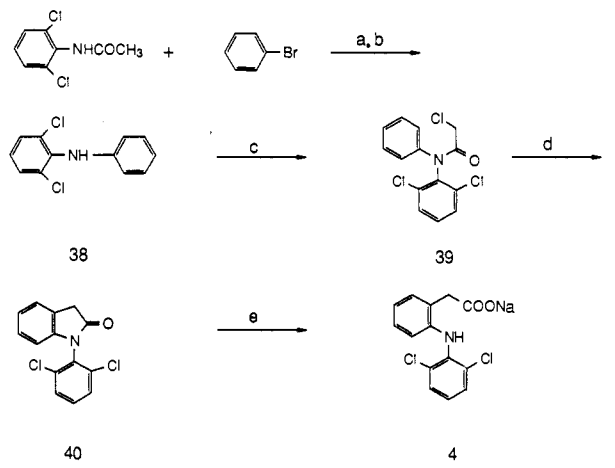


Figure 1. Upper left: general formula I of the phenylacetic acids used in this study. Upper right: diclofenac. Bottom left: Topography of diclofenac acid from X-ray analysis, side view showing the hydrogen bridge, δ = 2.00 Å. Bottom right: view from top showing angle of twist, α = 69°.

Scheme I[a]



[a] (a) K_2CO_3, Cu; (b) KOH, EtOH; (c) $ClCH_2COCl$; (d) $AlCl_3$, 160 °C; (e) NaOH, EtOH.

a clear maximum at log P = 1.0.[11]

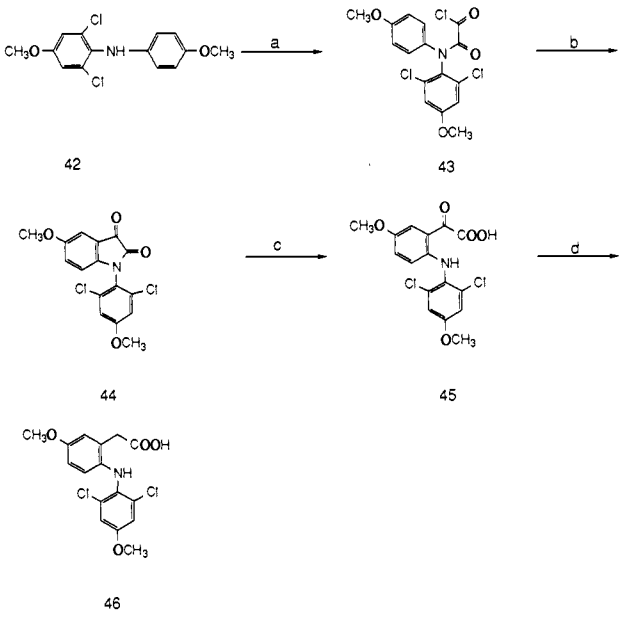
During the synthesis of the planned analogues, care was taken to ensure that the molecules conformed to these basic physicochemical and structural prerequisites. Diclofenac has a dissociation constant near pH 4 and a partition coefficient near 10. The X-ray analysis of diclofenac (Figure 1) shows a substantial angle of twist (α) between the two aromatic rings but also an intramolecular hydrogen bond (δ) between the carboxyl oxygen and the amino hydrogen.[12]

In the present paper we report the synthesis and physicochemical properties as well as the antiinflammatory potency and the inhibition of prostaglandin synthetase in vitro for a series of 36 analogues of diclofenac. Out of 120 analogues, representing a broad substitution pattern, the compounds used in this study were chosen according to

(11) Rodriguez, L.; Zecchi, V.; Tartarini, A. *Pharm. Acta Helv.* 1984, *59*, 95.

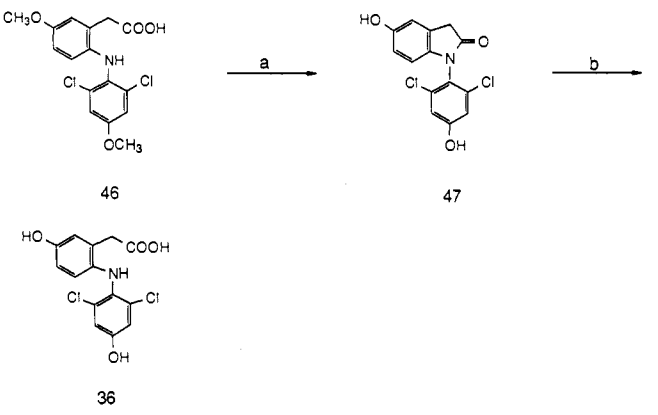
(12) Sallmann, A. The history of diclofenac. *Am. J. Med.* 1986, *80*, (*suppl. 4B*), 29.

Scheme II[a]



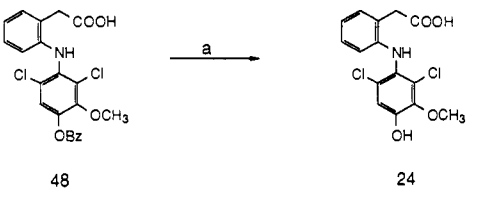
[a] (a) ClCOCOCl, benzene; (b) $AlCl_3$, $Cl_2CHCHCl_2$; (c) NaOH, EtOH, and HCl; (d) CH_3ONa, $NH_2NH_2 \cdot H_2O$, $CH_3OCH_2CH_2OH$, and HCl.

Scheme III[a]



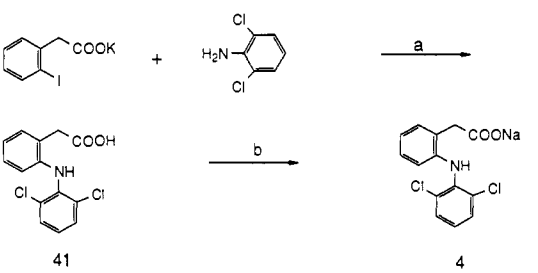
[a] (a) Pyridine·HCl, 170 °C; (b) NaOH, n-C_4H_9OH, and HCl.

Scheme IV[a]



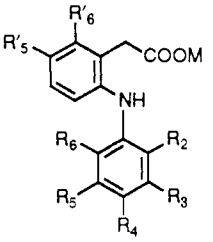
[a] (a) H_2, Pd–C (5%), THF, 1,2-dichlorobenzene.

Scheme V[a]



[a] (a) K_2CO_3, Cu powder, N-methylpyrrolidone, 120 °C, 2 N HCl; (b) aqueous Na_2CO_3.

Table I. Substituted 2-Anilinophenylacetic Acids



compd	R_2	R_3	R_4	R_5	R_6	R'_5	R'_6	M	method[a]	% yield	mp, °C	formula[g]
1	Cl		F		Cl	F		Na	E[b]	53	163-164	$C_{14}H_8Cl_2F_2NO_2Na$
2	Cl				Cl	F		Na	E[b]	44	180-182	$C_{14}H_9Cl_2FNO_2Na$
3	Cl				Cl	Cl		Na	A[c]	47	181-183	$C_{14}H_9Cl_3NO_2Na$
4	Cl				Cl			Na	A,[c] B,[c] E[d]	46, 64, 56	181-183	$C_{14}H_{10}Cl_2NO_2Na$
5	Cl		OMe		Cl			H	B[c]	42	129-132	$C_{15}H_{13}Cl_2NO_3$
6	Cl				Me			H	B[c]	54	143-147	$C_{15}H_{14}ClNO_2$
7	Cl		F		Cl			Na	E[d]	51	150-154	$C_{14}H_9Cl_2FNO_2Na$
8	Cl				Br			Na	A[c]	42	>250	$C_{14}H_{10}BrClNO_2Na$
9	Me	Me						H	E[d]	40	112-113	$C_{16}H_{17}NO_2$
10	Cl				I			H	B[c]	53	165-175	$C_{14}H_{11}ClINO_2$
11	Me	Me		Me	Me			H	F[e]	32	118-120	$C_{18}H_{21}NO_2$
12	Me				Me			H	B[c]	53	125-128	$C_{16}H_{17}NO_2$
13	Cl	Cl						H	F[e]	61	136-138	$C_{14}H_{11}Cl_2NO_2$
14	Cl	OH			Cl			H	B,[c] C	45	159-162	$C_{14}H_{11}Cl_2NO_3$
15	Me	Cl						H	B[c]	47	125-126	$C_{15}H_{14}ClNO_2$
16	Cl	OMe			Cl			H	B[c]	57	180-184	$C_{15}H_{13}Cl_2NO_3$
17	Cl	Me			Cl			Na	A[c]	45	147-149	$C_{15}H_{12}Cl_2NO_2Na$
18	Cl				Cl		Br	H	A[c]	17	178-180	$C_{14}H_{10}BrCl_2NO_2$
19	Cl	Me						H	E[d]	52	130-132	$C_{15}H_{14}ClNO_2$
20	Cl				F			Na	A[c]	62	265-270	$C_{14}H_{10}ClFNO_2Na$
21	Me	Me		Me	Me	Cl		H	B[c]	35	100-104	$C_{18}H_{20}ClNO_2$
22	Et				Et			H	B[c]	28	90-92	$C_{18}H_{21}NO_2$
23	Cl		Cl					H	A[c]	59	159-162	$C_{14}H_{11}Cl_2NO_2$
24	Cl	OMe	OH		Cl			H	F,[e] D	45	166-168	$C_{15}H_{13}Cl_2NO_4$
25		Cl		Cl				H	F[e]	62	138-140	$C_{14}H_{11}Cl_2NO_2$
26	Cl			Cl				H	E[d]	48	150-153	$C_{14}H_{11}Cl_2NO_2$
27	F		F					Na	F[e]	25	138-140	$C_{14}H_{10}F_2NO_2Na$
28	Cl		OH		Cl			H	B,[c] C	31	185-188	$C_{14}H_{11}Cl_2NO_3$
29		Cl	Cl					H	F[e]	61	148-150	$C_{14}H_{11}Cl_2NO_2$
30	Cl							H	F[e]	28	115-117	$C_{14}H_{12}ClNO_2$
31	Cl				Cl		OH	Na	B,[c] C	15	163-164	$C_{14}H_{10}Cl_2NO_3Na$
32	F				F			Na	A[c]	72	156-159	$C_{14}H_{10}F_2NO_2Na$
33								H	A[f]	49	114-116	$C_{14}H_{13}NO_2$
34	Cl	OH	OMe		Cl			H	F,[e] D	47	173-175	$C_{15}H_{13}Cl_2NO_4$
35	Cl		OH		Cl	OH		H	B,[c] C	50	217-219	$C_{14}H_{11}Cl_2NO_4$
36	Cl				Cl	OH		H	B,[c] C	37	193-195	$C_{14}H_{11}Cl_2NO_3$

