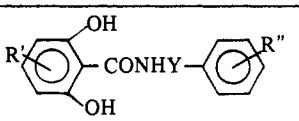
Table IV. Influence of an Enlargement of the DHB-anilides by Phenoxy Substituents on $K_{I\text{-}SDH\text{-}R}$ and $K_{I\text{-}SDH\text{-}F}$ and on Biological Data



R'	Y	R"	$K_{I\text{-}SDH\text{-}R}$, mol/l.	$K_{I\text{-}SDH\text{-}F}$, mol/l.	MTD[c] ($\times 10^{-5}$ mol/kg)	Q[d]	Effectiveness in sheep[e] ($\times 10^{-5}$ mol/kg)	
3,5-di-Cl		4'-Cl	18.00×10^{-8}	6.00×10^{-9}	6	30	0.6	+++
3,5-di-Cl	2-Cl-C_6H_4O	4'-Cl	0.87×10^{-8}	1.7×10^{-9}	5.4	5	0.65	+++
3-NO_2		4'-Cl	58.30×10^{-7}	9.73×10^{-7}	19.4	6	3.24	+++
3-NO_2	C_6H_5O	4'-Cl	0.82×10^{-7}	0.45×10^{-7}	24.9	1.8	2.5	++
3-NO_2	2-Cl-C_6H_4O	4'-Cl	0.46×10^{-7}	0.46×10^{-7}	22.9	1.0	2.3	+
3-NO_2		3',5'-di-CF_3	48.7×10^{-8}	29.3×10^{-8}	3.41	1.7	0.6	+++
3-NO_2	C_6H_5O	3',5'-di-CF_3	6×10^{-8}	6×10^{-8}	20	1	2	+++
3-NO_2	2-Cl-C_6H_4O	3',5'-di-CF_3	3×10^{-7}	1.1×10^{-7}	15	1	3.7	+++
Rafoxanide[f]			7.68×10^{-9}	5.6×10^{-9}		1.4	1.35	+++

[c-f] See footnotes of Table I.

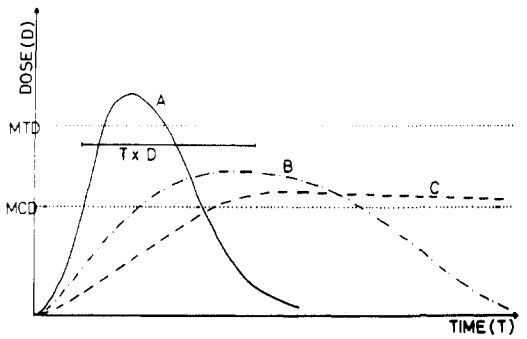


Figure 1. Dose-response curve of differently substituted DHB-anilides as fasciolicides: MTD = maximum tolerated dose; MCD = minimal curative dose; A, B, C = concentrations in the blood of compounds with different pharmacokinetic properties (see text).

constants ($K_{I\text{-}SDH\text{-}F}$) and Q values of the compounds, presumably by increasing the lipophilicity. The *in vivo* effectiveness is increased but to a lesser degree than might be expected from the decrease in $K_{I\text{-}SDH\text{-}F}$ values. These findings might indicate that the compounds are less easily absorbed or that their unspecific binding to body lipids is considerably stronger (Figure 1, type C). The use of these compounds as fasciolicides might involve residue problems.

In summary, then, although for good *in vivo* fasciolicide activity with DHB anilides one must have potent (low $K_{I\text{-}SDH\text{-}F}$) and preferably selective (high Q values) compounds, it would appear that in many cases the factors determining usefulness are biopharmaceutic or pharmacokinetic in nature.

Acknowledgments. We thank Professor Dr. H. Ruschig, J. König, Dr. H. Loewe, Dr. A. Sinharay, and Dr. E. Druckrey for making the compounds available to us and Miss R. Maier and Mr. R. Reisenleiter for their excellent technical assistance. The authors also thank Professor A. Burger and Dr. R. Allen for editorial assistance.

