Structure–Activity Relationships among Some Antiinflammatory 3-(5-Aryl-2-tetrazolyl)alkanoic Acids and Derivatives

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The Hansch and Free–Wilson mathematical models were used to examine the structure–activity patterns among the title compounds and their primary amides. The results of both were in agreement and were used to define the steric, electronic, lipophilic, and geometrical requirements for antiinflammatory activity. These requirements were quite exact in contrast to the known nonspecific nature of the physiological response elicited by other antiinflammatory acids.

Two mathematical models for analyzing structure–activity relationships have been developed within the last 10 years. The Hansch method uses combinations of lipophilic, electronic, and occasionally steric substituent parameters to correlate changes in chemical structure with changes in biological response. It is usually expressed mathematically as

$$\log \text{biological response} = -a\pi^2 + b\pi + c\sigma + dE_s + e \qquad (1)$$

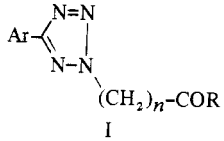
where π, the lipophilic substituent constant, is defined as $\log P_X - \log P_H$ in the 1-octanol–water system.[1] The variables σ and E_s are the Hammett and the Taft electronic and steric constants, respectively.[2,3] The numerical solution of eq 1 provides a measure of the relative influence of each of these physicochemical properties on the biological activity.

The second method is that of Free and Wilson, and it treats the total biological activity of a series as the sum of the incremental activities contributed by each part of every member of the series.[4] It takes the form

$$\text{biological response} = \mu + \Sigma a_i R_i \qquad (2)$$

where μ is the contribution to the biological activity made by the nucleus of the series and a_i that made by the substitutents R_i. The Free–Wilson model requires no substitutent constants for its application. However, it suffers the disadvantage of lacking any concrete basis for interpreting the results of the analysis in terms of a drug–receptor interaction.

Previous studies on the 2-alkanoic acids of the 5-aryltetrazoles, Ia and Ib, had established what structural geometry was necessary for antiinflammatory activity.[5] These two



I

a, n = 1; R = OH
b, n = 2; R = OH
c, n = 1; R = NH_2
d, n = 2; R = NH_2

models were used to determine the optimum substitutents.

5-Aryltetrazolylacetic and -propionic Acids and Amides. The propionic acids used in the Hansch analysis are listed in Table I together with their substitutent constants and antiinflammatory activities. The ortho-substituted compounds were not included because of possible distortion of the aryltetrazole interplanar angle that might introduce another variable into the situation.

All of the acids and amides were prepared by standard methods and most of the acids were reported previously.[5] Those that were not, along with all the amides, are new compounds. The pertinent experimental data for these are listed in Tables II and III.

The various substitutents in Table I represent a substantial range in lipophilic character: π values vary from −0.91 for the 3-amino compound **12** to 1.84 for the 3,5-dibromo compound **10**. These values were largely taken from the phenoxyacetic acid system.[1b] Those not appearing in this list were determined from the partition coefficient of the compounds themselves according to the published procedure.[6]

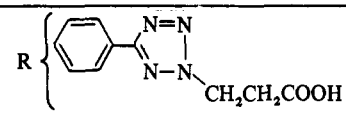
Biological Response. The antiinflammatory activities of these compounds were measured by their ability to protect rats against pleurisy induced by the irritants Evans Blue and carrageenin. The introduction of a mixture of these irritants (0.05 ml of a 0.316% solution) into the rat pleural cavity causes an inflammation that results in an increase in the volume of pleural fluid in the following 6 hr. This increase can be inhibited by a wide variety of antiinflammatory compounds including steroids and gold salts, and the inhibition is dose dependent.[7]

Each test compound is administered orally to six rats 1 hr before the irritants at a dose of 1-mmole per kg. Six hours after the irritants are given the animals are killed and the volumes of their pleural fluid measured. These values are compared with those of control groups of untreated animals as well as animals treated with 1-mmole doses of aspirin and phenylbutazone.[8] The results are expressed as an activity index (AI) which is a value equal to ten times the ratio of the mean pleural exudate volume (ml) of the fluid from the test rats divided by that from the six animals treated with the reference drug. Initially aspirin was used as the reference drug but was later dropped in favor of phenylbutazone. The response of the control rats to aspirin often varied a great deal from day to day while the effect due to phenylbutazone was much more reproducible. This may be due to the better solubility of the latter drug. Since many of the compounds in the Free–Wilson analysis were comparatively old, AI values based on aspirin were used. For the Hansch analysis, the AI values were calculated relative to phenylbutazone.[8]

