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Antitumor 1-(X-Aryl)-3,3-dialkyltriazenes. 2. On the Role of Correlation Analysis in Decision Making in Drug Modification. Toxicity Quantitative Structure–Activity Relationships of 1-(X-Phenyl)-3,3-dialkyltriazenes in Mice[1]

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A series of 11 triazenes ($X—C_6H_4N{=}NNRCH_3$) was characterized for toxicity in mice (LD_{50}). The quantitative structure–activity relationship (QSAR) obtained for toxicity was compared with the QSAR for antitumor activity. The close correspondence of the two QSAR leaves essentially no means for the synthesis of more potent, less toxic triazenes.

In the previous paper in this series,[2] eq 1 was formulated

$$\log 1/C = 0.10 \log P - 0.04 (\log P)^2 - 0.31 \Sigma\sigma^+ - 0.18 \text{ MR-2,6} + 0.39 E_s\text{-R} + 4.12 \quad (1)$$
$$n = 61;\ r = 0.836;\ s = 0.191;\ \log P_0 = 1.18$$

for the antitumor activity of $X\text{-}C_6H_4N{=}N\text{-}NR_1R_2$ acting against L1210 leukemia in mice. C in eq 1 is the concentration (moles per kilogram) producing a T/C of 140, MR-2,6 is the sum of molar refractivity of substituents in the two ortho positions, and E_s-R is the Taft steric parameter for the larger of R_1 and R_2. The log P_0 of 1.18 sets the upper limit of potency which can be obtained in this series by manipulation of the lipophilic/hydrophilic balance. Essentially the same log P_0 was found for imidazolyl- and pyrazolyltriazenes.[2] The only advantage to be gained from the MR-2,6 term is obtained when both ortho positions are unsubstituted. For practical purposes, the E_s-R term limits one to the $N(CH_3)_2$ since $NHCH_3$ compounds are so unstable.

At first glance, one presumes that more activity could be obtained by introducing more electron-releasing groups (large negative $\Sigma\sigma^+$); however, the QSAR of eq 2 effectively

$$\log k_X/k_H = -4.42\,\sigma - 0.16 \quad (2)$$
$$n = 14;\ r = 0.995;\ s = 0.171$$

limits this avenue. Equation 2 correlates the rate of hydrolysis of phenyltriazenes.[2] This is so enormously promoted by electron-releasing groups that it is not possible in practice to go beyond the 4-OCH_3 (half-life = 12 min) in the use of electron-releasing functions. Attempts to increase potency through steric and/or hydrogen-bonding effects of ortho substituents have reached

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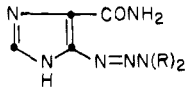
Table I. Constants for LD_{50} vs. Rats of $X\text{-}C_6H_4N{=}N\text{-}N(CH_3)_2$

X	Log 1/C Obsd[a]	Log 1/C Calcd[b]	|Δ log 1/C|	Log P[c]	σ^+
4-NO_2	2.06	2.15	0.09	2.71	0.79
H	2.54	2.49	0.05	2.59	0.00
4-CH_3	2.62	2.74	0.12	2.93	−0.31
4-I	2.68	2.75	0.07	3.70	0.14
4-Cl	2.71	2.65	0.06	3.33	0.11
4-F	2.71	2.55	0.16	2.67	−0.07
4-OCH_3	2.72	2.78	0.06	2.30	−0.78
4-Br	2.73	2.68	0.06	3.48	0.15

[a] From ref 3. [b] Calculated using eq 4. [c] Log P values calculated using eq 7 in ref 2 except for the experimentally determined value for the unsubstituted compound.

Table II. Constants for LD_{50} vs. Mice of 

R	Log 1/C Obsd[a]	Log 1/C Calcd[b]	|Δ log 1/C|	Log P
Methyl	2.44	2.41	0.03	−0.24
Ethyl	2.75	2.63	0.12	0.76
Propyl	2.70	2.86	0.16	1.76
Butyl	2.91	3.08	0.17	2.76
Pentyl	3.49	3.31	0.18	3.76

[a] From ref 4. [b] Calculated using eq 5.

the point where no obvious opportunities present themselves for exploitation.

The single remaining route to more effective triazenes appeared to be that of decreasing their toxicity and thereby obtaining congeners with better therapeutic ratios. The purpose of this report is to consider this possibility.