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2,6-Dihydroxybenzoic Acid Anilides Active against Liver Flukes. A Hansch Analysis

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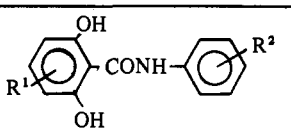
With the aid of Hansch analysis quantitative structure-activity relations are established between 2,6-dihydroxybenzanilides substituted in both phenyl rings and their inhibitory activity on the enzyme succinate dehydrogenase, isolated from rats and liver flukes.

The liver fluke (*Fasciola hepatica*) infests sheep and cattle and represents a major problem in veterinary medicine.[1,2] The most probable point of attack of several active substances against *F. hepatica* is that enzyme system which controls the transformation of fumarate into succinate and vice versa.[3] Since liver flukes are very much dependent on this enzyme,[4-6] its inhibition must greatly reduce the viability of the parasites.

Table I. Structure-Activity Parameters



No.	R^1	R^2	pK_i-R[a]	pK_i-L[a]	$\Sigma\pi_1$[b]	$\Sigma\pi_2$[b]
1	H	3-Cl	5.120	4.889	0.00	0.76
2	H	4-Cl	5.120	5.023	0.00	0.70
3	H	4-Br	5.188	5.284	0.00	1.02
4	H	4-J	5.550	5.509	0.00	1.26
5	H	3,5-$(CF_3)_2$	6.562	6.261	0.00	2.14
6	H	4-$CONH_2$		4.532	0.00	−1.49
7	H	4-Phenothiazinyl	7.629	6.629	0.00	4.15
8	3-NO_2	4-OCH_3		5.121	0.11	−0.04
9	3-NO_2	H	4.535	5.740	0.11	0.00
10	3-NO_2	3-CF_3	5.420	6.212	0.11	1.07
11	3-NO_2	4-Cl	5.234	6.012	0.11	0.70
12	3-NO_2	4-Br	5.434	6.344	0.11	1.02
13	3-NO_2	3,5-$(CF_3)_2$	6.312	6.533	0.11	2.14
14	3-NO_2	4-Phenothiazinyl	7.275	7.559	0.11	4.15
15	3,NO_2, 5-Cl	4-Cl	5.804	6.360	0.87	0.70
16	3-NO_2, 5-I	4-Cl	6.240	6.559	1.26	0.70
17	3-NO_2, 5-Br	4-Cl	6.190	6.889	1.05	0.70
18	3,5-Cl_2	4-Cl	6.742	8.202	1.52	0.70
19	3-$COCH_3$, 5-Br	4-Br	5.854	5.854	0.66	1.02
20	3-$COCH_3$, 5-I	4.Br	6.076	5.979	0.87	1.02
21	3-I	4-Br	6.407	7.638	1.15	1.02
22	3,5-Cl_2	4-Br	6.674	7.876	1.52	1.02
23	3,5-Br_2	4-Br	6.971	8.270	1.88	1.02
24	3-$COCH_3$	3,5-$(CF_3)_2$	6.007	5.680	−0.28	2.14
25	3-NO_2, 5-I	3,5-$(CF_3)_2$	6.775	6.827	1.26	2.14

[a] pK_i is the negative logarithm of K_i, the 50% inhibition constant. R refers to rat enzyme and L to liver fluke enzyme. [b] From ref 8. Suffix 1 refers to R^1 and suffix 2 to R^2.