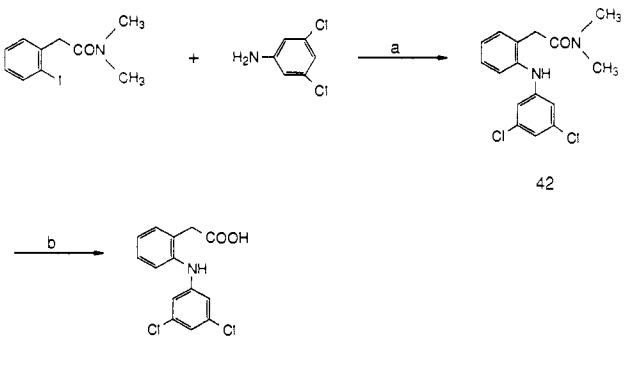
[a] See the Experimental Section. [b] From potassium 2-bromo-5-fluorophenylacetate. [c] From the corresponding diphenylamine. [d] From potassium 2-iodophenylacetate. [e] From *N,N*-dimethyl-2-iodophenylacetamide. [f] From diphenylamine. Stollé, R. J. *Prakt. Chem.* **1930**, *128*, 12. [g] Analysis for C, H, and N was within ±0.4% of the theoretical values.

the following criteria: the structural variations should be suitable to systematically explore the spacial and physicochemical requirements for the cyclooxygenase binding site for NSAID's.

Chemistry

The 2-(phenylamino)phenylacetic acids listed in Table I were synthesized following methods A–F, which are outlined in Schemes I–VI. Our initial synthesis involved the method of Stollé,[13] who described the preparation of **33**. Thus, condensation of the appropriate diphenylamines with refluxing chloracetyl chloride gave the substituted 2-chloro-*N*-phenylacetanilides, which could then be cyclized by heating in $AlCl_3$ in a melt at 160 °C to yield the substituted *N*-aryloxindoles. Hydrolysis with NaOH in refluxing ethanolic solution followed by acidification gave the 2-(phenylamino)phenylacetic acids[14] (**3**, **4**, **8**, **17**, **18**, **20**, **23**, **32**, **33**, method A, Scheme I). Since under the Friedel–Crafts conditions used in metod A ($AlCl_3$, 160 °C) alkyl migration and splitting of alkoxy groups can occur, method

Scheme VI[a]



[a] (a) K_2CO_3, Cu powder, CuI, toluene; (b) KOH, EtOH, and concentrated HCl.

(13) Stollé, R. J. *Prakt. Chem.* **1930**, *128*, 12.
(14) Sallmann, A.; Pfister, R. (Geigy, J. R., A.-G.) Brit. Pat. 1,132,318, 1968; *Chem. Abstr.* **1969**, *70*, 57455*b*.

B was found to be advantageous in certain cases: Treatment of substituted diphenylamines with oxalyl chloride in benzene followed by cyclization of the resulting *N*-phenyloxamic acid chloride with $AlCl_3$ in tetrachloroethane at room temperature gave *N*-arylisatins. Hydrolysis and acidification yielded the corresponding phenylglyoxylic acids, which were reduced in a Willgerodt–Kindler reaction to give, after acidification, the 2-(phenylamino)phenylacetic acids[15] (**4–6**, **10**, **12**, **14–16**, **21**, **22**, **46**, method B, Scheme II). Method A and B were general routes to 2-(phenylamino)phenylacetic acids, provided both ortho positions of one phenyl ring of the diphenylamines were occupied by halogen or alkyl substituents, thus avoiding the formation of positional isomers of the intermediate oxindoles[16] and isatins. Our attempts, as well as those of others,[16] to prepare 1-aryloxindoles by a one-step condensation of substituted anilines with *o*-chlorophenylacetic acid catalyzed with copper oxide[17] gave very poor results. A useful method has been developed by Coppola,[18] where *N*-arylisatins could be prepared by direct arylation of isatin with substituted aryl bromides and copper oxide in refluxing DMF [*N*-(4-methoxyphenyl)isatin and *N*-(3,5-dimethylphenyl)isatin are reported]. Two very attractive routes to 2-(phenylamino)phenylacetic acids, which avoid the formation of isomers, were described by Kato[19] and Nohara.[20] Following the method of Kato, potassium 2-iodophenylacetate[21] was reacted in a modified Ullmann reaction with substituted anilines in the presence of potassium carbonate and activated copper powder[22,23] in hot *N*-methyl-2-pyrrolidone. Acidification and crystallization yielded the 2-(phenylamino)phenylacetic acids (**1**, **2**, **4**, **7**, **9**, **19**, **26**, method E, Scheme V), along with varying amounts of the corresponding *N*-aryloxindoles as byproducts. The method of Nohara involved the condensation of *N*,*N*-dimethyl-2-iodophenylacetamide[20] and anilines in the presence of anhydrous potassium carbonate, copper, and cuprous iodide in refluxing toluene to yield the substituted *N*,*N*-dimethyl-2-(phenylamino)phenylacetamides, which were hydrolyzed with KOH in refluxing ethanol to give, after acidification, the 2-(phenylamino)phenylacetic acids (**11**, **13**, **25**, **27**, **29**, **30**, method F, Scheme VI). In several cases, we obtained better results with method F than with method E, because the *N*-aryloxindoles were not formed as byproducts in substantial amounts.

Hydroxylated 2-(phenylamino)phenylacetic acids (metabolites of diclofenac in humans[24,25]) were synthesized following methods C and D, outlined in Schemes III and IV. Thus the appropriately substituted methoxy-2-(phenylamino)phenylacetic acids (prepared following methods B and F) were treated with pyridine hydrochloride at 170 °C, which gave the hydroxy-substituted *N*-phenyloxindoles (e.g. **47**). These could be hydrolyzed to the hydroxy-2-(phenylamino)phenylacetic acids with NaOH in refluxing *n*-butanol (**14**, **28**, **31**, **35**, **36**, method C, Scheme III). Hydroxylated compounds with an additional methoxy group (**24**, **34**) were prepared by hydrogenation of the corresponding benzyloxy analogues with Pd–C in tetrahydrofuran and 1,2-dichlorobenzene (**24**, **34**, method D, Scheme IV).

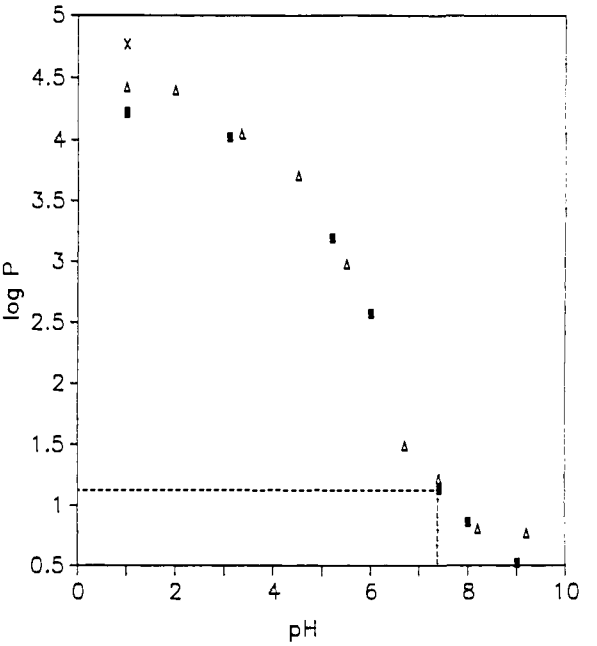


Figure 2. pH dependence of the *n*-octanol/aqueous phase partition (distribution) coefficients of diclofenac. Data from our laboratories (filled squares) were measured with the following aqueous phases: 0.1 N HCl at pH 1, 0.067 M glycine hydrochloride at pH 3.1, M/15 phosphate at pH 5.2, 6.0, 7.4, and 8.0, 0.06 M borax–NaOH at pH 9. The figure also includes the data reported by F. Barbato et al.[35] (triangles) and the calculated CLOGP value for the undissociated free acid (cross).

