Journal of Medicinal Chemistry

VOLUME 12, NO. 2 MARCH 1969

Potential Antitumour Agents. X. Bisquaternary Salts

B. F. CAIN, G. J. ATWELL, AND R. N. SEELYE

Cancer Chemotherapy Laboratory, Cornwall Geriatric Hospital, Auckland, New Zealand

Received May 9, 1968
Revised Manuscript Received September 27, 1968

It is suggested that the biological activity of a variety of cationic agents against experimental leukemias and trypanosomal species is dependent on (a) lipophilic–hydrophilic balance of the agents, (b) charge separation, (c) sufficient "contact" binding, this requirement varying with the biological test system involved, (d) a close over-all approach to planarity, and (e) capacity to fit a curved site of approximately 40 Å in diameter. That migration of these materials may be transport mediated is discussed. It is shown that a site equivalent to the minor groove in a helical polynucleotide would match the structural requirements.

Earlier papers[1] have described the experimental antileukemic effectiveness of a series of bisquaternary ammonium heterocycles and have shown that there is a marked dependence of biological activity on the lipophilic–hydrophilic balance of the molecules.[2] A convenient relative measure of this balance of physical properties consisted of the R_f values of the drugs in an immiscible solvent mixture.[2] The range of physical properties allowing demonstrable activity against the L1210 leukemia is very small. For example, in the N,N'-(6-quinolyl)terephthalamide quaternary salts (I) only the *n*-propyl, *n*-butyl, and *n*-amyl homologs show unequivocal activity; many examples of the sharp cut off in this type of agent have been presented.[1] It has also been shown that the R_f values of optimum members of structurally divergent series are extremely similar.[1,2] We draw attention to the fact that the biologically active cationic drugs listed in Table I have a similar balance of lipophilic–hydrophilic properties when examined by partition chromatographic methods.[3]

The agents listed in Table I have been selected by screening programs for activity against *Trypanosoma rhodesiense* or the L1210 leukemia. Discussion has been limited to those cationic drugs whose p*k* values are greater than 10.[4]

Where active transport mechanisms do not function it is generally accepted that the rate of drug translocation is intimately connected with partition properties (lipophilic–hydrophilic balance)[5–8] and, as a consequence, fully ionized drugs do not penetrate cellular membranes at very high rates. Thus, a cumulation of cellular barriers through which an agent must pass can effectively exclude fully ionized drugs.[9] Consequently, it appears remarkable that the bisonium members of Table I are able to influence disease processes considerably removed from the site of administration. Evidence has been presented that the phthalanilide[10] II will pass even the blood–brain barrier.[13]

The illustration that the inhibitory effects of the phthalanilides are proportional to the intracellular concentration of a drug–lipid complex,[14,15] and not extracellular free drug concentration, strongly suggests that a transport mechanism is operative. Evidence has been presented that a novel class of phospholipid is involved in this drug–lipid complex.[16] Involvement of a lipid carrier mechanism with quaternary ammonium salts has been described previously.[17–19] If the cationic drugs listed in Table I pass cellular

(1) Part IX: B. F. Cain, G. J. Atwell, and R. N. Seelye, *J. Med. Chem.*, **11**, 963 (1968), and references therein to earlier papers.

(2) G. J. Atwell and B. F. Cain, *ibid.*, **10**, 706 (1967).

(3) The reason for using dimidium (XXXV, R = CH_3) as an internal chromatographic standard[2] can now be seen.

(4) An admirable review of the current status of trypanosomal chemotherapy is presented by F. Hawking, *Exptl. Chemotherapy*, **1**, 131 (1963); **4**, 398 (1966).

(5) E. J. Ariëns, *Mol. Pharmacol.*, 7 (1964).

(6) W. Kunz, *Progr. Drug Res.*, **10**, 360 (1966).

(7) C. Hansch and S. N. Anderson, *J. Med. Chem.*, **10**, 745 (1967), and earlier papers quoted therein.

(8) E. J. Ariëns, *Progr. Drug Res.*, **10**, 429 (1966).

(9) The blood–brain barrier offers an excellent example of this; compare ref 8.

(10) The name phthalanilide in relation to experimental antitumor agents has come to cover a diverse group of compounds bearing amidinium functions. Examples II–XI show the principle members pertinent to our arguments. Full details of structural types are given in ref 11 and 12. While not agreeing with this terminology it is convenient to use in place of the cumbersome systematic nomenclature.