Two previous studies[3,4] of toxicity of triazenes have been reported; however, both suffer seriously from a poor selection of derivatives. We have formulated eq 3–5 from

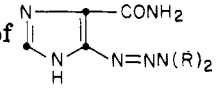
LD_{50} rats of $X\text{-}C_6H_4N{=}NN(CH_3)_2$ (Table I)

$$\log 1/C = -0.355\ (\pm 0.36)\ \sigma^+ + 2.60\ (\pm 0.15) \quad (3)$$

$$n = 8;\ r = 0.698;\ s = 0.175;\ F_{1,X} = 5.71$$

$$\log 1/C = -0.47\ (\pm 0.27)\ \sigma^+ + 0.29\ (\pm 0.24) \log P + 1.75\ (\pm 0.73) \quad (4)$$

$$n = 8;\ r = 0.906;\ s = 0.113;\ F_{1,X} = 9.22$$

LD_{50} mice[4] of  (Table II)

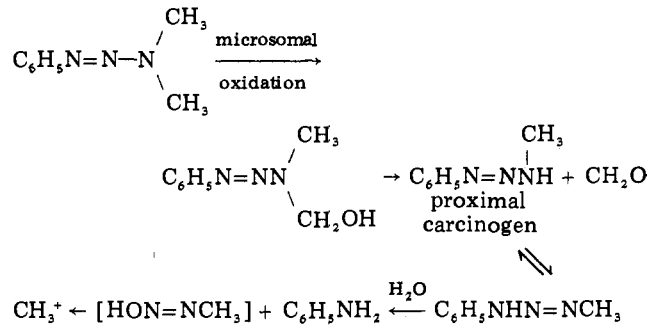
$$\log 1/C = 0.23\ (\pm 0.19) \log P + 2.46\ (\pm 0.42) \quad (5)$$

$$n = 5;\ r = 0.912;\ s = 0.185;\ F_{1,X} = 19.8$$

the data presented in these two studies. The data upon which eq 3 and 4 rest (Table I) contain a good spread in σ^+ of substituents but a poor spread in log P values. Equation 3 indicates that the electronic term is most important. The confidence limits on the log P term in eq 4 are broad so that we cannot place much confidence in this term. Adding a term in $(\log P)^2$ does not improve things. About all that one can conclude from eq 3 and 4 is that electron-releasing groups increase toxicity and that σ^+ gives a slightly better correlation than σ, suggesting a role for through resonance. The results with the imidazolyltriazenes (Table II) are also of little value because of the confidence limits on the log P term and the few data points.

We have now determined the LD_{50} values for 11 phenyltriazenes. In this set of 11 congeners, we have included compounds with a wide range of log P values (0.98–4.70), as well as a good spread in σ^+ values (−0.78 to 0.66).

Scheme I



Results

Equations 6 and 7 have been derived from the data in

$$\log 1/C = -0.0283\ (\pm 0.02)\ (\log P)^2 + 3.483\ (\pm 0.18) \quad (6)$$

$$n = 11;\ r = 0.731;\ s = 0.174;\ F_{1,X} = 10.31$$

$$\log 1/C = -0.0241\ (\pm 0.013)\ (\log P)^2 - 0.264\ (\pm 0.16)\ \sigma^+ + 3.490\ (\pm 0.12) \quad (7)$$

$$n = 11;\ r = 0.913;\ s = 0.110;\ F_{1,X} = 14.3$$

Table III on the LD_{50} of phenyltriazenes for mice. Equation 7 is a significant improvement over the best single-variable equation, eq 6 ($F_{1,9;\alpha=0.01} = 10.6$; $F_{1,8;\alpha=0.01} = 11.3$). Substituting σ for σ^+ gives the same quality correlation ($r = 0.910$). Adding a term in log P to eq 7 does not afford a significant reduction in the variance. C in these equations is the concentration producing the LD_{50}. Log P_0 represents the ideal lipophilicity for the most toxic compound; since its value is 0, making congeners either more or less lipophilic would reduce their toxicity in terms of the LD_{50}; however, because of the small coefficient with this term, significant changes are afforded only by large changes in log P. For example, substituting log P of 6 in eq 7 (with $\sigma^+ = 0$) yields log $1/C = 2.6$; substituting log P of 6 in eq 1 (with $\sigma^+ = 0$, MR-2,6 = 0.2, E_s-R = −1.24) yields log 1/C of 2.7; therefore, the therapeutic ratio is 1.04. Doing the same with log P of 1 (ideal log P for potency) yields a therapeutic ratio of 0.95; hence, there is virtually nothing to be gained therapeutically by the manipulation of log P. Furthermore, not only do the σ^+ terms of eq 1 and 7 cancel each other as far as the therapeutic index is concerned but, in addition, eq 2 tells us that trying to increase potency by using substituents more electron releasing than 4-OCH_3 will simply produce drugs too unstable with which to work. All paths appear to be blocked to better drugs in this series.