Table II.[a] Correlation Equations for Group 1-4

	n	r	s	p	p^b
1. pK_i-R = 0.764 (±0.069)π + 4.584	6	0.984	0.204	<0.001[c]	<0.001
pK_i-L = 0.416 (±0.070)π + 4.939	7	0.937	0.289	<0.01[d]	<0.005
2. pK_i-R = 0.646 (±0.048)π + 4.724	6	0.989	0.159	<0.001[e]	<0.001
pK_i-L = 0.489 (±0.071)π + 5.585	7	0.952	0.253	<0.001[f]	<0.001
3. pK_i-R = 1.008 (±0.086)π + 5.077	6	0.986	0.120	<0.001[g]	<0.001
pK_i-L = 1.501 (±0.404)π + 5.308	6	0.881	0.558	<0.05[h]	<0.05
4. pK_i-R = 0.929 (±0.035)π + 5.265	7	0.996	0.060	<0.001[i]	<0.001
pK_i-L = 1.496 (±0.374)π + 5.395	7	0.873	0.636	<0.02[j]	<0.02

[a] n represents the number of derivatives, r is the correlation coefficient, and s is the standard deviation from regression. The numbers in parentheses are the 95% confidence intervals. Significance data for the regression and correlation coefficients are given for each equation. [b] $r \neq 0$. [c] $t = 11.08$. [d] $t = 5.99$. [e] $t = 13.33$. [f] $t = 6.92$. [g] $t = 11.66$. [h] $t = 3.72$. [i] $t = 26.31$. [j] $t = 4.00$.

We tested this inhibition on the succinate dehydrogenase from liver flukes and also on the succinate dehydrogenase from the hearts of rats, which served as a model for the host organism.[7] In each case the 50% inhibition concentration, K_i (mol/l.), served as measurement for the inhibition. Since in the ideal case the chemotherapeutic index corresponds to the quotient of the inhibition constant of the same enzyme on the parasite and its host, we determined this quotient *in vitro*.

In practice one must, of course, also take into consideration the absorption of the drug and its metabolism. Therefore, a final evaluation of the effectiveness of a substance can be made only *in vivo*. Nevertheless, this *in vitro* test is a useful and time-saving method for choosing from a series of substances, those which on the basis of a good *in vitro* activity are also promising for the *in vivo* test.

We investigated 2,6-dihydroxybenzoic acid anilide derivatives with various substituents in the two aromatic rings. Four groups of derivatives were formed (Table I). In groups 1 and 2 the substitution in the aniline ring is varied and that in the dihydroxybenzoic acid ring is held constant; for groups 3 and 4 the reverse holds true.

Several equations were tested in each group to determine the variables which are essential for the response. The Hansch parameter π[8] served as the criterion for the influence on the lipohydrophilic behavior, the Hammett constant σ[9] for the electronic substituent influences, and the Taft parameter E_s[10] for steric substituent influences. The biological response was expressed each time by pK_i-R and pK_i-L, the negative logarithms of the inhibition constants K_i-R and K_i-L, whereby the indices R and L refer to rat and liver fluke enzyme, respectively. In all cases the best results were obtained by simple linear regression with the parameter π. The inclusion of other parameters gave no significant improvement, and, as is to be expected in an *in vitro* test, a parabolic expression offered no advantage over the linear one. From this one can conclude that electronic or steric substituent influences are not essential for the rate-determining step in the mechanism of action. Only the lipohydrophilic behavior is decisive. The best correlations for the individual groups are listed in Table II.

For the equations in groups 1 and 2, pK_i-R responds more sensitively to a variation of the substituents in the aniline ring than pK_i-L; the coefficients of π are larger in