Pharmacology

Cyclooxygenase Inhibition (PGS Activity) in Bovine Seminal Vesicle Microsomal Preparations. Conversion of [^{14}C]arachidonic acid to prostaglandins (PGE_2, $PGF_{2\alpha}$) was measured in the presence of cofactors and test compounds (30 min, 37 °C) according to the method of White and Glassman.[26] Compounds were tested in triplicate using log dilutions from 0.1 to 1000 μg/mL. Statistical analysis (Student's *t* test) showed significant effects at ≥20% inhibition of prostaglandin synthesis. IC_{50} values were determined graphically.

Adjuvant Arthritis in Rats (AdA Activity).[27] Adjuvant arthritis was induced in male Lewis rats (LEW/MOL, 145–170 g body weight, five animals per group) by intraplantar injection of 0.2 mg *Mycobacterium butyricum* (Difco) in 0.05 mL of paraffin oil into the left hind paw. The drugs (suspended in 0.75% methylcellulose) were given po once daily from day 11 to 14 after adjuvant injection. Paw volumes (injected paw) were measured on days 11 and 15 and the antiinflammatory effect was expressed as percentage change from arthritic controls. At least three different doses of active compounds were tested and ED_{40} values were calculated graphically. Edema inhibition reached significance at ≥20% (Student's *t* test).

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Table II. Physicochemical and Biological Data

compd	molec wt	expt: log P, pH 7.4	expt: pK_a	α[a]	σ[b]	B_1[c]	B_5[d] (sum)	inhibn of cyclooxygenase, log (1/IC_{50})[e]: obs	inhibn of cyclooxygenase, log (1/IC_{50})[e]: calc (eq 2)	inhibn of adjuvant arthritis, log (1/ED_{40})[f]: obs	inhibn of adjuvant arthritis, log (1/ED_{40})[f]: calc (eq 4)
indomethacin	357.8	1.00	4.20					−0.20	-	−0.15	-
1	354.1	1.62		69	2.62	1.80	3.60	0.25	0.25	0.25	0.32
2	336.1	1.48	4.02	69	2.56	1.80	3.60	0.13	0.32	0.05	0.31
3	352.6	1.97		69	2.56	1.80	3.60	0.10	−0.05	0.07	0.15
4	318.1	1.13	4.14	69	2.56	1.80	3.60	0.00	−0.67	0.03	−0.52
5	326.2	1.24	4.20	69	2.29	1.80	3.60	0.00	−0.66	−0.49	−0.54
6	275.7	1.17	4.20	69	1.57	1.80	3.84	0.00	−0.67	−0.74	−0.74
7	336.1	1.27		69	2.62	1.80	3.60	−0.05	−0.66	−0.47	−0.46
8	362.6	1.44	4.20	72	2.63	1.95	3.75	−0.28	−0.40	−0.14	−0.20
9	255.3	1.03		62	0.22	1.52	3.04	−0.30	−1.36	−1.59	−1.66
10	387.6	1.64	4.24	75	2.62	2.15	3.95	−0.30	−0.21	−0.11	−0.02
11	283.4	1.95	4.20	69	0.44	1.52	4.08	−0.78	−1.04	−1.25	−1.07
12	255.3	1.21	4.07	69	0.58	1.52	4.00	−0.78	−0.34	−0.29	−0.67
13	296.2	1.66	4.20	62	1.65	1.80	2.80	−1.00	−1.45		
14	312.2	0.72		69	2.68	1.80	3.60	−1.00	−1.50	−1.51	−1.70
15	275.7	1.54	4.30	62	0.66	1.52	3.04	−1.04	−1.39	−0.86	−1.40
16	326.2	1.18	3.95	69	2.68	1.80	3.60	−1.04	−0.67	−0.96	−0.48
17	332.2	1.69		69	2.49	1.80	3.60	−1.15	−0.81	−0.65	−0.48
18	375.1	1.87		69	2.56	1.80	3.60	−1.18	−0.96	−3.43	−3.43
19	275.7	1.59		62	1.21	1.80	2.80	−1.30	−1.41	−0.26	−1.28
20	301.7	0.56		62	2.21	1.80	3.15	−1.75	−1.71	−0.80	−1.64
21	317.5	2.93		69	0.44	1.52	4.00	−1.82	−1.86	−1.20	−1.61
22	283.4	1.91		69	0.82	1.52	6.34	−1.83	−1.00	−1.55	−0.94
23	296.2	1.33		62	1.51	1.80	2.80	−1.85	−1.33	−1.23	−1.23
24	342.2	0.51		69	2.31	1.80	3.60	−1.86	−1.71	−2.47	−1.98
25	296.2	1.98	4.31	58	0.74	1.00	2.00	−1.90	−2.12	−2.23	−1.85
26	296.2	1.64	4.10	62	1.65	1.80	2.80	−2.00	−1.44	−1.01	−1.19
27	285.2	0.55		60	0.99	1.35	2.35	−2.01	−1.91	−2.85	−2.09
28	312.2	0.70	4.40	69	2.19	1.80	3.60	−2.15	−1.51	−0.98	−1.78
29	296.2	1.80	4.30	58	0.60	1.00	2.00	−2.15	−1.93	−2.53	−1.78
30	261.7	0.91		62	1.28	1.80	2.80	−2.31	−1.41	−1.76	−1.49
31	334.1	1.73	4.48/11.1	69	2.56	1.80	3.60	−2.33	−2.50		
32	285.2	0.26	4.09	60	1.86	1.35	2.70	−2.36	−2.29	−2.24	−2.33
33	227.3	0.19	4.10	58	0.00	1.00	2.00	−2.46	−2.60	−2.94	−3.03
34	342.2	0.25	4.13/7.91	69	2.41	1.80	3.60	−2.47	−2.07	−2.47	−2.36
35	328.2	0.27		69	2.19	1.80	3.60	−3.04	−3.70	−2.78	−2.38
36	312.2	0.76	4.22	69	2.56	1.80	3.60	−3.35	−2.51	−0.98	−0.81

[a] Angle of twist of the two phenyl rings (degrees). [b] Sum of the Hammett σ constants of the substituents in ring B. [c] Verloop's size parameter (smallest cross section in Å units) for the larger one of the ortho substituents of ring B. [d] Sum of Verloop's size parameter (largest cross section) for both ortho substituents of ring B. [e] Bovine seminal vesicle microsomal preparations, IC_{50} in μmol/L. [f] Inhibition of inflammatory hind paw edema, induced by *Mycobacterium butyricum* in rats, ED_{40} in μmol/kg po.

Results and Discussion

Physical Chemistry. Similar to most other NSAID's, diclofenac is highly bound to human serum proteins (>99.5%), mostly to albumins.[28-30]

As has previously been described for phenylbutazone analogues[31] and other structural classes of NSAID's,[32-35] the log P of diclofenac has a dependence on pH which is characteristic for weak acids (Figure 2): in the pH region below the pK_a of about 4, e.g. in the stomach, diclofenac partitions between an organic and an aqueous phase as the undissociated free acid. The log P_u value (partition coefficient of the unionized acid between *n*-octanol and aqueous phase), calculated by the fragment procedure of Hansch and Leo[36] using the computer program CLOGP (Pomona[37]), agrees quite well with the measured log P at low pH (see Figure 2). When the pH is raised, a typical sigmoidal pH dependence of log P is observed: Within a range of about three pH units above the pK_a, log P decreases with pH according to eq 1, which describes the situation when only the unionized species partitions be-

$$\log P = \log P_u - \log (1 + 10^{pH-pK_a}) \quad (1)$$

Table III. Torsion Angles and Angles of Twist from X-ray Analyses

compd[a]	R_2	R_6	torsion angles[b]: ϕ_1	torsion angles[b]: ϕ_2	angles of twist,[b] α
4	Cl	Cl	16	−124	69
12	CH_3	CH_3	3	−114	69
30	Cl	H	7.7	−124	62
32	F	F	12.6	−131	60
33	H	H	9.8	−132	58

[a] Free acids were used in all cases. [b] Degrees.

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tween the aqueous and the organic phase, while the ionized fraction remains entirely in the water.

Above a pH of about 7, where more than 99.9% of the acid is deprotonated, the observed leveling off is caused by the finite partition coefficient of the ionized molecule itself (octanol contains about 26 mol % water and a certain amount of ionized species can dissolve in this modified lipid phase[35,38]) and ion pair extraction with counterions of the buffers used.[31,39] From the similarity of structures and pK's in this series, we can assume that all derivatives follow a similar log P/pH profile.

Topographical Aspects. From the results of the X-ray analyses, three topographical features emerged which are of particular interest in this study: The two torsion angles ϕ_1 ($C_{3'}$–$C_{2'}$–N–C_1) and ϕ_2 (C_2–C_1–N–$C_{2'}$) between the two phenyl rings and the nitrogen and the angle of twist, α, between the planes of the phenyl rings (Table III).

