the grouped Student's t test. Behavioral observations were made during the course of these experiments.

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Structure–Activity Relationship of Aniline Mustards Acting against B-16 Melanoma in Mice[1]

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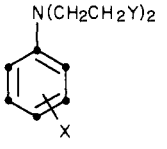
A set of 23 aniline mustards [X-$C_6H_4N(CH_2CH_2Cl)_2$] have been tested for their activity against B-16 melanoma in mice. The following quantitative structure–activity relationship (QSAR) correlates the data well: $\log 1/C = -2.06\sigma - 0.15\pi - 0.13\pi^2 + 4.13$ ($r = 0.936$). When this equation is compared with those formulated for aniline mustards acting against leukemia, it is found that $\log P_0$ (ideal lipophilicity) is higher for solid tumors. The QSAR brings out the unique activity of phenylalanine aniline mustard.

In studying the structure–activity relationships of various types of drugs acting against leukemia, we have been struck by the fact that the more effective anti-leukemia drugs appear to be unusually hydrophilic.[2] It is our belief that for solid tumors the more hydrophobic drugs in a given series should be more effective; that is, there is evidence in hand that $\log P_0$ will be higher for solid tumors than for leukemias. If our present evidence can be extended and generalized, this will be of great importance to those attempting to design better antitumor agents. The aniline mustards (I), which are widely used



I

in cancer chemotherapy, provide a good system for a study of this problem.

Equation 1 correlates the percent hydrolysis (reaction

% hydrolysis in 30 min at 66 °C in 50:50 acetone–water

$$\log \%\ \text{hyd} = -1.42\sigma + 0.45I_0 + 0.70I_{Br} + 1.21 \quad (1)$$

$$n = 42;\ r = 0.952;\ s = 0.157$$

with a nucleophile) of congeners of type I under standard conditions.[3] This equation, from the work of Ross, can be compared with eq 2 and 3 for the antitumor activity of

T/C 125 L-1210 leukemia in mice

$$\log 1/C = -0.31\pi - 0.96\sigma + 0.86I_0 + 4.07 \quad (2)$$

$$n = 19;\ r = 0.926;\ s = 0.315$$

T/C 180 P-388 leukemia in mice

$$\log 1/C = -0.34\pi - 1.39\sigma + 0.30I_0 + 4.15 \quad (3)$$

$$n = 16;\ r = 0.914;\ s = 0.311$$

other sets of aniline mustards[3] acting as antitumor agents. In eq 2 and 3, C is the molar concentration (mol/kg) producing a 25% (T/C 125) increase in life span of the mice. The indicator variable I_0 takes the value of 1 when ortho substituents are present and zero for all other cases. I_{Br} takes the value of 1 when Y of structure I is Br and zero when Y is Cl; that is, Br is the better leaving group for nucleophilic substitution. The coefficients with σ in eq 2 and 3 are in reasonable agreement with eq 1, suggesting that Ross' nucleophilic substitution model is a good one for predicting the electronic effect of substituents on the in vivo activity in chemotherapy. A most interesting feature of eq 2 and 3 is that both contain equivalent terms in π, each having a negative coefficient. Since adding a term in π^2 does not improve these two equations, this suggests that $\log P_0$ is lower than the lowest $\log P$ of any aniline mustard in these two sets (i.e., greater than −1.00 when X = $NH_3^+CHCH_2COO^-$). However, this point needs further study (see Discussion).