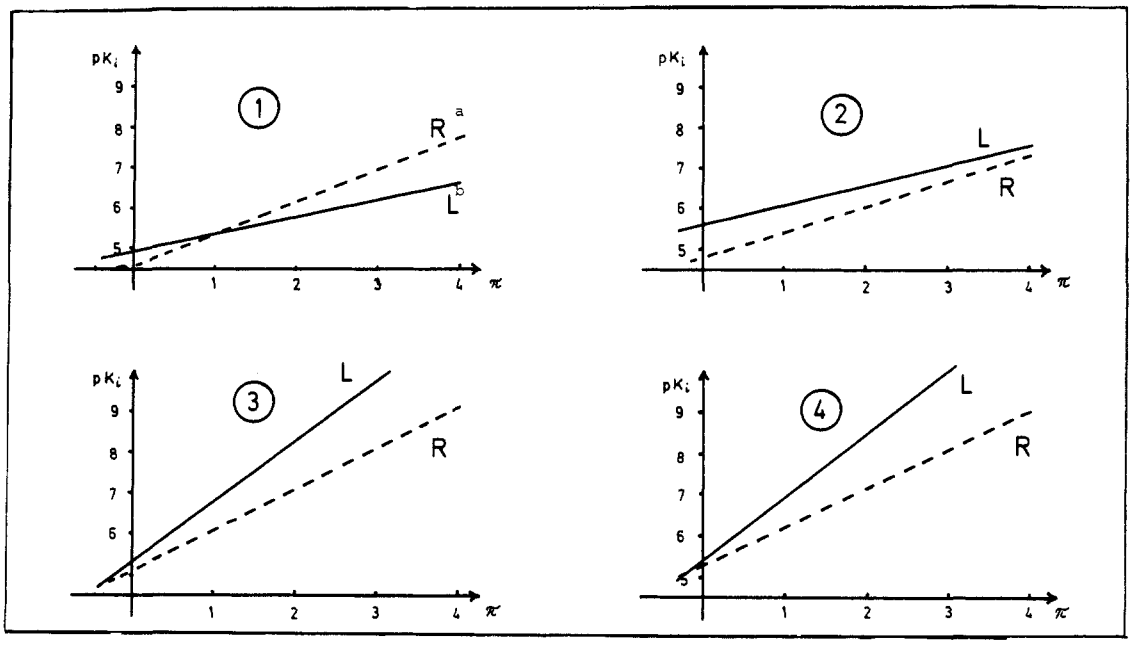


Figure 1. Dependency of pK_i on π in groups 1–4: a, rat enzyme; b, liver fluke enzyme.

the first case (0.764 > 0.416 and 0.646 > 0.489).

An analogous comparison in groups 3 and 4 shows that for a variation of the substituents in the dihydroxybenzoic acid ring the opposite holds true; here pK_i-L reacts more sensitively to a variation of π than pK_i-R (1.501 > 1.008 and 1.496 > 0.929). These facts can be very clearly shown in graphs (Figure 1).

In groups 1 and 2 the function pK_i-R has the steeper slope. With increasing values for π, the straight lines approach one another, and at the intersection the inhibition effect becomes the same for both enzymes. For higher π values the difference pK_i-L – pK_i-R, which corresponds to the (ideal) chemotherapeutic index, takes on negative values. This means that the compounds become more toxic for the host organism than for the parasite.

Favorable values, *i.e.*, a large difference ΔpK_i, would be obtained in groups 1 and 2 with negative π values. One recognizes, however, that with this the pK_i values become so small that they are of no interest chemotherapeutically.

In groups 3 and 4 one sees that with growing values for π the two straight lines diverge. The larger π becomes, the greater also the difference pK_i-L – pK_i-R. The compounds become increasingly more specific, *i.e.*, more toxic for the parasite than for the host organism.

Since a simple linear dependence of π is found for the relation between the substitution in the aniline ring and pK_i as well as for the relation between the substitution in the dihydroxybenzoic acid ring and pK_i, both relations may be combined in one equation.

Thus one obtains the following relations for pK_i-R and pK_i-L which are now equally valid for all of the derivatives studied.

$$pK_i\text{-R} = 0.856\ (\pm 0.066)\pi_1 + 0.677\ (\pm 0.041)\pi_2 + 4.666\ (\pm 0.085) \quad (1)$$

$$n = 23;\ s = 0.191;\ r = 0.972;\ F_{2,20} = 171.78;\ p < 0.001$$

$$pK_i\text{-L} = 1.312\ (\pm 0.158)\pi_1 + 0.427\ (\pm 0.085)\pi_2 + 5.145\ (\pm 0.167) \quad (2)$$

$$n = 25;\ s = 0.485;\ r = 0.891;\ F_{2,22} = 42.36;\ p < 0.001$$

Index 1 refers to substituents in the dihydroxybenzoic acid ring and index 2 to those in the aniline ring. Both equations show that substitution in the dihydroxybenzoic acid ring has a greater influence on the activity than substitution in the aniline ring. While this difference can be more or less neglected in the effect on the rat enzyme, it is considerable for pK_i-L.