Discussion

In order to assess more clearly what remains to be done, it behooves us at this point in our analysis to take a broader view of the work which has been done with triazenes. Although the antitumor activity of the triazenes was discovered[5] in 1955 and a large amount of work has been done with these substances in the past 20 years, the mechanism of their antitumor action is still poorly understood. Since the triazenes are also carcinogenic, a considerable effort has been made to establish how they react in mammals.

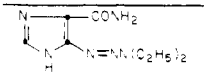
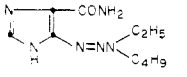
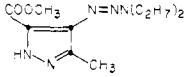
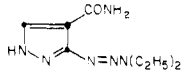
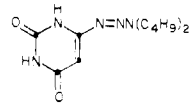
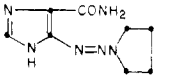
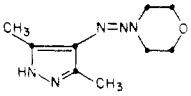
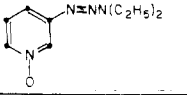
Preussmann and his colleagues[6] made one of the first

Table III. Constants for LD_{50} of X-C_6H_4N=$NNCH_3R$ vs. CDF_1 Mice

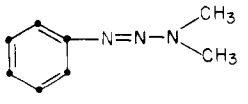
X	R	Log 1/C Obsd	Log 1/C Calcd	|Δ log 1/C|	Log P	σ^+	NSC no.
4-NHC(=O)NH_2	CH_3	3.68	3.63	0.05	1.25	−0.69	268 492
4-OCH_3	CH_3	3.55	3.57	0.02	2.30[a]	−0.78	515 460
4-$CONH_2$	C_4H_9	3.46	3.25	0.21	2.46	0.36	87 429
H	CH_3	3.43	3.33	0.10	2.59	0.00	3 094
4-$CONH_2$	CH_3	3.32	3.36	0.04	1.20	0.36	86 441
4-CH_3	CH_3	3.26	3.37	0.11	2.93[a]	−0.31	48 821
4-$CONH_2$	C_2H_5	3.24	3.33	0.09	1.70	0.36	276 375
4-SO_2NH_2	CH_3	3.20	3.32	0.12	0.98	0.57	157 030
4-CN	CH_3	3.20	3.18	0.02	2.39	0.66	157 034
4-CF_3	CH_3	3.05	3.00	0.05	3.69[a]	0.61	157 033
4-$CONH_2$	C_8H_{17}	2.80	2.87	0.07	4.70	0.36	276 741
DTIC		2.68			−0.24		45 388

[a] Log P calculated using techniques and eq 7 in ref 2.

Table IV. Activity against L1210 Leukemia

Compd	Dose, mg/kg	Activity (T/C)	NSC no.
	100	127	52371
	200	124	145928
	265	131	123150
	200	144	145927
	32	145	75947
	250	126	406801
	20	124	123151
	450	141	136074

studies of the action of microsomes on the simple phenyltriazene I. They postulated the following mechanism



I

for the generation of carbonium ions which they considered to be the carcinogenic agent (Scheme I). They also suggested that the monomethyltriazene is the actual proximal carcinogen. They showed that, under their experimental conditions, $HN(CH_3)_2$ was not dealkylated by microsomes and the benzene diazonium ion was not reduced to aniline; this was interpreted to mean that any $C_6H_5N_2^+$ produced via hydrolysis is not the active carcinogen. Their belief that CH_3^+ is the active moiety is supported by the findings that triazenes alkylate DNA and RNA in vitro and in vivo to produce 7-methylguanine and other methylated nucleic acid bases.[7,8] C_6H_5N=N-NH-C_2H_5 produces 7-ethylguanine. An important difference between carcinogenicity and antitumor action of the triazenes is that C_6H_5N=$NN(C_2H_5)_2$ is carcinogenic but has been reported as having no antitumor activity (however, see below). This suggests that Scheme I may not be the mechanism responsible for antitumor activity.