(11) R. Hirt, "Chemotherapy of Cancer," Pl. A. Plattner, Ed., Elsevier Publishing Co., Amsterdam, 1964, p 228.

(12) J. H. Burchenal, ref 11, p 233.

(13) W. I. Rogers, I. M. York, and C. J. Kensler, *Cancer Chemotherapy Rept.*, **19**, 67 (1962).

(14) D. W. Yesair, W. I. Rogers, P. E. Baronowsky, I. Wodinsky, P. S. Thayer, and C. J. Kensler, *Cancer Res.*, **27**, 314 (1967).

(15) D. W. Yesair, F. A. Kolner, W. I. Rogers, P. E. Baronowsky, and C. J. Kensler, *ibid.*, **26**, 202 (1966).

(16) D. W. Yesair, W. I. Rogers, J. T. Funkhouser, and C. J. Kensler, *J. Lipid Res.*, **7**, 492 (1966).

(17) R. R. Levine, Proceedings of the First International Pharmacological Meeting, Stockholm, 1961, Vol. 4, Hogben, Ed., Pergamon Press, Oxford, 1962.

(18) R. R. Levine and E. W. Pelikan, *J. Pharmacol. Exptl. Therap.*, **131**, 319 (1961).

(19) R. R. Levine and A. F. Spencer, *Biochem. Pharmacol.*, **8**, 248 (1961).

TABLE I

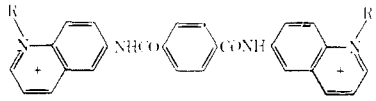
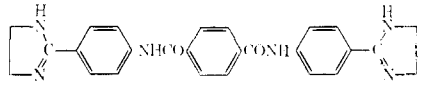
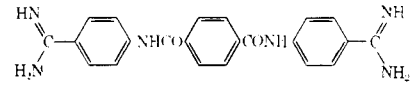
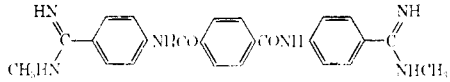
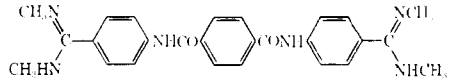
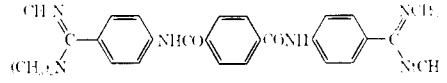
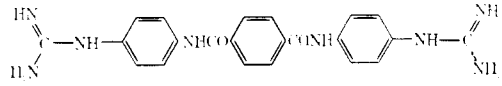
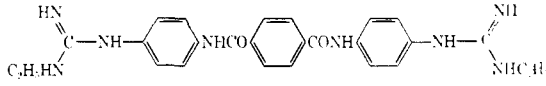
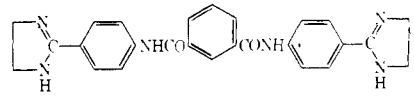
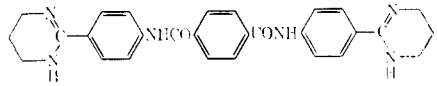
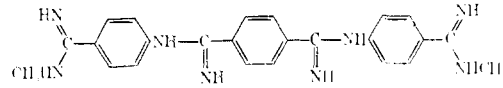
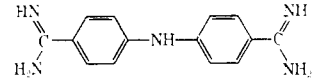
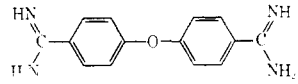
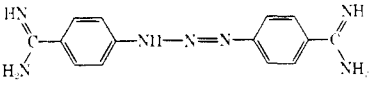
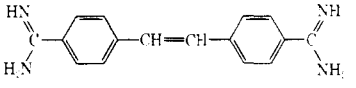
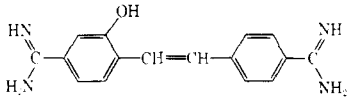
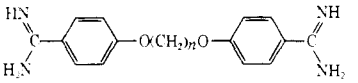
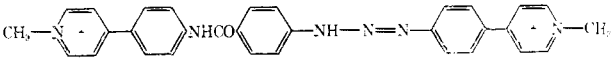
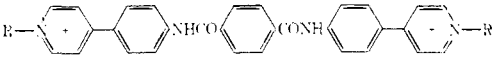
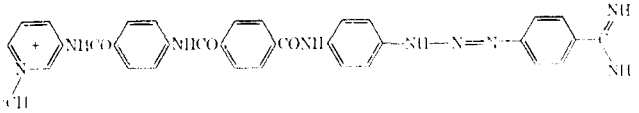
No.	Structure		R_f[c]	LD50[b]	Tx[c]	GS, V[d]
I[e]		R = CH_3	0.61	—		18
		R = C_2H_5	0.68			
		R = $CH_3(CH_2)_2$	0.80	+		
		R = $CH_3(CH_2)_3$	0.82	++		
		R = $CH_3(CH_2)_4$	0.87	++		
		R = $CH_3(CH_2)_5$	0.92	—		
II			0.88	++[f]	g	19
III				—[f]	g	19
IV				++[f]	g	19
V			0.90	++[f]	g	19
VI				—[f]	g	19
VII				—[f]	g	19.5
VIII			0.93	++[f]	g	19.5
IX			0.91	++[f]	g	19
X			0.90	++[f]	g	19
XI			0.92	++[f]	g	19
XII			0.96	—[h]	+	9
XIII			0.99	—[h]	±	9
XIV			0.94	±[i]	+	13
XV			0.95	—[h]	+	12.5
XVI			0.94	—[h]	+	12.5
XVII		n = 5	1.05	—[h]	+	19
XVIII			0.83	++[f]		23.5
XIX		R = CH_3	0.74	++[f]		23.5
		R = C_2H_5	0.84	++[f]		
XX			0.88	++[f]		28.5