In the development of eq 2 and 3 and in our recent study,[3] we have used π for the neutral form of $(CH_2)_nCOOH$ even though carboxylic acids are almost

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Table I. Data Used to Derive Equation 1 for Aniline Mustards of the Type X-$C_6H_4N(CH_2CH_2Cl)_2$ Acting against B-16 Melanoma

no.	X group	log 1/C obsd	log 1/C calcd[a]	$\|\Delta \log 1/C\|$	π	σ	highest T/C attained[b]	dose for highest T/C, mg/kg
1	4-Cl	2.74	3.48	0.74	0.71	0.23	108	400.00
2	4-CH=CHPh	2.86[c]	2.93	0.07	2.68	−0.07	101	100.00
3	4-CH=C(CN)$_2$	3.00	2.68	0.32	0.05	0.70	108	200.00
4	4-CONHPr	3.25	3.41	0.16	−0.19	0.36	109	100.00
5	4-OPh	3.35	3.31	0.04	2.08	−0.03	139	200.00
6	4-N-Bu	3.44	3.60	0.16	2.05	−0.16	125	100.00
7	4-CN	3.57[c]	2.81	0.76	−0.57	0.66	108	25.00
8	3,5-NHCONH$_2$	3.69	3.75	0.06	−2.60	−0.06	108	25.00
9	4-CH$_3$	3.86	4.35	0.49	0.56	−0.17	137	50.00
10	H	4.38	4.13	0.25	0.00	0.00	159	12.50
11	3,4-(CH$_3$)$_2$	4.45	4.29	0.16	1.12	−0.24	167	50.00
12	3,5-NH$_2$	4.45	4.36	0.09	−2.46	−0.32	131	6.25
13	3,4-(OCH$_3$)$_2$	4.52	4.44	0.08	−0.04	−0.15	162	25.00
14	4-OBu	4.56	4.24	0.32	1.55	−0.32	148	25.00
15	4-NHCONH$_2$	4.63	4.59	0.04	−1.30	−0.24	147	12.50
16	4-OCH$_3$	4.68	4.68	0.00	−0.02	−0.27	163	25.00
17	4-(CH$_2$)$_3$COOH	4.70	4.44	0.26	0.21	−0.17	133	8.00
18	4-OEt	4.87	4.54	0.32	0.38	−0.24	197	50.00
19	4-OH	4.93	4.93	0.00	−0.67	−0.37	147	8.00
20	4-NH$_2$	5.35	5.47	0.12	−1.23	−0.66	124	1.00
21	4-NH$_2$, 3-CH$_3$	5.50	5.67	0.17	−0.67	−0.73	142	2.00
22	4-CH$_2$CH(NH$_2$)COOH	6.18[c]	3.13	3.05	−3.56	−0.07	236	4.00

[a] Calculated using eq 7. [b] These are the highest T/C reached at the doses given and they are not directly proportional to the activity. [c] These points not included in deriving eq 7.

completely ionized under physiological conditions. The partition coefficient for ionic species not only depends on the kind of counterion involved, but also on the concentration of counterion. It is not yet clear how to model physiological partitioning (of ions) with the octanol/water system.

Equation 4, from the work of Bardos, shows that the ED_{90} of aniline mustards vs. Walker tumor

$$\log 1/C = -1.19\sigma^- + 0.75I_{Br} - 1.00\pi - 0.53\pi^2 + 3.84 \quad (4)$$

$$n = 14;\ r = 0.940;\ s = 0.291;\ \log P_0 = 1.95$$

situation for *neutral* mustards acting against solid tumor (Walker 256 rat) is much different[3] from the leukemia cases. I_{Br} in eq 4 takes the value of 1 when Y = Br and 0 when Y = Cl or I; that is, for in vivo action, Br is the best substituent for Y. Again we see a negative coefficient with σ; however, we now find that optimum lipophilicity for *neutral* aniline mustards attacking the solid Walker tumor is 1.95.

In order to further explore the relationship of lipophilicity with tumor type, we have now tested the series of aniline mustards in Table I against the solid tumor B-16 melanoma.