The method used here of expressing the biological activity as the variable response due to a constant molar dose differs from the usual procedure of examining the variable molar dose necessary to evoke a constant response (ED_{50}, LD_{50}, etc.). The method is not novel however, and a comparison of the two expressions has been discussed elsewhere.[9]

Hansch Analysis. At first, all the compounds in Table I except the 4-amino analog (**34**, which was not tested) were examined against only the π values. The first- and second-order π terms gave a poor correlation (eq 3) which was only

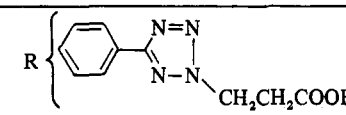
Table I. Observed and Calculated Acute Antiinflammatory Activities for Some 3-(5-Aryl-2-tetrazolyl)propionic Acids by the Method of Hansch



No.	Substitutent	π[b]	σ_m[c]	σ_p[c]	E_s[d]	Log AI[a] Obsd	Log AI[a] Calcd[e]	Δ log AI
1	H	0.00	0.00	0.00	1.24	0.91	0.73	0.18
2	3-F	0.13	0.06	0.34	1.24	1.03	0.79	0.24
3	4-F	0.15	0.34	0.06	0.49	0.81	0.80	0.01
4	3-Cl	0.76	0.23	0.37	1.24	1.06	0.92	0.14
5	4-Cl	0.71	0.37	0.23	0.18	0.77	0.91	−0.14
6	3,5-Cl_2	1.52	0.75[f]	0.75[f]	1.24	1.04	0.74	0.30
7	3,4-Cl_2	1.46	0.53[f]	0.53[f]	0.18	0.90	0.77	0.13
8	3-Br	0.94	0.23	0.39	1.24	1.06	0.91	0.15
9	4-Br	1.02	0.39	0.23	0.00	0.96	0.90	0.06
10	3,5-Br_2	1.88	0.72[f]	0.72[f]	1.24	0.42	0.54	−0.12
11	3-I	1.15	0.28	0.35	1.24	0.93	0.87	0.06
12	3-NH_2	−0.91[g]	−0.66	−0.16	1.24	−0.47	0.04	−0.51
13	3-CH_3CONH	−0.79	−0.02	0.21[h]	1.24	0.02	0.16	−0.14
14	4-CH_3CONH	−0.79[i]	0.21[f]	−0.02	0.99[j]	0.45	0.16	0.29
15	3-$C_6H_5N{=}N$	1.71[g]	0.64	0.64	1.24	0.68	0.64	0.04
16	4-$C_6H_5N{=}N$	1.71	0.64	0.64	−0.75[k]	0.64	0.64	0.00
17	3-NO_2	0.11	0.78	0.71	1.24	0.00	0.78	−0.78
18	4-NO_2	0.24	0.71	0.78	−0.75	0.04	0.83	−0.79
19	3,5-$(NO_2)_2$	0.22	1.40[f]	1.40[f]	1.24	0.60	0.82	−0.22
20	4-OH	−0.61	0.00	−0.36	0.99[j]	0.70	0.32	0.38
21	3-CH_3O	0.12	−0.27	0.12	1.24	0.62	0.78	−0.04
22	4-CH_3O	−0.04	0.12	−0.27	0.99	0.70	0.71	0.01
23	3,5-$(CH_3O)_2$	0.24	0.50[f]	0.50[f]	1.24	0.59	0.83	−0.24
24	3,4-$(CH_3O)_2$	−0.23	−0.12[f]	−0.12[f]	0.99	0.40	0.60	−0.20
25	3-CH_3	0.51	−0.17	−0.07	1.24	0.89	0.89	0.00
26	4-CH_3	0.52	−0.07	−0.17	0.00	0.50	0.90	−0.40
27	4-$(CH_3)_3C$	1.68	−0.12	−0.20	−1.54[l]	0.53	0.53	0.00
28	3-CF_3	1.07	0.55	0.42	1.24	0.75	0.90	−0.15
29	4-CF_3	1.07[i]	0.42	0.55	−1.16	0.57	0.90	−0.33
30	3-CH_3CO	−0.28	0.52	0.31	1.24	0.40	0.57	−0.17
31	4-CH_3CO	−0.37	0.31	0.52	−0.75[k]	0.50	0.51	0.01
32	3-pyridyl	−0.90[g]	0.93[m]	0.64[m]	1.24	−0.08	0.05	−0.13
33	4-SO_2NH_2	−0.58[g]	0.46[h]	0.57	−0.75[k]	0.72	0.35	0.37