TABLE I (*Continued*)

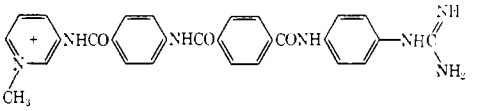
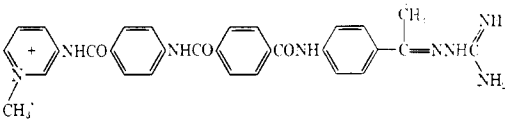
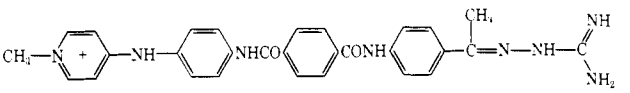
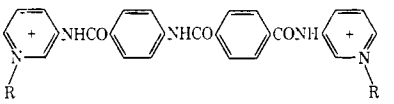
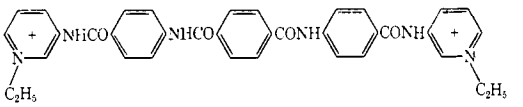
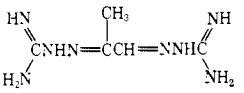
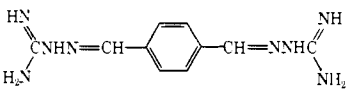
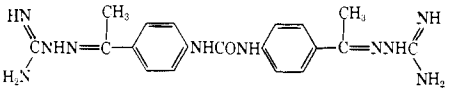
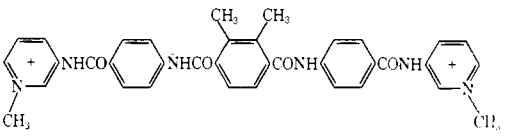
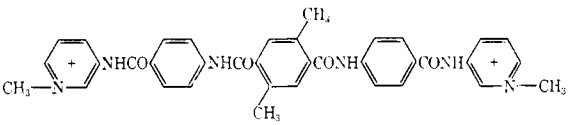
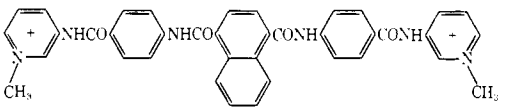
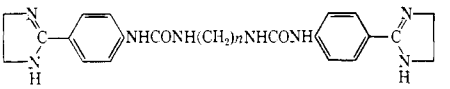
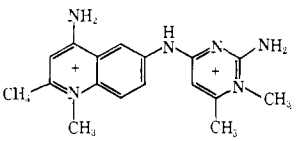
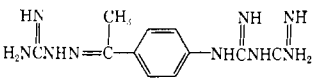
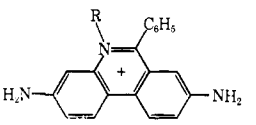
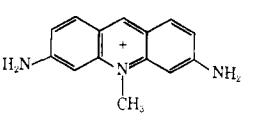
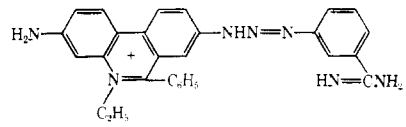
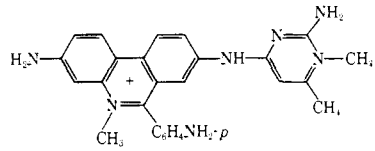
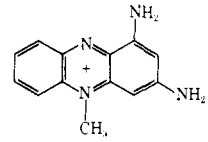
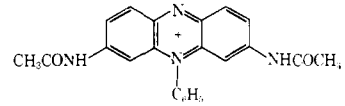
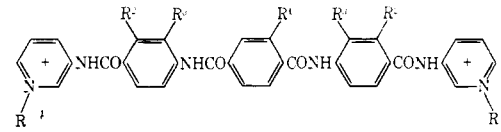
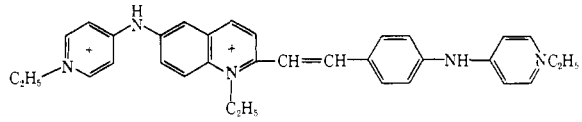
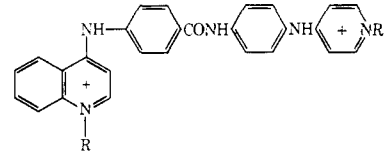
No.	Structure		R_D[a]	L1210[b]	Ty[c]	CS, Å[d]
XXI			0.82	++[k]		21.5
XXII			0.84	+[k]		24.5
XXIII			0.89	++[k]		24
XXIV[e]		R = CH_3 R = C_2H_5 R = $CH_3(CH_2)_2$	0.83 0.91 1.04	– – –		19.5
XXV			0.91	++[l]		26
XXVI			0.90	+		8.5
XXVII			0.91	+[m]		12.5
XXVIII			0.93	++[n]		19.5
XXIX			0.96	+[i]		26
XXX			0.95	–[i]		26.5
XXXI			0.94	+[i]		26
XXXII		n = 0 n = 3 n = 8	0.93 0.94 1.04	+[f] +[f] +[f]	*g* *g* *g*	17 20.5 27
XXXIII			0.90	–[o]	+	8.5
XXXIV			0.92	–[h]	+	12.5
XXXV		R = CH_3 R = C_2H_5	1.00 1.02	±[h] +[h]	+ +	
XXXVI			0.96	±[h]	+	