Since the compounds are dissolved in an aqueous environment and interact with a rather lipophilic cyclooxygenase enzyme system, it seemed interesting to compare the geometrical situation in D_2O and in a lipophilic solvent, $CDCl_3$, by ^1H NMR. In D_2O the sodium salts were used and in chloroform the free acids were used. In D_2O, the H-3′ signal of the 2,6-dichloro derivative (4) appears at rather high field (6.47 ppm) due to the shielding of the aryl ring B, suggesting a similar torsion angle to that observed in the crystalline state. In agreement with the X-ray data, a smaller torsion angle can be deduced for the compounds lacking 2,6-disubstitution since in their NMR spectra the H-3′ signal appears at much lower field (7.08 ppm for **33** and 7.11 ppm for **30**). In $CDCl_3$ the H-3′ protons were found at 6.57 ppm for **4** and again at much lower field for **33** (7.36 ppm) and for **30** (7.36 ppm), suggesting that the twisting of the phenyl rings depends very little on the lipophilicity of the environment.

The experimentally determined angles of twist of the five compounds shown in Table III were used to estimate, by analogy, the corresponding angles of the remaining derivatives of this series to be used as independent variables in the QSAR analysis (Table II). Such an estimation appeared permissible since, as can be seen in Table III, these angles correspond directly to the size of the substituents in ortho position. In addition, molecular mechanics calculations (program MACROMODEL, Columbia University version 2.5, with the MM2 force field by Allinger) have shown a surprisingly good agreement with the angles of twist determined by X-ray (Table III): α = 67.8° for **4**, 68.0° for **12**, 61.8° for **30**, 59.7° for **32**, and 56.4° for **33**.

From the results of the X-ray analyses of the five compounds (coordinates are given in the supplementary material), we concluded that in the crystals of the free acids the double-bonded oxygen of the carboxylic group must be hydrogen bonded to the proton of the diphenylamine nitrogen: Distances in the order of 0.30 nm are measured between the centers of the oxygen and the nitrogen atoms, and 0.20–0.22 nm are the distances between the oxygen and the experimentally determined positions of the H on the nitrogen. Interestingly, this position does not coincide with the expected position assuming a sp^2 nitrogen; instead, the hydrogen is shifted between 13° (for **30**) and 20° (for **4** and **32**) out of the C_1–N–$C_{2'}$ plane toward the double-bonded oxygen.

In contrast, Reck et al.,[40] who have succeeded in obtaining single crystals of diclofenac acid hydrate, came to the conclusion, that, judging from the O···H distances of between 0.21 and 0.22 nm and the steric situation of the 7-ring formed, a N–H···O hydrogen bond could not exist. However, they were not able to localize the hydrogen atoms experimentally and apparently had to estimate their positions which, in the case of this particular diphenyl N–H, may not be correct.

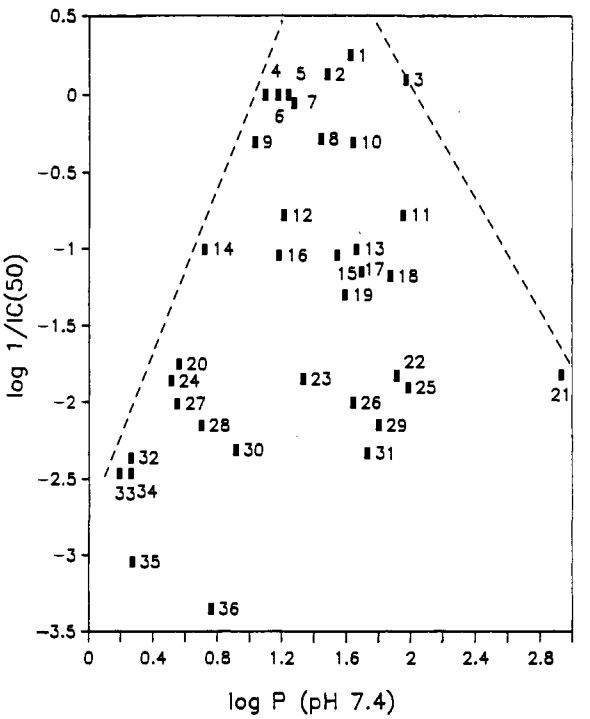

Figure 3. Plot of log ($1/IC_{50}$) for cyclooxygenase inhibition versus lipophilicity (experimental log P at pH 7.4). Diclofenac is compound 4.

Quantitative Structure–Activity Relationships (QSAR) of the PGS Data. All 36 compounds of this series which had been tested for cyclooxygenase inhibition were subjected to a QSAR analysis. From a crude inspection of the plot of log $1/C$ vs log $P_{pH\,7.4}$ (Figure 3) there would seem to be no clear-cut dependence on lipophilicity; however, it is immediately obvious that all very active compounds are in the log P region between 1 and 2 (dashed lines). Besides log P and the electronic parameter (sum of Hammett σ of all substituents on ring B) several variables were tested in the regression equations which describe the geometrical situation of the two aromatic rings: α, the angle of twist between the two phenyl rings, B_1 and B_5, Verloop's STERIMOL parameters for the smallest and largest cross sections of the 2- and 6-substituents.[41]

Several indicator variables for structural features suspected to influence activity were also used in the QSAR equations:

I_{usu26}	only H or F in position 2 and 6 of ring B
I_{usu6}	H or F in position 6, a larger substituent than F in position 2
I_{OHA}	OH in position 5′ or 6′ of ring A
I_{OHB}	OH in position 3 or 4 of ring B
$I_{hal5'}$	halogen in position 5′ of ring A
I_{su4}	substituent larger than F in position 4 of ring B
$I_{su6'}$	substituent in position 6′ of ring A

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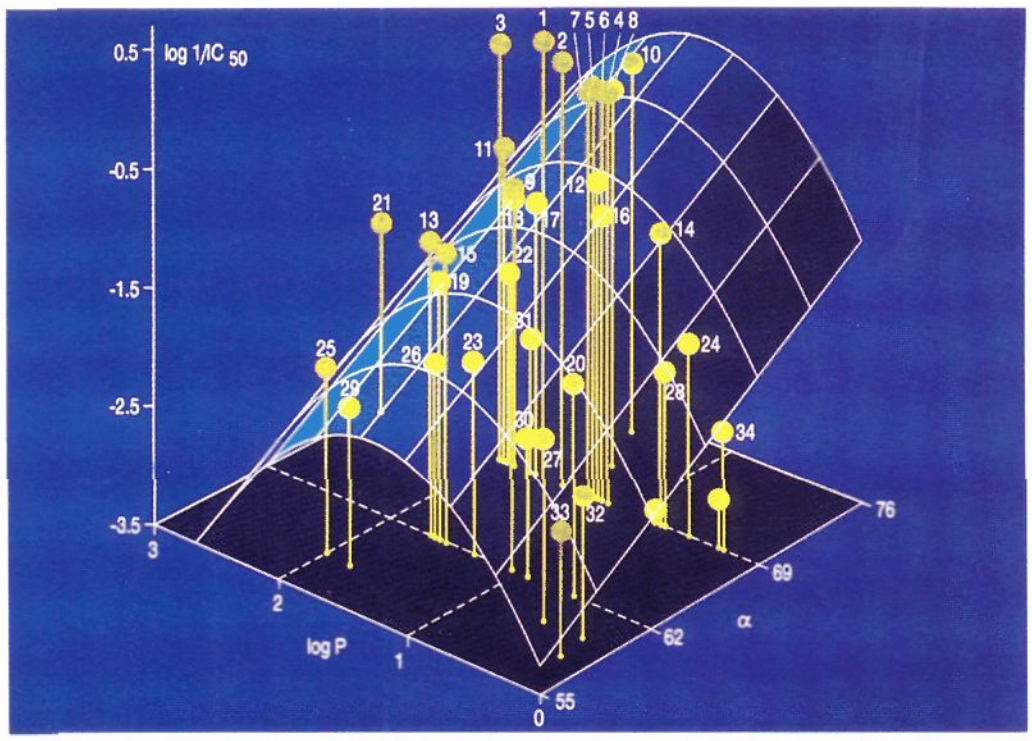

Figure 4. Cyclooxygenase inhibition versus lipophilicity (log P at pH 7.4) and angle of twist between the phenyl rings, α. The surface was calculated by the continuous variables of eq 2: $\log (1/IC_{50}) = 8.43 + 1.99 \log P - 0.79 (\log P)^2 + 0.094\alpha$.

The correlation coefficients between the independent variables are given in the supplementary material. Except for those variables which essentially describe the same situation, namely the ortho substitution in ring B (variables α, B_1, B_5, I_{usu26}), but were not used together in the same regression equations, the correlation coefficients are all below 0.6.