With the aid of these two equations new derivatives can now be planned rationally. What is strived for is the strongest possible effect on the parasite with only a slight effect on the host organism, *i.e.*, the highest possible value for pK_i-L with a large difference pK_i-L – pK_i-R. If this difference ΔpK_i is correlated with the substituent parameters π the following equation is obtained.

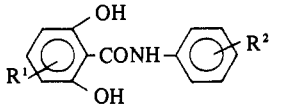
$$\Delta pK_i = 0.448\ (\pm 0.168)\pi_1 - 0.258\ (\pm 0.104)\pi_2 + 0.501\ (\pm 0.214) \quad (3)$$

$$n = 23;\ s = 0.485;\ r = 0.695;\ F_{2,20} = 9.37;\ p < 0.005$$

Although this correlation is not as good as in the preceding cases, it shows clearly that in order to achieve a large difference ΔpK_i, π_1 should be as positive as possible and π_2 negative.

Therefore, effective and selectively active derivatives can be expected if the dihydroxybenzoic acid ring substituents are very lipophilic and at the same time the aniline ring

Table III. Structure-Activity Parameters



No.	R^1	R^2	pK_i-R[a]	pK_i-L[a]	$\Sigma\pi_1$[b]	$\Sigma\pi_2$[b]
26	3,5-Cl_2	4-OCH_3	6.242	7.237	1.52	−0.04
27	3,5-Cl_2	4-CO_2CH_3	6.455	7.510	1.52	0.38[c]
28	3,5-Cl_2	4-CN	5.907	7.062	1.52	−0.32
29	3,5-Cl_2	4-$CONH_2$	4.971	6.056	1.52	−1.49
30	3,5-Cl_2	4-$COCH_3$	5.911	7.085	1.52	−0.37
31	3,5-Cl_2	4-$NHCOCH_3$	4.827	6.647	1.52	−0.79
32	3,5-Cl_2	4-*n*-C_4H_9	7.356	8.699	1.52	2.00

[a] See Table I, footnote *a*. [b] See Table I, footnote *b*. [c] As no π value is reported for CO_2CH_3 in the phenoxyacetic acid series, we used the mean value from the benzene (−0.01), phenol (0.50), and aniline series (0.65).

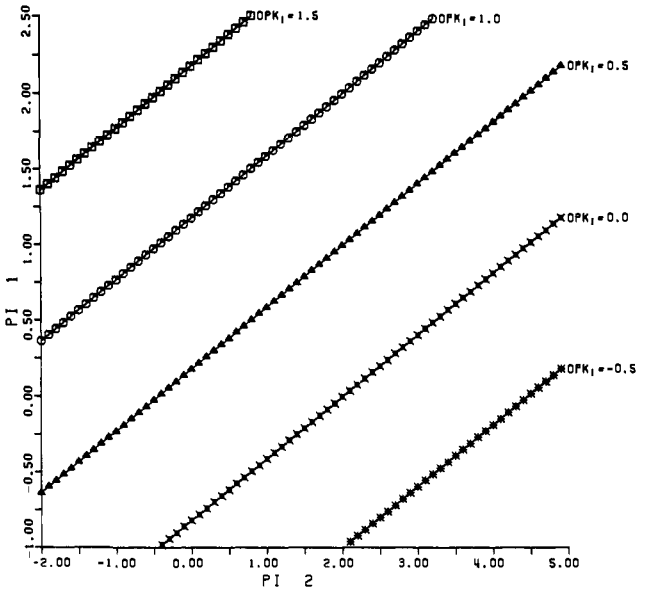


Figure 2. Diagram of isoergs.

substituents hydrophilic or only very slightly lipophilic.