TABLE I (*Continued*)

No.	Structure		R_f[a]	L1210[b]	Ty[c]	CS, Å[d]
XXXVII			0.96	++[e]	+	14
XXXVIII			0.98	−[f]	+	8.0
XXXIX			0.98	+[g]		
XL			1.01	+[g]		
XLI						
XLII			0.92	+[h]		8.5 12 20
XLIII		R = CH_3 R = C_2H_5 R = $CH_3(CH_2)_2$	0.87 0.96 1.03	++[i] ++ +		18.5

[a] R_f relative to dimidium (XXXV, R = CH_3); see ref 2. [b] A relative gauge of activity against the L1210 leukemia: no activity, −; increase in life span 25–50%, ±; 50–100%, +; 100% ++. [c] Unequivocal activity against *Trypanosoma rhodesiense* denoted by +; see ref 4. [d] Charge separations measured from Courtald models (*vide infra*). In amidinium compounds charge is taken to be midway between the two nitrogen atoms comprising the resonant system. Charge in the quaternary salts is taken to be at the quaternary nitrogen atom; no allowance for delocalization has been made. Where several geometrical isomers are possible, that lying closest to a curve of 20-Å radius has been considered (see discussion). See ref 24 for a further list of charge separations to slightly different criteria. [e] Reference 2. [f] References 11, 12, 24. [g] It has been stated that certain phthalanilides have trypanosomal activity,[11] but as far as we are aware full details of these tests have not been published. [h] Tests under comparable conditions in this laboratory. See J. H. Burchenal, M. S. Lyman, J. R. Purple, V. Coley, S. Smith, and E. Bucholz, *Cancer Chemotherapy Rept.*, **19**, 19 (1962), for details of tests of homidium (XXXV, R = C_2H_5) and isometamidium (XXXVII) against L1210 and further murine leukemias. [i] Berenil (XIV) in our hands has occasionally given statistically significant life extension with the L1210 but activity is of an extremely low order. [j] Biological data of this paper. [k] Reference 27. [l] Reference 30. [m] Reference 28. [n] A. Marser, *Experientia*, **23**, 173 (1967), gives the background leading to the development of this compound. E. Mihich presented details of the screening results at the Ninth International Cancer Congress, Tokyo, 1966. [o] Personal communication from Dr. J. L. Hartwell, CCNSC, Bethesda, Md. [p] L. R. Duvall, *Cancer Chemotherapy Rept.*, **23**, 61 (1962). [q] L. R. Duvall, *ibid.*, **23**, 63 (1962).