Results

We have derived eq 5–7 from the data in Table I. In aniline mustards of type I vs. B-16 melanoma T/C 125

$$\log 1/C = -2.07(\pm 0.64)\sigma + 3.92(\pm 0.22) \quad (5)$$

$$n = 19;\ r = 0.856;\ s = 0.419;\ F_{1,7} = 46.6$$

$$\log 1/C = -1.99(\pm 0.64)\sigma - 0.10(\pm 0.16)\pi + 3.93(\pm 0.22) \quad (6)$$

$$n = 19;\ r = 0.870;\ s = 0.413;\ F_{1,16} = 1.55$$

$$\log 1/C = -2.06(\pm 0.48)\sigma - 0.15(\pm 0.12)\pi - 0.13(\pm 0.07)\pi^2 + 4.13(\pm 0.20) \quad (7)$$

$$n = 19;\ r = 0.936;\ s = 0.303;\ F_{2,15} = 8.79;\ \pi_0 = -0.57\ (-1.35\ \text{to}\ -0.11)$$

these equations, σ is the most important term, showing that electron-releasing substituents make the most potent antitumor agents. The value of $F_{1,16}$ shows that eq 6 is not a significant improvement over eq 5; however, $F_{2,15}$ shows that eq 7 is a significant improvement over eq 5 ($F_{2,15}$; $\alpha = 0.005 = 7.7$). From the value of π_0 in eq 7, we calculate log P_0 to be 2.33, which is in good agreement with the value for eq 4. Hence, for *neutral* aniline mustards acting against solid tumors, we find that log P_0 is definitely higher than for the same type of compound acting against leukemia. It is of interest to note that a log P of 2 is the optimum value for lipophilicity for *neutral* compounds to enter the central nervous system.[4] In fact, the value of 2 may well turn out to be about the ideal value for neutral compounds to diffuse into the lipophilic compartments of the body.

The phenylalanine mustard (**22**, Table I) is grossly mispredicted by eq 7, although compounds of this type are well predicted in the leukemia system by eq 2 and 3. The fact that this molecule is 1000 times more potent than eq 7 predicts is good evidence for a different type of transport from the site of injection of drug (ip) to the site of the melanoma. One could conclude that either the octanol/water log P value measured in water with the absence of plasma is not a good model for passive transport or that active transport is involved; we favor the latter view. In any case, compound **22** of Table I has unique activity when compared with the neutral aniline mustards.

One aniline mustard (X = 4-SO_2NH_2) was found to be completely inactive against B-16 melanoma and is not included in Table I. Its activity is predicted by eq 7 to be 2.85, which is extremely low. Possibly more thorough testing might uncover some slight activity for this congener. Three compounds (**2**, **7**, and **22**) in Table I were not used in formulating eq 7. Compounds **2** and **7** had such low T/C values and such poor patterns of activity that they were excluded. Compound **2** is well fit and compound **7** is poorly fit. If all compounds except **22** are included, we obtain eq 8 which is not significantly different from eq 7.

$$\log 1/C = -1.84(\pm 0.44)\sigma - 0.17(\pm 0.11)\pi - 0.14(\pm 0.06)\pi^2 + 4.20(\pm 0.19) \quad (8)$$

$n = 21$; $r = 0.931$; $s = 0.323$; $\pi_0 = -0.62$ (−1.40 to −0.21)

Actually, there are six compounds in Table I that did not achieve a T/C of 125. These are compounds of borderline activity and, knowing the difficulties involved in getting reproducible antitumor data, one should be concerned with the validity of these log $1/C$ values. Therefore, a correlation (eq 9) was made dropping these

$$\log 1/C = -1.71(\pm 0.79)\sigma - 0.16(\pm 0.12)\pi - 0.14(\pm 0.07)\pi^2 + 4.27(\pm 0.29) \quad (9)$$

$n = 15$; $r = 0.937$; $s = 0.236$; $\pi_0 = -0.60$ (−1.51 to −0.14)

data points. The collinearity between π and σ in eq 7 and 9 is low, $r^2_{\pi,\sigma} = 0.04$ and 0.23, respectively. The results embodied in eq 7 and 9 clearly support our previous findings[2] that the most effective drugs for leukemia are more hydrophilic than those one would want for solid tumors.