No satisfactory correlations ($r^2 < 0.55$) were obtained in preliminary analyses where only these indicator variables were used in Free–Wilson type regression calculations. The correlations improved greatly when the continuous variables were brought into the equations in so-called "mixed Hansch models". The different possibilities to express the twisting of the phenyl rings (by α, by B_1, B_5, or by the first two indicator variables) produced quite similar statistical results. However, the following equation explained the PGS data most satisfactorily:

$$\log [1/IC_{50}(PGS)] = -8.43 (\pm 1.44) + 1.99 (\pm 0.56) \log P - 0.79 (\pm 0.20) (\log P)^2 + 0.094 (\pm 0.024)\alpha + 1.02 (\pm 0.34) I_{hal5'} - 1.66 (\pm 0.33) I_{OHA} - 0.61 (\pm 0.35) I_{OHB} \quad (2)$$

$$n = 36 \qquad s = 0.525 \qquad r = 0.848$$

The standard deviation of 0.5 of the regression corresponds to the reliability of the biological data and thus cannot be further improved. The calculated optimum lipophilicity, log $P_{opt} = 1.26$, corresponds well with the maximum observed visually on the plot of log $(1/IC_{50})$ versus log P (Figure 3). Some indicator variables also entered eq 2 in a significant manner ($p < 0.05$). They show that a halogen substituent in position 5′ of ring A increases PGS activity by a factor of 10 while the OH groups of the known metabolites **28** and **36** decrease the activity by 0.61 and 1.66 log units, respectively.

In the three-dimensional plot (Figure 4), the grid surface was calculated by the continuous variables of eq 2 (log P, $(\log P)^2$, and α) and their coefficients. This figure demonstrates how the PGS activity can be explained to a great deal by lipophilicity and ring twisting. It shows particularly well how the most active compounds **1–10** are closely clustered.

QSAR of the Adjuvant Arthritis (AdA) Test. Two of the compounds tested for PGS activity (**13** and **31**) could not be used in the adjuvant arthritis test due to lack of material.

A plot of the adjuvant arthritis vs the PGS activity tests (Figure 5) reveals a reasonable correlation

$$\log [1/ED_{40}(AdA)] = -0.36 (\pm 0.21) + 0.69 (\pm 0.13) \log [1/IC_{50}(PGS)]$$

$$n = 34 \qquad s = 0.75 \qquad r = 0.687 \quad (3)$$

suggesting that the inhibition of the cyclooxygenase is indeed the underlying mechanism in this arthritis model, at least for more potent compounds. There are two noteworthy outlyers, compounds **18** and **36**. The first one, with a large Br substituent in position 6′, was unexpectedly devoid of activity in the AdA test, while reasonably active in the PGS test. The other one, with a OH group in position 5′, showed medium activity in the AdA test and only marginal activity in the PGS test. Excluding **18** and **36**, the correlation coefficient improves to $r = 0.813$ ($n = 32$). Compounds with an additional halogen in position 5′ (**2**, **3**) and in positions 4 and 5′ (**1**) were even more active in vitro and in vivo than diclofenac: These compounds were designed to inhibit the metabolism of diclofenac by blocking potential metabolic sites and thus enhance in vivo activity.

With the same set of independent variables as for the PGS data the following "best" QSAR equation was obtained for log $(1/ED_{40})$ of the adjuvant arthritis data:

$$\log [1/ED_{40}(AdA)] = -9.05 (\pm 1.37) + 2.20 (\pm 0.52) \log P - 0.80 (\pm 0.18) (\log P)^2 + 0.10 (\pm 0.02)\alpha - 0.80 (\pm 0.32) I_{OHB} - 2.65 (\pm 0.52) I_{su6'} + 0.93 (\pm 0.33) I_{hal5'} \quad (4)$$

$$n = 34 \qquad s = 0.492 \qquad r = 0.873$$

The lipophilicity optimum which is apparent in the plot of the AdA activities vs log P (Figure 6) is almost the same (calculated log $P_{opt} = 1.38$) as for the PGS data and thus

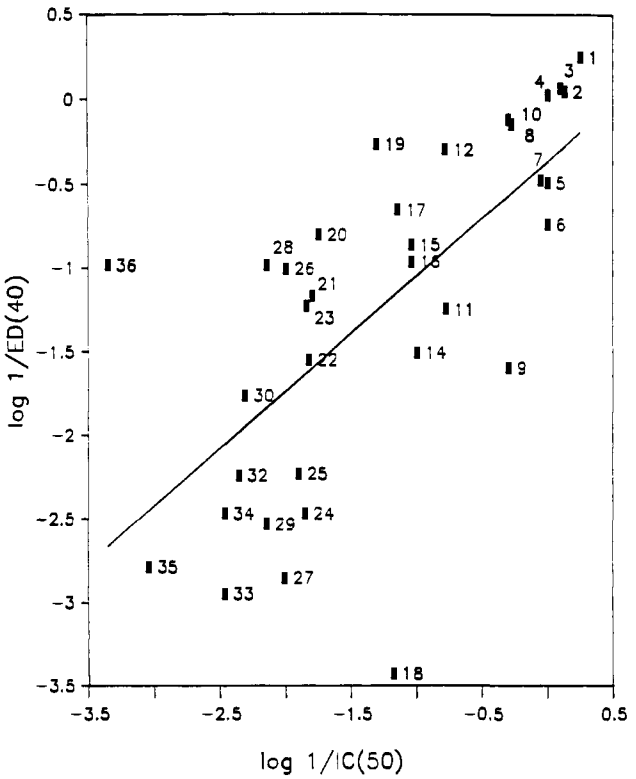

Figure 5. Plot of log ($1/ED_{40}$) for activity in adjuvant arthritis versus log ($1/IC_{50}$) for inhibition of cyclooxygenase.

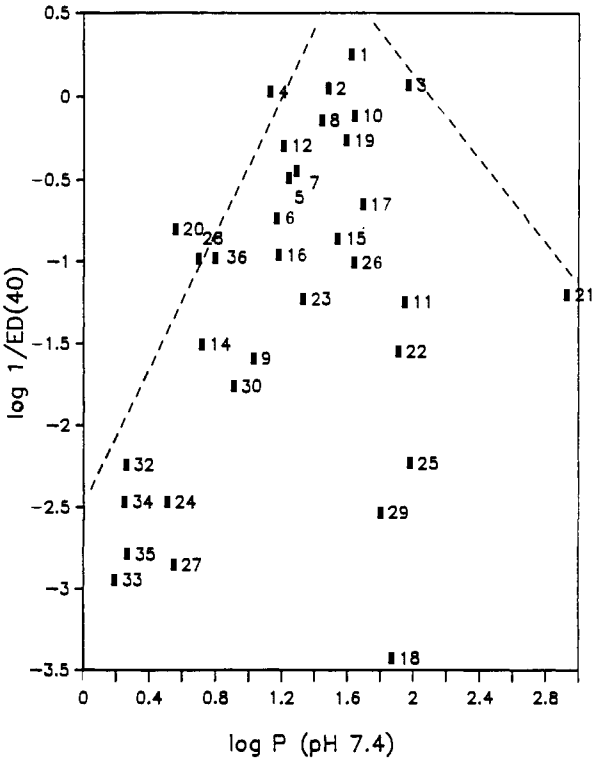

Figure 6. Plot of log ($1/ED_{40}$) for activity in adjuvant arthritis versus lipophilicity. Diclofenac is compound 4.

again in the range initially identified for good antiinflammatory activity. Ring twisting by large ortho substituents in ring B is also a promoting factor. Furthermore, it can be seen that OH substitution in ring A does not decrease AdA activity because the corresponding indicator variable does not enter into the regression equation. Halogen substitution in position 5′ enhances activity by 0.93 log units, while a bromine in position 6′ leads to a significant loss of activity.

Conclusions

This study has shown that the two pharmacological models used, inhibition of cyclooxygenase at the enzyme level and in vivo inhibition of rat adjuvant arthritis, correlate reasonably well with one another. For 36 congeners of diclofenac both activities can largely be explained by the same simple physicochemical and geometrical parameters: lipophilicity and twisting of the two aromatic rings due to ortho substitution of ring B.

Within the range of substituents investigated, we have found no clear evidence that group electronic properties were of decisive importance. In the ortho position of ring B, chlorine and methyl substituents have both provided analogues (1–8) of high activity, with Cl derivatives appearing most effective. Since the *o,o′*-difluoro compound (**32**) was found considerably less active, the twist of the two rings seems to be an essential prerequisite for high activity.

The same conclusion has been drawn by Kaltenbronn et al.[42] from the results found in a large series of *N*-arylanthranilic acids tested for antiinflammatory activity: 2,6-dichloro substitution plus an additional substituent in position 3 gives the most active examples (e.g. meclofenamic acid). A remarkable difference is, however, that the disubstituted analogue 4 of our series is considerably more active than the 2,3,6-trisubstituted compounds **16** and **17**.

Some of the compounds with OH groups in addition to two ortho Cl substituents (**28**, **34–36**) are metabolites of diclofenac in rats and men, as has been demonstrated in pharmacokinetic studies.[24] Their intrinsic activity in the in vitro PG assay is more than 100 times lower than that of diclofenac. This may be the reason for their weak activity in rat adjuvant arthritis. One possible explanation for the low intrinsic activity of these compounds may be their low partition coefficient.