barriers as a phospholipid complex, it becomes easier to understand the distribution of these positively charged materials through the tissues. The lack of parallelism between *in vitro* and *in vivo* experiments has already led Sivak, *et al.*,[20] to state that "Cell permeability to the phthalanilides may be one of the most important factors responsible for the efficacy of some phthalanilides and the ineffectiveness of others that are structurally similar."

From the above discussions it appears likely that the observed dependence on lipophilic–hydrophilic properties of these drugs is either associated with the binding of the drugs to the phospholipid carrier, movement of the drug to areas where the phospholipid occurs, and/or migration of the drug–lipid complex.[21]

(20) A. Sivak, W. 1. Rogers, I. Wodinsky, and C. J. Kensler, *Cancer Res.*, **25**, 902 (1965).

(21) Drug distribution could be visualized as follows. (a) Free drug moves by a partition-dependent process through a necessarily limited number of cellular barriers to a site where interaction with the lipid component takes place. (b) Drug–lipid complex moves to the site of action. Movement of drug–lipid complex could be passive and depend on the lipophilic–hydrophilic balance of the complex (and hence the drug) or active when cellular transport mechanisms could be invoked.

Examination of the retention or efflux of a phthalanilide–lipid complex in sensitive and resistant tumor cells[22] suggests that drug effectiveness is dependent on time of intracellular residence. Thus, selectivity of action could be intimately related to transport.

If a common transport-mediated distribution is accepted and this operates over the diverse set of compounds listed in Table I, differing in number of charges, charge separation, and other structural features, there can be little specificity of the carrier system except a requirement for a cationic charge[23] and possibly a particular lipophilic–hydrophilic balance.[23]

Regardless of the mode of distribution, the lipophilic–hydrophilic balance of these cationic agents is a prime determinant for biological activity.

While the agents listed in Table I have similar partition properties and biological activity, it was felt, in the early stages of this investigation, that some further evidence of generic relationship was required. Reference to Table I shows there is a close relationship among the quaternary salts we have prepared[1,2] (*cf.* I, XIX, XXV) and the phthalanilides (*cf.* II–XI). The screening results[11,12,24] from a very large array of phthalanilides leave no doubt that there is a similar relationship between biological activity and physical properties to that observed in the quaternary salts. This can be seen, for example, in the changes in biological activity on successive N-alkylation from III to VI and the homologation of the quaternary salts represented by I. The R_f values for active members of the phthalanilides that we have been able to examine (II, V, VIII–XI) support this thesis. However, attention has been drawn to the requirement for an N-amidinium substituent in the phthalanilides in order to obtain antileukemic activity,[11,24] and that these, therefore, represent a class of compound different from the usual bisamidines (XII–XVII). On the basis of our simple partition hypothesis, the alkyl substituent on these molecules merely serves to adjust the lipophilic–hydrophilic balance into the critical range required for activity. To obtain proof of this point we first examined a compound containing a diazoamino function (XVIII) where an amide had previously been used (XIX). This compound (XVIII) proved to be active against the L1210 leukemia, demonstrating that the diazoamino function is compatible with activity. Comparison of the R_f values of the methyl quaternary salts of XVIII and XIX shows that replacement of the amide function by diazoamino causes an increase in R_f approximating that observed between bismethyl and bisethyl quaternary salts in one series (XIX, R = CH_3 and C_2H_5), *i.e.*, equivalent to an increase due to two methylene groups. Accordingly, the diazoamino compound XX, bearing an unsubstituted amidine function, was prepared and shown to be convincingly active against the L1210.