An important difference in the dependence of activity on hydrophobicity in eq 4 and 7 should be noted. Although the log P_0 values are essentially the same, two different types of parabolic relationships are involved. A much broader parabola is defined in eq 7. The steeper slopes of the π terms in eq 4 show a greater dependence of activity in the Walker tumor on lipophilic character. Penetration to and into the Walker tumor would appear to be more π controlled than penetration into the melanoma.

The meaning of this difference comes more into focus when one considers the details of the B-16 melanoma test, especially in relation to the Walker tumor test. In the B-16 melanoma test, ground melanoma tissue is injected ip into the mouse followed by ip injection of the drug. The tissue pieces develop into solid tumors. That is, this kind of test is regarded by the National Cancer Institute as a good model for a solid tumor. However, it is quite different from the Walker 256 tumor test where the tumor is grafted onto the hind leg of a rat. The random walk process for the injected drug in melanoma is much simpler than the random walk for the drug in the Walker tumor. Using drugs where the log P value is considerably different from log P_0 will not result in much change in activity with the melanoma but will be quite important with the Walker tumor.

Focusing on the hydrophobic dependence alone, we can arbitrarily assign zero values to the σ^- and I_{Br} terms in eq 4; then, using an ideal π value (−0.6), we calculate log $1/C$ to be 3.05. If instead we use a π value of −2.00, log $1/C$ is calculated to be −0.28. The difference between these two figures represents over a 1000-fold change in activity when ideal hydrophobic character is off by 2. Using the same assumptions in eq 7, we calculated a log $1/C$ of 4.0 and 3.3; here, the difference represents only a fivefold change in activity. One must remember that two different types of tumors are being compared; however, we believe that it is primarily the difference in the two random walk processes that accounts for the different dependence of antitumor activity on lipophilicity.

One must also keep in mind that the definition of biological response (log $1/C$) is of course an important determinant of the shape of the correlation equation. For example, we showed in the case of nitrosoureas acting against leukemia that as one uses progressively higher T/C values to define log $1/C$ the value of log P_0 increases and that more lipophilic drugs are better able to produce a larger increase in life span.[5] Unfortunately, most of the aniline mustards yielded rather low T/C values so that it was not possible to formulate QSAR at higher T/C levels without making large extrapolations.

Although the evidence now in hand is not by any means conclusive, it does suggest directions for future research. Equation 7 clearly demonstrates the unique character of an amino acid moiety in assisting transport of aniline mustards into solid tumors. Increasing the lipophilic character of the phenylalanine mustard in such a way that deleterious steric or electronic effects are not involved might yield even more potent agents for solid tumors. Also, more potent neutral aniline mustards can be designed following the leads suggested by eq 2, 3, and 7.

Method

The substituent constants used in the formulation of eq 7 are from our recent compilation.[6] π for $-CONH\text{-}n\text{-}C_3H_7$ is calculated from $\pi\text{-}CONHCH_3$. The σ value for $-CONH\text{-}n\text{-}C_3H_7$ is taken to be the same as that of $CONHCH_3$.

All of the aniline mustards of Table I have been previously reported, except compound 4. Standard procedures[7-13] were used for their preparation. One new compound [4-n-$C_3H_7NHCOC_6H_4N(CH_2CH_2Cl)_2$] was prepared by dissolving 4-$ClCOC_6H_4N(CH_2CH_2Cl)_2$ in dry ether at 10 °C. To this was added an ether solution of n-propylamine. After the addition of the amine, the solution was stirred for 15 min. The mixture was then washed with water several times and dried over magnesium sulfate. Evaporation of the ether and recrystallization of the residue from benzene gave white crystals, mp 93–94 °C. The product gave a satisfactory carbon, hydrogen analysis.

The compounds were administered intraperitoneally to BDF mice bearing the tumor in nine consecutive (daily) injections. The T/C (test/control) were evaluated from the median survival time of the treated and control animals. A T/C value of 125 means a 25% increase in life span of the test animal compared to the untreated control animals. The T/C values were plotted against dose, and the best straight line was used to make a short extrapolation to a T/C of 125. C in eq 2–7 is molar concentration (mol/kg).

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