The findings of the QSAR analyses allowed the rationalization of the high activity of diclofenac. Although there are three close analogues with even higher activity, all with halogen substituents in position 5′ of ring A (**1–3**), the choice of a compound for clinical evaluation depends on a number of other factors besides high activity: chemical stability (e.g. resistance to ring formation to the 2-indolinone), synthetic simplicity, and tolerability. Diclofenac, while not the most active derivative, has proven superior in these other equally important aspects.

Experimental Section

Proton NMR spectra were determined on a Bruker AM 300 and a Varian VXR 400 S spectrometer with Me_4Si as internal standard, and all J values are in hertz. Melting points were taken on a Reichert melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed with silica gel 60 F_{254} plates (Merck). The system used for TLC was benzene–ethyl acetate–acetic acid 90:5:5 with spot location by spraying with a solution prepared by dissolving 0.5 g of $K_2Cr_2O_7$ in 80 mL of H_2O and adding 20 mL of concentrated H_2SO_4. All the organic phases were dried over anhydrous $MgSO_4$.

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Method A. Preparation of 2-(Phenylamino)phenylacetic Acids from Diphenylamines (via Oxindoles). 1. 2-Chloro-*N*-(2′,6′-dichlorophenyl)-*N*-phenylacetamide (39). A mixture of 40.0 g of 2,6-dichlorodiphenylamine[14] (0.17 mol) and 56.5 g of chloroacetyl chloride (0.5 mol) was refluxed for 16 h, cooled, and evaporated. The residue was dissolved in 500 mL of chloroform–ether (1:2). The organic phase was washed with 100 mL of 2 N $KHCO_3$ solution and 100 mL water and evaporated. The residue was recrystallized from MeOH to give 35.0 g of **39** (66%). Mp: 143–144 °C. Anal. ($C_{14}H_{10}Cl_3NO$): C, H, N, Cl.

2. 1-(2,6-Dichlorophenyl)oxindole (40). A 31.5-g portion of 2-chloro-*N*-(2′,6′-dichlorophenyl)-*N*-phenylacetamide (**39**, 0.1 mol) and 30.0 g of $AlCl_3$ were mixed, and the mixture was heated at 160 °C, for 2 h (at 100 °C melting occurs). The molten mass was cooled and poured onto 300 g of crushed ice while the mixture was stirred. The precipitated oil was dissolved in 300 mL of chloroform. The organic phase was washed with 50 mL of 2 N $KHCO_3$ and 50 mL of water and evaporated. Recrystallization from MeOH gave 19.9 g of **40** (75.4%). Mp: 124–125 °C. ^1H NMR ($CDCl_3$): δ 7.51 (A_2 part of A_2B, H-3 and H-5), 7.38 (B part of A_2B, H-4), 7.34 (dm, H-6′), 7.21 (tm, H-4′), 7.10 (td, H-5′), 6.41 (dm, H-3′), 3.78 (s, CH_2).

3. Sodium [2-[(2,6-Dichlorophenyl)amino]phenyl]acetate (4). A solution of 18.6 g of 1-(2,6-dichlorophenyl)oxindole (**40**), 66.0 mL of 2 N NaOH, and 66.0 mL of EtOH was refluxed for 4 h. The clear solution was cooled in an ice bath for 4 h. The precipitated crystals were filtered off and recrystallized from 80 mL of water to yield 19.5 g of **4** (93%). Mp: 280–283 °C. ^1H NMR (DMSO-d_6): δ 10.47 (s, b, NH), 7.44 (A_2 part of A_2B, H-3 and H-5), 7.05 (B part of A_2B, H-4), 7.03 (dd, H-6′), 6.91 (td, H-4′), 6.72 (td, H-5′), 6.22 (db, H-3′), 3.37 (s, CH_2). Anal. ($C_{14}H_{10}Cl_2NO_4Na$): C, H, N.

Method B. Preparation of 2-(Phenylamino)phenylacetic Acids from Diphenylamines (via Isatins). 1. *N*-(4-Methoxyphenyl)-*N*-(2′,6′-dichloro-4′-methoxyphenyl)oxaniloyl Chloride (43). A 62.44-g portion of oxalyl chloride (0.49 mol) was added dropwise at 5 °C to a solution of 83.44 g of 4′-methoxy-2,6-dichloro-4-methoxydiphenylamine (**42**, 0.28 mol) in 378 mL of benzene. The mixture was stirred for 2 h at room temperature and evaporated. The residue was dissolved in 400 mL of benzene and the solution was again evaporated to dryness to obtain **43** as a brown oil (108.8 g, 100%).

2. 1-(2,6-Dichloro-4-methoxyphenyl)-5-methoxyisatin (44). To a solution of 108.8 g of *N*-(4-methoxyphenyl)-*N*-(2′,6′-dichloro-4′-methoxyphenyl)oxaniloyl chloride (**43**, 0.28 mol) in 595 mL of tetrachloroethane was added portionwise 38.36 g of $AlCl_3$. The mixture was stirred for 20 h at room temperature and was poured onto a mixture of 800 g of ice and 200 mL of 2 N HCl. The organic phase was washed with water, 2 N $KHCO_3$ solution, and again with water and evaporated. Crystallization from ether afforded 76.3 g of **44** (77.4%). Mp: 145–146 °C. ^1H NMR ($CDCl_3$): δ 7.25 (d, H-6′), 7.12 (dd, H-4′), 7.05 (s, H-3 and H-5), 6.45 (d, H-3′), 3.86 (s, OCH_3), 3.82 (s, OCH_3).

3. [2-[(2,6-Dichloro-4-methoxyphenyl)amino]-5-methoxyphenyl]glyoxylic Acid (45). A solution of 76.3 g of 1-(2,6-dichloro-4-methoxyphenyl)-5-methoxyisatin (**44**, 0.216 mol), 215.5 mL of 1 N NaOH, and 2100 mL of ethanol was heated under reflux for 10 min. The solution was cooled and evaporated. The residue was dissolved in 2000 mL of water, washed with ether, and acidified with 2 N HCl. The yellow precipitate was extracted with ether. The organic extract gave, after washing with water and evaporation, the crude product. Crystallization from ether–petroleum ether afforded 69.8 g of **45** (87.3%) as yellow crystals. Mp: 123–125 °C dec. ^1H NMR (DMSO-d_6): δ 11.3 (b, COOH), 9.40 (s, NH), 7.27 (s, H-3 and H-5), 7.20 (dd, H-4′), 7.05 (d, H-6′), 6.28 (d, H-3′), 3.85 (s, OCH_3), 3.70 (s, OCH_3). Anal. ($C_{16}H_{11}Cl_2NO_4$): C, H, N.

4. 2-[(2,6-Dichloro-4-methoxyphenyl)amino]-5-methoxyphenylacetic Acid (46). Hydrazine hydrate (53.45 g, 1.07 mol) was added to a solution of 80.25 g of [2-(2,6-dichloro-4-methoxyanilino)-5-methoxyphenyl]glyoxylic acid (**45**, 0.217 mol) in 900 mL of 2-methoxyethanol. The temperature of the mixture was increased to 60 °C and 125.4 g of $NaOCH_3$ (2.32 mol) was added portionwise. The mixture was slowly heated to 150 °C, whereby methanol, water, hydrazine, and part of the solvent evaporated. The mixture was kept at 150 °C for 1 h, cooled, and poured onto 8 kg of crushed ice. The aqueous phase was extracted with 800 mL of ether and acidified with concentrated HCl at 0 °C. The precipitated oil was extracted with ether. The ether solution was washed with water and evaporated. The residue was crystallized from ether–petroleum ether to yield 64.4 g of **46** (83.4%). Mp: 145–150 °C dec. ^1H NMR (DMSO-d_6): δ 12.5 (b, COOH), 7.11 (s, H-3 and H-5), 6.82 (d, H-6′), 6.64 (dd, H-4′), 6.54 (s, b, NH), 6.17 (d, H-3′), 3.78 (s, OCH_3), 3.67 (s, OCH_3 and CH_2). Anal. ($C_{16}H_{15}Cl_2NO_4$) C, H, N.

Method C. Preparation of Hydroxy-Substituted 2-(Phenylamino)phenylacetic Acids. 1. 1-(2,6-Dichloro-4-hydroxyphenyl)-5-hydroxyoxindole (47). [2-[(2,6-Dichloro-4-methoxyphenyl)amino]-5-methoxyphenyl]acetic acid (**46**; 31.5 g, 0.088 mol) was added in portions to a melt of 200 g of pyridine hydrochloride (1.73 mol) at 170 °C. The mixture was heated at 180 °C for 3 h and poured onto 2000 mL of ice–water while hot. The precipitated product was filtered off, washed with water, and dissolved in 1000 mL of ethyl acetate. The organic phase was washed with 200 mL of 1 N HCl and water (2 × 100 mL) and evaporated. The residue was crystallized twice from methanol–water, yielding 21.2 g of **47** (77.3%). Mp: 260–263 °C. ^1H NMR (DMSO-d_6): δ 10.6 (b, OH), 9.1 (b, OH), 7.06 (s, H-3 and H-5), 6.84 (d, H-6′), 6.62 (dd, H-4′), 6.19 (d, H-3′), 3.72 (s, CH_2). Anal. ($C_{14}H_9Cl_2NO_3$): C, H, N.