Thus, one major difference between the phthalanilides and the trypanosomal bisamidines is a more hydrophilic amide backbone in the former which requires a compensatory adjustment of the hydrophilic–lipophilic balance by N substitution. It is thus not surprising to find that the phthalanilides are stated to have trypanosomal activity.[25] Attempts to prepare phthalanilides with more lipophilic functions in place of amide but retaining a Δ^2-imidazoline as basic function has given rise to inactive materials as expected from this thesis.[1,11,24] N substitution in the trypanosomal bisamidines (XII–XVII) has proved dystherapeutic:[26] such substitution would be expected to produce more lipophilic and hence less active materials.

Since a guanidine function is somewhat more lipophilic than an amidine, it was possible to construct an amide-linked molecule (XXI) with an unsubstituted guanidine function active against the L1210 leukemia. This is in contrast to the usual N-alkylation considered necessary in bisguanidine members of the phthalanilides (*cf.* VII and VIII).

Experience with a guanylhydrazone[27] as basic terminus has shown this to be more lipophilic than either an amidine or a guanidine. The preparation of L1210 active hybrid species containing one quaternary salt and one guanylhydrazone function (XXII, XXIII) leaves little doubt that the bisguanylhydrazones and the bisquaternary salts should be considered as analogs and, hence, from the preceeding arguments, also as analogs of the amidines and guanidines. With small molecules such as methylglyoxal bisguanylhydrazone (XXVI), which has partition figures close to that of peak members of other series (Table I), replacement of the methyl group by higher alkyl or aryl functions or substitution of the guanidine with N-alkyl groups would be expected to lead to more lipophilic and therefore less active agents. This is in fact the case.[28,29]

The structure–activity relationships in the phthalanilides led to the hypothesis that there is an apparent dependence on intercharge separation, highest activity being observed with a separation of close to 20 Å.[11] With the recognition of the critical importance of the lipophilic–hydrophilic balance in this area, we were able to construct a series of bisquaternary salts whose whose activity showed a more flexible dependence on intercharge separation.[27] The inactive bisquaternary salts (XXIV) appeared to be anomalous in that the intercharge separation (19.5 Å) corresponds closely to that in the active phthalanilides (19–19.5 Å) and the partition properties appear to be in the correct range (Table I). However, extension of XXIV by one *p*-aminobenzoate unit gave XXV which proved highly active against L1210 even though the charge separation (26 A) differed markedly from that in the phthalanilides.[30] This led to the realization that the members of the bisquaternary salts and the phthalanilides, highly active against the L1210, have three essentially coplanar rings between the two basic func-

(22) D. W. Yesair and C. Ho-Fook, *Cancer Res.*, **28**, 314 (1968).

(23) The presumption that cationic charge is a necessary requirement follows from the anionic nature of the phospholipid carrier. We make the assumption at this point in our argument that there is little specificity in the carrier. It should be remembered throughout the remainder of the discussion that the structure–activity relationships derived might apply to the transport system if this had specific requirements.

(24) L. Lee Bennett, Jr., *Progr. Exp. Tumor Res.*, **7**, 259 (1965).

(25) Quoted in ref 12. As far as we are aware full details of these tests have not been published.

(26) J. N. Ashley, H. J. Barber, A. J. Ewins, G. Newberry, and A. D. H. Self, *J. Chem. Soc.*, 103 (1942).

(27) Part VIII: G. J. Atwell, B. F. Cain, and R. N. Seelye, *J. Med. Chem.*, **11**, 690 (1968).

(28) E. Mihich, *Cancer Res.*, **23**, 1375 (1963).

(29) E. G. Podrebarac, W. H. Nyberg, F. A. French, and C. C. Cheng, *J. Med. Chem.*, **6**, 283 (1963).

(30) G. J. Atwell, B. F. Cain, and R. N. Seelye, *ibid.*, **11**, 295 (1968).

tions.[31] If it were concluded that these three rings made an essential contribution in binding to the site surface it could explain the nonactivity of the trypanosomal amidines (XII–XVII) against the L1210 leukemia.[32] The doubly charged trypanosomal agent, isometamidium (XXXVII), that is active against L1210, has considerably more planar ring area than the bisamidines.

The L1210 active guanylhydrazones listed in Table I (XXVI–XXVIII) are notable exceptions to the idea of ring contact. However, it is not inconceivable that these narrow molecules could penetrate further into the irregularities of a site surface and thus, through closer approach to anionic functions, gain increased electrostatic binding in addition to multiple "contact" interactions.[33] Each type of guanylhydrazone would then have to be considered separately since interpolation of an aromatic residue as in XXVII might then hold one or both hydrazone functions away from the site surface.