Another confusing finding of Preussmann and Hengy[6] is that dealkylation occurred with liver or lung microsomes but not with brain or kidney microsomes; yet triazenes do not cause liver or lung cancer but do produce brain and kidney tumors. They suggested that the intermediates [C_6H_5N=$NN(CH_3)CH_2OH$ or C_6H_5N=$NNHCH_3$] are produced in the liver and then transported to other parts of the body where they decompose to produce carbonium ions. They found that the half-life of C_6H_5N=$NNHCH_3$ at pH 7.4 and 37 °C in the presence of serum is 6 min; while this might explain tumors in other parts of the body, it is indeed strange that liver tumors are not found. Since triazenes alkylate guanine, it may be that guanine, in some stage of DNA synthesis, is more exposed to alkylation than in liver tissue.

Pittillo et al.[9] found that in bacterial cells, DTIC [4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide] is more effective in inhibiting dividing cells than resting cells (>56 to 1). In this respect it differs from alkylating agents such as nitrogen mustards, which are only three times as effective against dividing cells, and $ClCH_2CH_2N(NO)CONHCH_2CH_2Cl$, which is only 13 times more effective against dividing cells. Again, the evidence points out that triazenes do not behave as typical alkylating agents in their antitumor activity.

Gerulath et al.[10] have studied the effect of DTIC with reference to the phases of the cell cycle in Chinese hamster ovary cells in vitro. They conclude that DTIC kills cells most effectively in the G_1 and early S phase and that it is only moderately lethal to cells in other phases of the cell cycle. This evidence in mammalian cells also supports a different mode of action of DTIC from typical alkylating agents.

Hradec and Kolar[11] have shown that various triazenes promote the formulation of aminoacyl-tRNA in cell-free systems. This was also found for other carcinogens.

A recent report by Connors et al.[12] suggests that the fact that substituents in the phenyltriazene series may be strongly electron releasing or withdrawing has little or no effect on antitumor activity of phenyltriazenes acting against TLX5 lymphoma. This, of course, is not in line with our findings on L1210 leukemia or Dunn et al.[13] findings on Sarcoma 180 tumor. In addition to noting that the electronic effect of ring substituents had no effect on antitumor activity, Connors et al. concluded, as others have, that an NCH_3 group is essential for activity. Data in Table IV from the National Cancer Institute indicate that this point is not yet settled.

Another conclusion of Connors et al. is that when a

methyl group and a larger alkyl group are on the 3-nitrogen atom, the larger alkyl group is more rapidly dealkylated. Their own work shows that $N(Et)_2$ is dealkylated only half as fast as $N(CH_3)_2$. McMahon's work,[14] which has been treated in QSAR terms,[15] shows that N-demethylation occurs in preference to dealkylation of larger alkyl groups. If the larger group were always dealkylated more rapidly, then one might surmise that the biologically active species is the CH_3^+ fragment; the results in Table IV do not support this.

In summary, we can say that neither the mechanism of antitumor activity nor the mechanism of carcinogenicity of the triazenes is well understood; however, there is no doubt that these compounds are both quite toxic in a nonspecific model (LD_{50}) and highly carcinogenic. Since we have not been able to separate the structural features for toxicity from those for efficacy, there is little support to encourage further work on triazenes as antitumor agents. Dunn[13] has also concluded that "...the separation of toxic and antitumor activities would be difficult..." on the basis of two QSAR, one for efficacy and one for LD_{50}.

One might argue that most of our evidence in support of discontinuing work on triazenes comes from the X-$C_6H_4N{=}NN<$ series and that exploration of aromatic heterocycles might uncover new leads. While there is no absolute way to refute this argument, the QSAR for pyrazolyl- and imidazolyltriazenes[2] does not suggest any leads in this direction. In addition, Hutchinson[16] has shown that, as far as increased survival time in mice is concerned, DTIC, $C_6H_5N{=}NN(CH_3)_2$, and $C_6H_5N{=}NNHCH_3$ all gave the same increase in life span, although about three times as much DTIC was needed. This follows from its suboptimal log P and the relatively greater electronegativity of the imidazole ring compared with the phenyl ring. The important element is the electronic factor since phenyltriazene has a superoptimal log P value.

Since there are almost an infinite number of conceivable aryltriazenes, it is impossible to state now, or even after several thousand more congeners have been made and tested, that a better derivative cannot be found. However, it is our belief that there are many much more interesting lead compounds for antitumor activity in which it would be more profitable to invest one's resources. Unless one had new biochemical or molecular biological information suggesting that a new triazene might be more effective in some specific way, we would not recommend the synthesis and testing of new congeners.

Method

The log P values and σ constants used in this study are the same as those in paper 1 in this series.[2]

The drug administration regimen for determination of LD_{50} values consisted of daily injections on days 1–9.[17] The LD_{50} values were calculated by plotting percent mortality vs. log dose utilizing the probit method.[18]

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