[a] Activity index = ten times the ratio of the mean pleural exudate volumes of the test compound at 1 mmole/kg over 1 mmole/kg of phenylbutazone. [b] From the phenoxyacetic acid series, ref 1b, Table I unless noted. [c] From ref 2, Table VII except where noted. [d] From ref 3, p 228 unless noted. [e] Calcd from eq 10. [f] From ref 2, Table XVI. [g] Determined in 1:1 octanol-water. [h] See ref 24. [i] The value for the meta isomer was used. [j] Estimated as equal to methoxy. [k] Estimated as equal to nitro. [l] See ref 25. [m] From ref 2, Table XVIII.

Table II. 3-(5-Aryl-2-tetrazolyl)propionic Acids



No.	R	Mp, °C	Formula	% yield[a]	Recrystn solvent	Analysis
34	4-NH_2	182	$C_{10}H_{11}N_5O_2$	94	MeOH	N basic[b]
14	4-CH_3CONH	218	$C_{12}H_{13}N_5O_3$	40	MeOH	C, H
16	4-$C_6H_5N{=}N$	234	$C_{16}H_{14}N_6O_2$	65	MeOH-THF	C, H
21	3-CH_3O	91	$C_{11}H_{12}N_4O_3$	12	C_6H_6	C, H
23	3,5-$(CH_3O)_2$	139	$C_{12}H_{14}N_4O_4$	27	MeOH-H_2O	C, H
27	4-$(CH_3)_3C$	170	$C_{14}H_{18}N_4O_2$	22	C_6H_6	C, H
30	3-CH_3CO	107	$C_{12}H_{12}N_4O_3$	26	MeOH-H_2O	C, H

[a] Yield calcd from the starting tetrazole. [b] Calcd 6.00; found, 5.84.

slightly improved by the addition of an electronic term (eq 4).† Numerous other electronic substitutent constants such as pK_a, σ_p, σ^+, the group dipole moment μ,[11] the electric polarizibility P_E,[12] the molar refractivity R,[13] and the molar attraction constant F[14] were examined as well. None of them was significant. It was obvious simply by inspection, however, that the meta-substituted compounds were always more active than their para counterparts, and for this reason the series was divided into two subseries and each looked at separately.

The meta-substituted compounds with the π values alone gave eq 5. This is a substantial improvement over the previous equations in spite of the observation that the nitro compounds **17** and **19** were still poorly fit. On the premise that their less than expected activity was a consequence of *in vivo* reduction, they were deleted from the set.‡ The 16

†r = multiple correlation coefficient, n = number of members of the set, and s = standard error of the estimate.[10]

‡An experiment was devised to see if this was really the case. For optimum effect, the test drugs are normally given 1 hr before the irritants. If the time lapse between the administration of the drug and the Evans Blue-carrageenin is greater than this, the effectiveness of the drug is diminished. The dinitro compound 19 and the chloro compound 5, which are about equally active, were given 1, 2, and 4 hr before the irritants. If the dinitro compound was being metabolically deactivated, then the effectivensss of 19 should have dropped off more rapidly than the chloro compound. It did not. No other speculation is offered for the poor fit of the nitro-substituted compounds.

remaining meta- and dimeta-substituted phenyltetrazolylpropionic acids gave eq 6. This is significant at the 99.95% level ($F = 54.47$).† None of the electronic parameters were able to improve on this.