In order to check this statement, several 3,5-dichloro-2,6-dihydroxybenzoic acid anilides were synthesized and tested (Table III). By incorporating the new derivatives into the overall correlation we obtained

$$pK_i\text{-R} = 0.883\ (\pm 0.066)\pi_1 + 0.688\ (\pm 0.039)\pi_2 + 4.645\ (\pm 0.088) \quad (4)$$

$$n = 30;\ s = 0.219;\ r = 0.963;\ F_{2,27} = 173.08;\ p < 0.001$$

$$pK_i\text{-L} = 1.371\ (\pm 0.123)\pi_1 + 0.472\ (\pm 0.068)\pi_2 + 5.080\ (\pm 0.148) \quad (5)$$

$$n = 32;\ s = 0.449;\ r = 0.908;\ F_{2,29} = 67.92;\ p < 0.001$$

A comparison of these equations with those originally obtained shows that the new derivatives can be incorporated well into the existing scheme.

The correlation of ΔpK_i with π_1 and π_2 was also repeated with the inclusion of the new derivatives and yielded the improved equation

$$\Delta pK_i = 0.501\ (\pm 0.137)\pi_1 - 0.204\ (\pm 0.080)\pi_2 + 0.411\ (\pm 0.181) \quad (6)$$

$$n = 30;\ s = 0.453;\ r = 0.765;\ F_{2,27} = 19.04;\ p < 0.001$$

For this equation a diagram of isoergs† was plotted, which permits us to read ΔpK_i for each desired parameter combination (Figure 2). All points on one of the straight lines (isoergs) have the same (ideal) chemotherapeutic index. Below the straight line for $\Delta pK_i = 0$ the toxicity is greater for the host organism than for the parasite. The farther one goes up to the left from this critical line, the more favorable the ratio between the two inhibition constants becomes, and one sees that a combination of a high π_1 value and a negative π_2 value will provide the best results.‡

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†By analogy with the terms "isotherms" or "isobars," we suggest the term "isoergs" for lines denoting the same biological response.

‡After the work for this manuscript had been completed, some additional compounds were synthesized and tested in the enzyme assay. They are listed in the preceding paper with their K_i values. Some of them could not be used here, either because of structural changes or because of missing substituent parameters. But there are still some 20 congeners of this series that can be included. However, since their activity could be predicted reasonably well with the existing regression equations, we did not think it necessary to include them in the Hansch analysis.

Studies on the Stereospecificity of Closely Related Compounds Which Block Postganglionic Acetylcholine Receptors in the Guinea-Pig Ileum

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Esters of dimethylamino- diethylamino-, pyrrolidino-, and piperidinoethanols have been prepared with the resolved forms of mandelic, cyclohexylphenylacetic, cyclohexylphenylglycolic, and α-methyltropic acids and converted to their hydrochlorides, methiodides, and ethiodides. Enantiomeric pairs of quaternary derivatives of hyoscyamine, hyoscine, and homatropine have also been made and the affinity constants of all the compounds have been measured for the postganglionic acetylcholine receptors of the guinea-pig ileum. The derivatives of mandelic and cyclohexylphenylacetic acids had only low stereospecificity but those of cyclohexylphenylglycolic and α-methyltropic acids had considerable stereospecificity as did the derivatives of hyoscyamine and hyoscine. Even though the asymmetric center is at the other end of the molecule, changes in the composition of the onium group produce considerable changes in stereospecificity in these series of enantiomers and possible reasons for this are discussed.

Relationships between chemical structure and binding to acetylcholine receptors have been investigated by Abramson, Barlow, Mustafa, and Stephenson[1] who measured the affinities of over 100 compounds related to acetylcholine for the postganglionic acetylcholine receptors of the guinea-pig ileum. From the results they concluded that any change in structure which could lead to increased affinity, such as an increase in size, was likely to lead also to a disturbance in