2. [2-[(2,6-Dichloro-4-hydroxyphenyl)amino]-5-hydroxyphenyl]acetic Acid (36). To a solution of 8.0 g of 1-(2,6-dichloro-4-hydroxyphenyl)-5-hydroxyoxindole (**47**, 0.026 mol) in 200 mL of *n*-butanol were added 7.0 g of NaOH (0.18 mol) and 1.0 g of KOH (0.018 mol), and the reaction mixture was refluxed for 24 h and evaporated in vacuo. The residue was dissolved in 700 mL of water and the purple aqueous solution was extracted with ether (2 × 200 mL), cooled to 0 °C, and acidified with concentrated HCl. The precipitate was taken up in 300 mL of ether. The organic phase was washed with 30 mL water, 0.5 N $NaHCO_3$ (5 × 80 mL), and 80 mL of 2 N $KHCO_3$ solution. The combined $NaHCO_3$ extracts were cooled to 0 °C and acidified with 2 N HCl, and the precipitate was dissolved in 200 mL of ether. The organic layer was washed with 30 mL of water and evaporated to yield crude product. Recrystallization from ether–methylene chloride gave 6.3 g of **36** (75.6%). Mp: 217–219 °C. ^1H NMR (DMSO-d_6): δ 12.5 (b, COOH), 10.0 (b, OH), 8.78 (s, b, OH), 6.87 (s, H-3 and H-5), 6.62 (d, H-6′), 6.47 (dd, H-4′), 6.25 (s, b, NH), 6.06 (d, H-3′), 3.59 (s, b, CH_2). Anal. ($C_{14}H_{11}Cl_2NO_3$): C, H, N.

Method D. Preparation of Hydroxymethoxy-Substituted 2-(Phenylamino)phenylacetic Acids. [2-[(2,6-Dichloro-3-methoxy-4-hydroxyphenyl)amino]phenyl]acetic Acid (24). A mixture of 9.78 g of [2-[2,6-dichloro-3-methoxy-4-(benzyloxy)anilino]phenyl]acetic acid (**48**), 100 mL of THF, 10 mL of 1,2-dichlorobenzene, and 1.0 g of Pd–C (5%) was hydrogenated at normal pressure for 25 min at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated to leave a concentrated solution of the product in 1,2-dichlorobenzene. Addition of petroleum ether afforded pure **24** (98%). Mp: 166–168 °C. ^1H NMR (CD_3OD): δ 7.17 (dd, H-6′), 7.03 (td, H-4′), 6.96 (s, H-5), 6.82 (td, H-5′), 6.28 (dd, H-3′), 3.84 (s, OCH3), 3.73 (s, CH_2). Anal. ($C_{15}H_{13}Cl_2NO_4$): C, H, N.

Method E. Preparation of 2-(Phenylamino)phenylacetic Acids (via Ullmann Reaction of 2-Iodophenylacetic Acid). 1. Sodium [2-[(2,6-Dichlorophenyl)amino]phenyl]acetate (4). Potassium 2-iodophenylacetate[21] (7.11 g, 23.7 mmol) was added to a mixture of 19.2 g of 2,6-dichloroaniline (118.5 mmol), 11.2 g of anhydrous potassium carbonate (81.15 mmol), 0.75 g of activated copper powder[22,23] (11.8 mmol), and 28 mL of *N*-methylpyrrolidone while the inner temperature was kept at 120 °C. The mixture was kept at 120 °C for 22 h with stirring, while water (14.5 mL) was distilled off through a descending condenser. The hot reaction mixture was treated with hot (90 °C) water and filtered while hot through 5 g of Celite. The filtrate was cooled to room temperature and the *N*-methylpyrrolidone layer was removed. The aqueous layer was treated with 60 mL of chloroform. The precipitated potassium 2-(2,6-dichloroanilino)-phenylacetate was collected by filtration (4.97 g of off-white crystals) and suspended in a mixture of 100 mL of 2 N HCl and 200 mL of ether. The mixture was vigorously shaken and filtered,

and the layers were separated. The ether phase was washed with water and concentrated to leave a yellow, crystalline residue which was dissolved in a hot solution of 4.56 g of Na_2CO_3 and 21 mL of water. The solution was cooled to 0 °C and the precipitated crystals were collected by filtration to give 4.22 g of **4** (56%). Mp: 280–283 °C. Similarly, potassium 2-bromo-5-fluorophenylacetate gave the fluoro analogues **[2-[(2,6-dichloro-4-fluorophenyl)-amino]-5-fluorophenyl]acetic acid (1) and [2-[(2,6-dichlorophenyl)amino]-5-fluorophenyl]acetic acid (2).**

2. (2-Bromo-5-fluorophenyl)acetonitrile. A mixture of 23.2 g of 2-bromo-5-fluorobenzyl bromide[42] (86.4 mmol) of 17.3 g of potassium cyanide (260 mmol) in 160 mL of ethanol and 35 mL of water was refluxed for 3 h, cooled, and evaporated. A 300-mL portion of water and 300 mL of ether were added to the residue. The aqueous layer was separated and washed with ether (3 × 50 mL). The combined organic phases were washed with 100 mL of water and evaporated. Crystallization from methylcyclohexane afforded 8.5 g of product (46%). Mp: 77–79 °C. ^{1}H NMR ($CDCl_3$): δ 7.57 (dd, $J_{3,F}$ = 5, $J_{3,4}$ = 9, H-3), 7.30 (dd, $J_{6,F}$ = 9, $J_{4,6}$ = 3, H-6), 6.97 (td, $J_{4,F} = J_{3,4}$ = 9, $J_{4,6}$ = 3, H-4), 3.82 (s, CH_2). Anal. (C_8H_5BrFN): C, H, N.

3. Potassium 2-Bromo-5-fluorophenylacetate. To a suspension of 8.5 g of (2-bromo-5-fluorophenyl)acetonitrile (39.7 mmol) in 9.2 mL of acetic acid was added a mixture of 9.2 mL of concentrated H_2SO_4 and 9.2 mL of water. The solution was refluxed for 2 h, cooled, and poured onto 400 g of crushed ice. The precipitate was filtered, washed with 200 mL of water, and dried in vacuo for 10 h. The crystals were dissolved in 19 mL of 2 N KOH. The solution was evaporated in vacuo at 20 °C. The residue was crystallized from ethanol to yield 9.5 g (95%) of the potassium salt. Mp: 276–278 °C. ^{1}H NMR (DMSO-d_6): δ 7.48 (dd, $J_{3,F}$ = 5.5, $J_{3,4}$ = 9, H-3), 7.15 (dd, $J_{6,F}$ = 10, $J_{4,6}$ = 3, H-6), 6.90 (td, $J_{4,F} = J_{3,4}$ = 9, $J_{4,6}$ = 3, H-4), 3.27 (s, CH_2). Anal. (C_8H_5BrFOK): C, H, Br, F, K.

Method F. Preparation of 2-(Phenylamino)phenylacetic Acids (via Ullmann Reaction of *N*,*N*-Dimethyl-2-iodophenylacetamide). 1. *N*,*N*-Dimethyl[2-[(3,5-dichlorophenyl)amino]phenyl]acetamide (42). A mixture of 8.6 g of *N*,*N*-dimethyl-2-iodophenylacetamide[20] (29.75 mmol), 10.0 g of 3,5-dichloroaniline (61 mmol), anhydrous potassium carbonate (23.48 mmol), 1.0 g of powdered activated copper, 0.3 g of cuprous iodide, and 140 mL of toluene was heated under reflux with stirring for 100 h, while water was removed with a Dean–Stark apparatus filled with molecular sieve type 4 Å. The mixture was filtered while hot. The filtrate was evaporated and the resultant brown oil was subjected to steam distillation to remove excess 3,5-dichloroaniline. The aqueous residue was extracted with ethyl acetate (500 mL). The organic phase was washed with water (100 mL) and evaporated. The residue was purified by flash column chromatography (250 g of silica gel, 2:1 hexane–ethyl acetate) to yield the pure product **42** (7.1 g, 73.7%). Mp: 97–99 °C. ^{1}H NMR ($CDCl_3$): δ 8.42 (s, b, NH), 7.40 (dd, H-3′), 7.24 (td, H-4′), 7.19 (dd, H-6′), 6.99 (td, H-5′), 6.89 (d, H-2 and H-6), 6.78 (t, H-4), 3.71 (s, CH_3), 3.20 (s, NCH_3), 2.97 (s, NCH_3). Anal. ($C_{16}H_{16}Cl_2N_2O$): C, H, N.