	r	n	s	
$\log AI = -0.22\pi^2 + 0.42\pi + 0.61$	0.697	33	0.27	(3)
$\log AI = -0.22\pi^2 + 0.42\pi - 0.06\sigma_m + 0.62$	0.701	33	0.27	(4)
$\log AI = -0.30\pi^2 + 0.56\pi + 0.62$	0.860	18	0.25	(5)
$\log AI = -0.37\pi^2 + 0.58\pi + 0.63$	0.945	16	0.16	(6)
$\log AI = -0.10\pi^2 + 0.19\pi + 0.61$	0.427	15	0.26	(7)
$\log AI = -0.14\pi^2 + 0.23\pi + 0.66$	0.609	14	0.20	(8)
$\log AI = -0.85\sigma_p^2 + 0.77\sigma_m + 0.58$	0.841	14	0.14	(9)
$\log AI = -0.31\pi^2 + 0.48\pi + 0.73$	0.851	28	0.20	(10)
$\Delta \log AI = -0.24\sigma_m + 0.21$	0.662	10		(11)
$\Delta \log AI = -0.42\sigma_p + 0.30$	0.676	10		(12)
$\Delta \log AI = -0.008E_s + 0.17$	0.466	10		(13)
$\log AI$ (para) = 0.79 $\log AI$ (meta) + 0.79	0.850	10		(14)

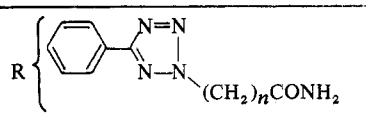
The 15 para compounds were left to be examined (the unsubstituted acid **1** was considered a nonpara isomer since it lacked the seemingly deleterious para-substitutent). As seen from eq 7, these gave no correlation at all with π. Other combinations of π, E_s, μ, R, and P_E were no better. Deletion of the nitro compound **18** gave eq 8, a faint improvement. The best fit of all possible combinations to the para subset was the parabolic electronic relationship of eq 9 ($F = 14.60$).

A comparison of eq 6 and 9 demonstrates the difference between the meta- and para-substitution patterns except for one drawback. The chief offenders against eq 8 are the 4-methyl and the 4-trifluoromethyl compounds **26** and **29**. If they are dropped and the rest of the para subset combined with the meta compounds, eq 10 results. This is a respectable fit ($F = 32.86$), and the regression coefficients resemble those of eq 6 quite closely. From this it could be argued that the difference between the two types of substitution is not real at all but only due to bad experimental data for **26** and **29**. That the difference between the two is real was proved by the following experiment.

There are 11 pairs of aryltetrazolylpropionic acids in Table I that bear the same substitutent in both the meta and the para positions. They are listed separately in Table IV. The Δ log AI values (meta minus para) for these pairs were examined against the substitutent constants σ_m, σ_p, E_s, $\Delta\pi$, $\Delta\sigma$, μ, R, and P_E to see if the meta/para difference was due to a regular variation in some substitutent effect. No significant correlation was found. Although the acetamido pair **13** and **14** deviated badly, their deletion from the set failed to help. The three best fits to the Δ log AI values are eq 11, 12, and 13. A linear relationship was obtained, however, by simply plotting the activities of the para isomers against the meta isomers giving eq 14 ($F = 20.78$, $s = 0.12$). Thus there is a real difference in the way that the meta- and para-substituted compounds produce the observed physiological effects; each type of isomer must interact with the receptor in a different way.[15] Why this is so is not clear. The slope of eq 14 amounts to a rather large difference in activity for so subtle a structural change. Only in a few other instances has biological activity been shown to so greatly affected by the positioning but not the bulk of the attached groups.[16] In the 5-aryl-2-tetrazolylpropionic acid series this positioning effect seems independent of both the size and the electrical nature of the attached group. The compounds are biologically stable, ruling out the possibility of one type of isomer being preferentially metabolized. Such a dependence on position recalls some recent work on steric effects in charge-transfer complex formation.[17] It was found that in some instances certain highly substituted benzene compounds acting as donors were sterically reduced to interacting with acceptors at only one of the two faces of the molecule. As a result, they were only half as effective as complexing agents as were lesser substituted analogs. So it may be the geometry of the meta-substituted compounds that allows the better fit with the receptor, while groups in the para position somehow impede the drug-receptor combination.