Our investigations with an extensive series of quaternary salts led us to conclude that a close approach to over-all planarity was necessary for high antileukemic activity.[1,2] Similar statements have been made with regard to the phthalanilides.[11,24] Proceeding from this point we prepared the two isomeric dimethyl-substituted derivatives XXIX and XXX since accurate models[34] showed that interaction of the methyl substituents with the NH of the terephthalanilide function would be expected to constrain these into *syn* and *anti* forms[35] on approach to a planar site. Only the *ortho*-disubstituted derivative (XXIX) was active against the leukemia. The naphthalene derivative XXXI, where similar steric effects are operative, was also active. Models of these active materials, in the *syn*[35] configuration, suggest that the three rings of the terephthalanilide system would bind to a surface which could be described as a sector of an annulus. Inner and outer radii of the annulus were, respectively, 20 and 25 Å.

The isophthaloyl derivative IX is highly active against L1210.[11,12,24] A model of IX in a planar configuration where both carbonyl oxygens and the 2-H atom of the isophthalanilide system lie to one side of the molecule shows the same 20-Å inner radius of curvature. Superposition of this model onto that of the *syn* configuration of the corresponding terephthaloyl derivative (II) shows that the ring areas match closely, although the carbonyl oxygens lie on opposite sides of the curve.[36,37] No other set of rotamers of these models shows matching ring areas.

The linear charge separations[38] listed in Table I can be grouped into four sets: 8–9, 12.5–14, 18.5–21.5, and 23.5–26.5 Å. If these distances are considered against a curve of approximately 20-Å radius it is found that intercepts close to 8 Å are made around the curve. Thus, binding could be to an ordered polynucleotide[39] where the phosphates are located at intervals of 8 Å. Inspection of an accurate scale model[40] of a section of a twin-stranded deoxyribonucleic acid helix[41] shows that small lengths of the minor groove have a narrow slot-like appearance, the floor and ceiling of the slot approximating to sections of an annulus bounded by radii of 20 and 25 Å. The linear distances measured are from one phosphate to the next approximately 8 Å, from the first to the third 14 Å, first to fourth 20 Å, to the fifth 25 Å, and to the sixth 27.5 Å. Trial placement of models of the various bisonium drugs listed in Table I in the narrow groove in the model DNA helix showed an excellent fit in all cases.[42] Thus the dibasic drugs listed in Table I could all act at such a site.

The agents homidium (XXXV, R = C_2H_5) and trypaflavin (XXXVI) have been shown to intercalate between the base pairs of a DNA helix.[43–47] The effects of intercalation may be responsible in part for the biological activity of such species. Lodgement in the narrow groove could precede the final step of intercalation. Bis-charged variants of the phen-

(31) The heterocyclic ring bearing the quaternary charge being taken as charge alone and not providing contact equivalent to the aromatic nuclei comprising the backbone structure.

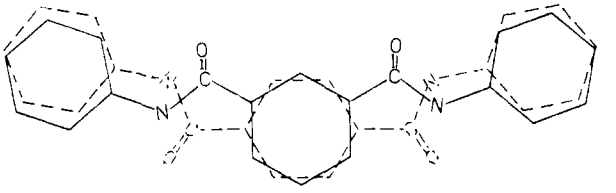
(32) Although the bisamidines (XII–XVII) do not themselves have experimental antileukemic activity, there are reports that some of these may show synergism with the phthalanilides [J. H. Burchenal, M. S. Lyman, J. E. Purple, V. Coley, S. Smith, and E. Bucholz, *Cancer Chemotherapy Rept.*, **19**, 19 (1962)]. More interestingly, two inactive members administered together produce increases in survival time in the L1210 system equivalent to that produced by the phthalanilides [E. Mihich and A. I. Mulhern, *Proc. Am. Assoc. Cancer Res.*, **7**, 49 (1966)]. It is tempting to suggest that site–time–occupancy is an important factor with these materials; either one species is sufficiently strongly bound at the site for adequate residence or a combination of less firmly bound components can serve.

(33) A bigoanide serves as an acceptable basic terminus in place of a guanylhydrazone[2] but confers considerable hydrophilic character on the resultant molecule and this must be compensated for.

(34) Courtauld models, a series of space-filling models developed jointly by Courtaulds, Maidenhead, U. K., and Grillin and George Ltd., Wembley, U. K.