2. [2-[(3,5-Dichlorophenyl)amino]phenyl]acetic Acid (25). A mixture of 7.1 g of *N*,*N*-dimethyl[2-[(3,5-dichlorophenyl)-amino]phenyl]acetamide (**42**, 21.94 mmol), 6.0 g of KOH (96 mmol), and 100 mL of ethanol was refluxed for 10 h. The solvent was distilled off under reduced pressure. The residue, dissolved in 100 mL water, was washed with ether, cooled to 0 °C, and acidified with concentrated HCl. The precipitate was extracted with ether. The organic extract gave, after washing with water, filtration, and evaporation, the crude product. Crystallization from ether–petroleum ether afforded 5.46 g of **25** (84%). Mp: 138–140 °C. ^{1}H NMR ($CDCl_3$): δ 10.0 (b, COOH), 7.25–7.40 (m, H-3′, H-4′, and H-6′), 7.12 (m, H-5′), 6.78 (t, H-4), 6.71 (d, H-2 and H-4), 3.67 (s, CH_2). Anal. ($C_{14}H_{11}Cl_2NO_2$): C, H, N.

Dissociation Constants. Since all of the analogues of this series are phenylacetic acids of the same type differing only in the substitution patterns of the aromatic rings, the pK_a's do not vary much. Most of the compounds are not soluble enough in water for direct potentiometric titration. One alternative method used was to extrapolate apparent pK's determined by potentiometric titration in methylcellosolve–water mixtures of varying compositions to 100% water.[31] The other method was by spectrophotometric titration as described by Albert and Serjeant[44] using a flow-through spectrophotometric cell in a Cary 118 connected to a microtitration setup essentially as described by Simon.[45]

1-Octanol/Water Partition Coefficients. Octanol/aqueous phase partition (distribution) coefficients were determined at physiological pH 7.4 with M/15 phosphate buffer saturated with 1-octanol (Wang and Lien[46]). The experiments were carried out by the flask-shaking procedure according to established procedures (OECD method No. 107, OECD[47]). The log P values of five compounds, listed in Table II, were not measured but estimated from the calculated log P_u of the undissociated compound and the pK_a using eq 1.

QSAR Variables. For the aromatic ortho, meta, and para substituents of ring B, Hammett σ constants were used to model their electronic effects. The size of the ortho substituents of ring B was described by B_1 and B_5, the smallest and largest diameter of the van der Waals volume of the substituent (Verloop et al.[41]). We also used the sum of the B_1 values of the two ortho atoms or groups in order to combine in only one independent variable the total bulk of the ortho substituents.

Particular substitution patterns of the aromatic rings, such as the presence of halogen atoms in ring A or OH groups in either ring A or B, were described by indicator variables. A total of seven indicator variables were used in the final analysis.

X-ray Analysis. Crystal data for the five compounds measured are given in Table III. X-ray data were collected on an automatic four-circle diffractometer, ENRAF NONIUS CAD4, with graphite-monochromated Cu Kα radiation and 0/20 procedure. The measured intensities were corrected for Lorentz and polarization effects but not for absorption.

All structures were solved by direct methods (MULTAN 80[48]). Data for the hydrogen atoms were found from difference Fourier maps. Least-squares refinements were carried out with anisotropic thermal parameters for non-H atoms and isotropic ones for H atoms. Final fractional coordinates and bond lengths and angles with their standard deviations are given in the supplementary material. They agree in general with the standard values quoted in the literature. Thermal parameters and tables of observed and calculated structure factors are available on request from the authors.

Data Analysis. Multiple regression analyses were carried out with the program STATGRAPHICS using an IBM/PC/AT.

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Registry No. 1, 127792-42-1; 1 (free acid), 127792-20-5; **2**, 127792-43-2; **2** (free acid), 127792-21-6; **3**, 15307-80-9; **3** (free acid), 15307-85-4; 4, 15307-79-6; 4 (free acid), 15307-86-5; **5**, 118409-80-6; **6**, 23189-28-8; **7**, 127792-44-3; **7** (free acid), 127792-22-7; **8**, 127792-45-4; **8** (free acid), 127792-23-8; **9**, 64758-92-5; **10**, 127792-24-9; 11, 127792-25-0; **12**, 23189-27-7; **13**, 70172-32-6; 14,

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69002-85-3; **15**, 37984-36-4; **16**, 127792-26-1; **17**, 24542-90-3; **17** (free acid), 15307-71-8; **18**, 122794-97-2; **19**, 127792-27-2; **20**, 100754-91-4; **20** (free acid), 100754-93-6; **21**, 127792-28-3; **22**, 127792-29-4; **23**, 70172-31-5; **24**, 118423-38-4; **25**, 127792-30-7; **26**, 127792-31-8; **27**, 127792-46-5; **27** (free acid), 127792-32-9; **28**, 64118-84-9; **29**, 127792-33-0; **30**, 127792-34-1; **31**, 127792-47-6; **31** (free acid), 127792-35-2; **32**, 90233-41-3; **32** (free acid), 90233-40-2; **33**, 70172-33-7; **34**, 106610-60-0; **35**, 69002-86-4; **36**, 69002-84-2; **38**, 15307-93-4; **39**, 15308-01-7; **40**, 15362-40-0; **41**, 15307-81-0; **42**, 127792-36-3; **43**, 127792-37-4; **44**, 127792-38-5; **45**, 127792-39-6; **46**, 127792-40-9; **47**, 73328-72-0; **48**, 127792-41-0; $ClCH_2COCl$, 79-04-9; oxalyl chloride, 79-37-8; *N,N*-dimethyl-[2-[(3,5-dichlorophenyl)amino]phenyl]acetamide, 127792-48-7; 2,6-dichloroaniline, 608-31-1; potassium 2-iodophenylacetate, 100754-92-5; potassium cyanide, 151-50-8; 2-bromo-5-fluorobenzyl bromide, 112399-50-5; (2-bromo-5-fluorophenyl)acetonitrile, 127792-49-8; potassium 2-bromo-5-fluorophenylacetate, 127792-50-1; 3,5-dichloroaniline, 626-43-7; *N,N*-dimethyl-2-iodophenylacetamide, 75117-26-9; cyclooxygenase, 39391-18-9.

Supplementary Material Available: Tables listing the correlations between the parameters used in the regression equations, the crystal data, final atomic positional parameters, bond distances, and angles of compounds **4**, **12**, **30**, **32**, and **33** (18 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Evaluation of Some Cyclic Phosphoramidate Nucleoside Derivatives

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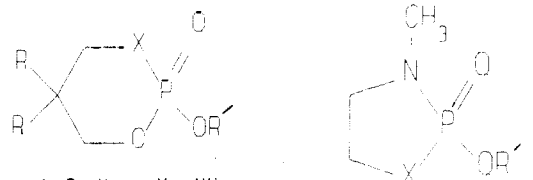
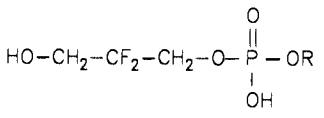
(*E*)-5-(2-Bromovinyl)-2′-deoxy-5′-*O*-(3-methyl-2-oxo-5-formyl-1,3,2-oxazaphosphacyclopentan-2-yl)uridine has been synthesized and, under physiological conditions and without the necessity for enzyme activity, has been shown to yield the 5′-nucleotide in vitro. Unfortunately this compound is not sufficiently stable in solution for it to be tested in vivo. The biological properties of this and some related derivatives of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine and acyclovir have been evaluated in in vitro and in vivo systems designed to show the effects of any intracellular liberation of the nucleotide. Although some of the derivatives are probably acting as prodrugs of the active nucleosides, there is no evidence for the liberation of meaningful concentrations of the 5′-nucleotide by any of the compounds.

Organic phosphomonoesters, such as nucleotides, do not readily enter living cells and in any case are often dephosphorylated before they can penetrate the membrane. Many drugs, particularly nucleoside analogues, owe their activity to the fact that in the living cell they are metabolized in the first instance to their phosphomonoesters, and it might be of therapeutic value if a class of neutral phosphomonoester prodrugs could be synthesized that may penetrate the cell wall and then subsequently be converted to a biologically active drug.

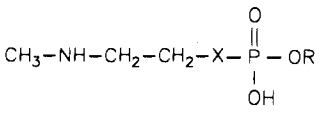
For the past decade, we have synthesized a number of cyclic phosphotriester and phosphoramidate derivatives of 5-substituted 2′-deoxyuridine analogues (**1–5**), in order to see if they would have such properties.[1,2] Similar compounds have been synthesized by other workers.[3,4]



These compounds (**1–5**) were evaluated for their ability (a) to inhibit the growth of leukemia L1210 cells in cell culture and (b) to act as thymidylate synthase inhibitors either in cell culture or against the isolated enzyme. Compounds **1** and **2**, which have stable ring systems, were essentially inactive whereas compounds **3–5**, which are hydrolyzed under physiological conditions to acyclic phosphorus intermediates, showed considerable inhibitory activity against L1210 cells. This activity appeared to be due to the inhibition of thymidylate synthase in the cells, although compounds **1c–2a,2b** themselves did not inhibit the isolated enzyme. However, these compounds were not active against a variant of L1210 cells which was resistant to 5-bromo-2′-deoxyuridine (presumably because of lack of thymidine kinase activity) and against which one might have expected to see some activity if indeed the 5′-mononucleotide was being liberated inside the cell. Thus, the activity seen in the normal L1210 cell line was probably due to the production of 5-fluoro-2′-deoxyuridine outside the cell which was then subsequently phosphorylated once inside the cell. Although compounds **3** and **4** are hydrolyzed at physiological pH, the products have been shown to be of the type **6** and **7**, respectively.



(6)



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