But this leads to a problem. If the meta/para distinction stems from a charge-transfer complex between drug and receptor that allows the receptor to discriminate between the two, such a process should be reflected by an electronic parameter in eq 6 and 10. It is not. However, if the free energy of such an interaction were small relative to the total drug-receptor interaction, it could be masked by the electrical component of the π term already present in the equations.[1b,18] And, if this is the case, the activities of compounds whose substitutents have similar π values but different σ values should be distinctly different.

Table III. 5-Aryltetrazolyl-2-acetamides and Propionamides



No.	R	n	Mp, °C	Formula	% yield	Recrystn solvent	Analysis
35	H	1	187	$C_9H_8N_5O$	42	EtOH-H_2O	C, H
36	4-Cl	1	242	$C_9H_8ClN_5O$	79	DMF-H_2O	C, H
37	3,4-Cl_2	1	215	$C_9H_7Cl_2N_5O$	74	EtOH	C, H
38	H	2	132	$C_{10}H_{11}N_5O$	79	EtOH-H_2O	C, H
39	3-Cl	2	146	$C_{10}H_{10}ClN_5O$	60	MeOH-H_2O	C, H
40	4-Cl	2	165	$C_{10}H_{10}ClN_5O$	50	MeOH-H_2O	C, H
41	3,5-Cl_2	2	165	$C_{10}H_9Cl_2N_5O$	72	MeOH-H_2O	C, H
42	3,4-Cl_2	2	155	$C_{10}H_9Cl_2N_5O$	78	EtOAc	C, H
43	3-Br	2	147	$C_{10}H_{10}BrN_5O$	70	EtOH-H_2O	C, H
44	4-Br	2	168	$C_{10}H_{10}BrN_5O$	28	*i*-PrOH	C, H
45	3,5-Br_2	2	202	$C_{10}H_9Br_2N_5O$	69	DMF-EtOH	C, H
46	4-NO_2	2	172	$C_{10}H_{10}N_6O_3$	50	MeOH	C, H
47	3,5-$(NO_2)_2$	2	171	$C_{10}H_9N_7O_5$	54	MeOH	C, H

This is confirmed in Table V. Here are listed the Δ log AI values (deactivating minus activating) of six pairs of acids from Table I whose substitutents have roughly the same π values but different σ constants. In every case the deactivating substitutent is the more active compound.

All of this points to the 5-aryl group on the tetrazole ring in structure I as the site of hydrophobic bonding and a steric factor as well as an electronic interaction. This last interaction seems to be of the charge-transfer type with the 5-phenyl moiety acting as the acceptor. This picture is consistent with the receptors sketched for other nonsteroidal antiinflammatory agents.[19]

Differentiation of eq 6 and 10 gives π_0 values of 0.79 and 0.77, respectively. Log P for the parent compound in the propionic acid series (1) was found to be 0.62, and this gives approximately 1.40 for log P_0. The optimum lipophilic character of this series of antiinflammatory acids can then be represented by an octanol-water partition coefficient of about 25.

Table IV. Differences in Antiinflammatory Activity between Meta- and Para-Substituted Isomers of the 3-(5-Aryl-2-tetrazolyl)propionic Acids

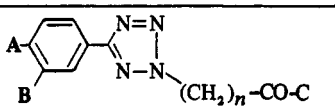
Pair No. (meta, para)	Substitutent	Δ log AI_{m-p}	$\Delta\sigma_{m-p}$	Δ E_s
2, 3	F	0.22	0.28	0.49
4, 5	Cl	0.29	0.14	0.18
6, 7	Cl_2	0.14	0.15	0.18
8, 9	Br	0.10	0.16	0.00
13, 14	CH_3CONH	−0.43	0.23	0.99
15, 16	$C_6H_5N{=}N$	0.04		−0.75
21, 22	CH_3O	−0.08	0.38	0.99
23, 24	$(CH_3O)_2$	0.19	0.38	0.99
25, 26	CH_3	0.39	0.10	0.00
28, 29	CF_3	0.18	−0.13	−1.16
30, 31	CH_3CO	−0.10	−0.21	−0.75

Table V. Differences in Antiinflammatory Activity between Pairs of Electronically Different 3-(5-Aryl-2-tetrazolyl)propionic Acids of Similar Lipophilic Character

Pair No.	Substitutents	Δ log AI	$\Delta\sigma_m$	$\Delta\sigma_p$	$\Delta\pi$
2, 21	3-F, 3-CH_3O	0.41	0.33	0.22	0.01
3, 22	4-F, 4-CH_3O	0.11	0.22	0.33	0.19
4, 25	3-Cl, 3-CH_3	0.17	0.40	0.44	0.25
5, 26	4-Cl, 4-CH_3	0.27	0.44	0.40	0.18
16, 27	4-$C_6H_5N{=}N$, 4-$C(CH_3)_3$	0.11	0.76	0.84	0.03
33, 20	4-SO_2NH_2, 4-OH	0.02	0.46	0.93	0.03

Free-Wilson Analysis. There were several reasons for trying this approach on the 5-aryl-2-tetrazolylalkanoic acid system. All that had been established so far was the proper configuration of the overall molecule and the most advantageous constitution of the aryl group, along with the total lipophilic requirement. The preference for a three carbon side chain was unproven. And the unexpected finding that many amides of the acids in Table I possessed antiinflammatory activity made it uncertain as to which functional

Table VI. Observed and Calculated Acute Antiinflammatory Activities for 27 5-Aryl-2-tetrazolylpropionic and Acetic Acids and Amides by the Method of Free and Wilson



	A				B				n		C		AI[a]		
No.	H	Cl	NO_2	Br	H	Cl	NO_2	Br	1	2	OH	NH_2	Obsd	Calcd (run 1)	Calcd (run 2)
48	1				1				1		1		7.7	7.32	7.43
35	1				1				1			1	5.6	6.39	5.83
49		1			1				1		1		8.3	5.95	6.74
36		1			1				1			1	5.3	5.02	4.67
50		1				1			1		1		6.9	6.92	7.49
37		1				1			1			1	3.8	5.99	5.89
1	1				1					1	1		11.0	11.21	11.31
38	1				1					1		1	9.3	10.31	9.49
4	1					1				1	1		12.6	12.17	12.31
39	1					1				1		1	9.1	11.24	10.61
5		1			1					1	1		7.9	9.83	9.93
40		1			1					1		1	9.5	8.90	8.33
6	1					2				1	1		15.5	13.52	13.44
41	1					2				1		1	12.2	12.59	11.84
7		1				1				1	1		13.2	10.80	11.15
42		1				1				1		1	8.4	9.87	9.55
8	1							1		1	1		12.9	10.20	13.57
43	1							1		1		1	12.9	9.27	11.97
9				1	1					1	1		10.6	10.21	10.55
44				1	1					1		1	8.9	9.28	8.95
10	1							2		1	1		3.8	9.58	
45	1							2		1		1	11.2	10.15	
17	1						1			1	1		5.7	7.95	8.18
18			1		1					1	1		1.2	2.56	2.90
46			1		1					1		1	3.0	1.64	1.39
19	1						2			1	1		6.0	5.08	5.18
47	1						2			1		1	4.3	5.15	3.58

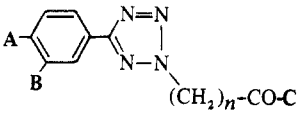
For run 1	Position
15 (H) + 8 (Cl) + 2 (NO_2) + 2 (Br) = 0	A
12 (H) + 10 (Cl) + 5 (NO_2) + 6 (Br) = 0	B
6 (n = 1) + 21 (n = 2) = 0	n
14 (OH) + 13 (NH_2) = 0	C

For run 2	Position
13 (H) + 8 (Cl) + 2 (NO_2) + 2 (Br) = 0	n
12 (H) + 10 (Cl) + 5 (NO_2) + 2 (Br) = 0	C
6 (n = 1) + 19 (n = 2) = 0	A
13 (OH) + 12 (NH_2) = 0	B

[a] Activity index = 10 times the ratio of the mean pleural exudate volumes of the test compound at 1 mmole/kg over 1 mmole/kg of aspirin.

group was better for activity. Some antiinflammatory acids are even known to give rise to amides which are inactive.[20] The Free-Wilson method also seemed to be a good way to confirm the difference between the meta and para isomers, as well as checking the prediction that the linear model would break down as the activity dependence on π becomes parabolic.[21]

The data in Table VI have been restricted to those compounds having H, Cl, NO_2, and Br substitutents in the meta and para positions of the phenyl ring (A and B in structure II); one or two methylene groups in the side chain; and to acids and primary amides. Dimeta-substituted compounds were given a coefficient of two for position B. Arithmetic values of the activity index referenced to aspirin served as the dependent variable. First the entire set of 27 compounds was run (run 1); then the dibromo compounds **10** and **45**



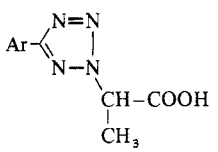
II

were dropped and the analysis repeated (run 2). For each compound in the set an equation is generated from Table VI. For each position of substitution another equation is generated by summing up all the substituents at that position and setting their total contributions to the activity equal to zero. These are the so-called constraint equations, and they are listed at the bottom of Table VI.

For the full set of 27 compounds there are 31 simultaneous equations in 13 unknowns. For these the regression produced a fit of $r = 0.834$; $s = 0.24$; $F = 5.15$; $n = 27$. The contributions of the nucleus μ and the substitutents are listed in Table VII. The dibromo pair was then dropped and the second analysis gave $r = 0.931$; $s = 0.16$; $F = 12.90$; $n = 25$. The mediocre correlation for the first run was markedly improved by dropping the highly lipophilic dibromo compounds **10** and **45**. With these present the set contains a lipophilic spread of 2.40 log units; without them it is 1.88. Most of the improvement in run 2 stems simply from the removal of the acid **10**. The amide is not badly fit at all. Still the results of run 2 tend to bear out the predicted inability of the linear Free-Wilson model to cope with a second-order dependence of biological activity on lipophilic character.[21]

A comparison of the group contributions at positions A and B (Table VII) confirms the meta/para distinction: attachment at the latter position always contributes less to the activity than at the former.

The preference for a three carbon atom side chain is confirmed by the superiority of $n = 2$ over $n = 1$, a difference of 3.66 log units of the activity index. This must be due to a geometrical rather than a lipophilic requirement since the structurally isomeric α-methylacetic acids III were much less active than the corresponding propionic acids Ib.[5]



III

The ranking of the aromatic substitutents at both positions is Br > Cl > H > NO_2, roughly in line with their π values. It is interesting to note that the nitro compounds are well handled by this model in contrast to their poor fit to the Hansch eq 6 and 10.

Of the 264 possible compounds represented by structure II in Table VI, the two predicted from run 2 to be the most active are the 3,5-dichloropropionic acid **6** and the 3-bromopropionic acid **8**. And they are, in fact, the most active 5-aryl-2-tetrazolylalkanoic acids yet made. The other unsynthesized analogs are predicted to be less active.

Conclusions

Two points of interest emerge from the structure-activity studies on these compounds: one pertains to method, the other to the results.

While the Hansch method provided much information about the influence of lipophilic character on activity, it also obscured the role of the electronic state of the aromatic portion of the molecule. This was due mainly to the conglomerate nature of π, but also to the poor choice of substitutents. Only seven of the 33 compounds in Table I have negative σ_m values. Although nothing can be done about the partial electronic nature of π, a selection of roughly equal numbers of activating and deactivating substitutents would probably have prevented masking the electrical effect in eq 6 and 10.

The second finding was the existence of closely defined steric and geometrical, as well as electronic, requirements for activity. Nonsteroidal antiinflammatory drugs are felt to be nonspecific in action, affecting a wide variety of biochemical and physiological processes.[22] Membrane stabilization, for instance, is suspected as a mechanism of action for many nonsteroidal antiinflammatory drugs.[23] While the aryltetrazolylpropionic acids may indeed do this, it is hard to see how such a process could be so strongly affected by the shape and electrical nature of the aryl group. The lipophilic character of the drugs may be involved with membrane stabilization, but the constitution of the phenyl ring must be affecting some other process. Perhaps eq 14 represents one kind of interaction, the electronic dependency another, and eq 6 and 10 a conglommerate of several different lipophilic requirements. Or perhaps there is only one physiological response really relevant to the antiinflammatory effect (at least the chronic effect), with the others being either unrelated to the problem or themselves manifestations of some more fundamental biochemical process.

Experimental Section§

Preparation of the 5-Aryltetrazolyl-2-acetic and -propionic Acids (Ia and Ib). Most of these were made by alkylating the sodium salt of the 5-aryltetrazoles with either ethyl bromoacetate or ethyl 3-bromopropionate as described.[5] See Table II.

Preparation of the 5-Aryltetrazolyl-2-acetamides and -propionamides (Ic and Id). These were made by refluxing the correspondng acids in a 1:1 v/v mixture of $CHCl_3$ and $SOCl_2$ (10 ml per g) for 6 hr. The solutions were evaporated to dryness under reduced pressure, the residues taken up in ether (10 ml per g), cooled to 0°, and saturated with NH_3 to give the primary amides. See Table III.

3-[5-(4-Aminophenyl)-2-tetrazolyl]propionic Acid (34). A solution of 18 g (0.069 mole) of 3-[5-(4-nitrophenyl)-2-tetrazolyl]propionic acid (**18**) in 350 ml of MeOH was hydrogenated over Pd/C at 5 atm. Recrystallization from MeOH gave 15 g (94%) of tan crystals, mp 182°.

§ All melting points are uncorrected and were determined with a Büchi capillary melting point apparatus (W. Büchi, Glasapparatefabrik, Flawil, Switzerland). Analyses indicated by symbols were within ±0.4% of the calculated values. The regression analyses were performed with an IBM 360-50 computer using either BMD 02R (UCLA) stepwise or BMD 03R (UCLA) multiple least-squares programs.

Table VII. Group Contributions as Calculated by the Free-Wilson Model from Structure II

Position	Group	Contribution Run 1	Contribution Run 2
	μ	8.39	8.46
A	H	1.12	1.07
A	Cl	−0.25	−0.09
A	NO_2	−7.52	−7.12
A	Br	0.13	0.53
B	H	0.38	−0.09
B	Cl	1.35	1.13
B	NO_2	−2.87	−3.00
B	Br	−0.62	2.39
n	1	−3.02	−2.78
n	2	0.86	0.88
C	OH	0.45	0.77
C	NH_2	−0.48	−0.83

3-[5-(4-Acetamidophenyl)-2-tetrazolyl]propionic Acid (14). A solution of 5 g (0.022 mole) of 3-[5-(4-aminophenyl)-2-tetrazolyl]-propionic acid 34 in 200 ml of Ac_2O was allowed to stand for 24 hr at room temperature. The reaction was diluted with 2 l. of water and the solid collected and dried. Recrystallization from MeOH gave 2.4 g (40%) of fine white needles, mp 218°.

3-[5-(4-Phenylazophenyl)-2-tetrazolyl]propionic Acid (16). A solution of 10 g (0.043 mole) of 3-[5-(4-aminophenyl)-2-tetrazolyl]-propionic acid (34) and 4.6 g (0.043 mole) of nitrosobenzene in 150 ml of warm glacial acetic acid was allowed to stand for 24 hr. The precipitate was collected, washed with MeOH, and recrystallized from THF–MeOH to give 9 g (65%) of red crystals, mp 234°.

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Synthesis and Structure–Activity Relationships of Disodium Cromoglycate and Some Related Compounds

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The synthesis of the antiasthmatic substance 1,3-bis(2-carboxychromon-5-yloxy)-2-hydroxypropane disodium salt (disodium cromoglycate) and a number of its analogs is described. The homologous passive cutaneous anaphylaxis (PCA) reaction in the rat, based on a reaginic antibody–antigen system, has been used as a routine screen to assess the activity of these compounds as potential antiasthmatic drugs. The structural requirements for biological activity in the PCA reaction are discussed with reference to the type and position of linkage of the two chromone nuclei. There is an indication that in this system coplanarity of the chromone nuclei is one requirement for activity.

Khellin (I)[1,2] is a naturally occurring oxygen heterocycle with vasodilator and smooth muscle relaxing properties, which has had limited clinical use in the treatment of angina and bronchial asthma. Our investigations led to a series of chromone-2-carboxylic acids which did not possess the biological properties associated with khellin. On administration to an asthmatic volunteer prior to antigen challenge, however, they inhibited in varying degrees the bronchoconstrictor response. The development of this discovery led to the introduction of disodium cromoglycate (II) (cromolyn sodium, USAN)† for the treatment of asthma.

†Intal, Lomudal.