(35) *syn* is used to signify that both carbonyl oxygens of the terephthalanilide lie to the same side of the molecule in a planar configuration.

(36) Diagram showing overlap of the ring areas in a terephthalanilide (broken line) and an isophthalanilide system. Bond angles and lengths are as used in Courtald model 34.



(37) We have assumed that the amide functions in these molecules when absorbed to a planar site would have the planar *trans* amide configuration shown by electron diffraction in crystalline amides.

(38) Charge separations have been measured from Courtald models when these are constructed with the most probable conformation, which allows closest approach to a curve of 20-Å radius.

(39) The interaction of the bisamidines and the phthalanilides with nucleic acids is well authenticated; leading references are given in the review articles.[11,12] See also H. M. Raven, K. Norpath, W. Unterberg, and H. Haar, *Experientia*, **21**, 300 (1965); A. Sivak, W. I. Rogers, I. Wodinsky, and C. J. Kensler, *Cancer Res.*, **25**, 902 (1965); A. Sivak, W. I. Rogers, and C. J. Kensler, *Biochem. Pharmacol.*, **12**, 1055 (1963).

(40) A model of a section of a twin-stranded DNA helix of the Watson–Crick structure as investigated by M. Wilkins and coworkers at the Medical Research Councils Biophysics Research Unit, King's College, London. Available from Griffin and George Ltd., Wembley, U. K.

(41) It is not implied that only a twin-stranded deoxyribonucleic acid helix will suffice. There is a lack of models of ordered ribose polynucleotides available for examination.

(42) Binding in the major groove was considered but it is difficult to visualize how the aromatic nuclei would make binding contact while at the same time having effective electrostatic interaction of the charged functions.

(43) L. Lerman, *J. Mol. Biol.*, **3**, 18 (1961).

(44) L. Lerman, *Proc. Natl. Acad. Sci. U. S.*, **49**, 94 (1963).

(45) V. Luzzati, F. Masson, and L. Lerman, *J. Mol. Biol.*, **3**, 634 (1961).

(46) W. Fuller and M. J. Waring, *Ber. Bunsenges. Phys. Chem.*, **68**, 805 (1964).

(47) M. J. Waring, *Biochim. Biophys. Acta*, **87**, 358 (1964).

anthridines such as isometamidium (XXXVII)[48] or prothidium (XXXVIII) could equally well reside in a site equivalent to the minor groove of a DNA helix or with the phenanthridine nucleus intercalated between the base pairs with the second basic function protruding and matching to a phosphate. It could well be that such charged species may reside in the narrow groove until such time as the central stack of purine–pyrimidine pairs opens because of the demands of some metabolic sequence. The phenanthridine moiety could then intercalate.[49]

The slot-like nature of the minor groove in DNA helices is such that only drugs of very narrow cross section could enter. This could explain the requirement for a close approach to planarity commented on earlier.[1,11]

It is believed that the details given in this paper should allow planned synthesis of trypanosomal and experimental antileukemic agents. We present two successful examples, XLII and XLIII, active against L1210, predicted from our site model concept. XLII uses three charges so that the lipophilic-styryl system could be brought into the correct range of hydrophilic–lipophilic properties. The use of three charges affords sufficient electrostatic binding so that three rings of contact need not be provided. Although active this compound could not be claimed to be strikingly so. XLIII is a novel variant predicted from the model which shows an extremely high order of activity. Further successful examples will be reported in due course.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Analyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal melting point apparatus using the maker's-supplied stem corrected thermometer. A heating rate of 2°/min from 20° below the melting point was used.

The symmetrical bis bases listed in Table II were prepared from the substituted terephthaloyl chloride and 3-(*p*-aminobenzamido)-pyridine in diethylene glycol dimethyl ether as detailed earlier.[30] Methods for quaternization, paper chromatography, etc., have been described adequately.[2] Intermediates not previously described in the literature follow.

1-Methyl-4-(*p*-aminophenyl)pyridinium *p*-Toluenesulfonate.—4-(*p*-Nitrophenyl)pyridine (1 g) was dissolved in $PhNO_2$ (2 ml) at 165°. Methyl *p*-toluenesulfonate (1.5 ml) was added to the hot solution, there was an exothermic reaction, and the quaternary salt crystallized from the hot solution. The salt was collected after cooling, washed (Et_2O), and dried. It proved to be excessively soluble in H_2O and since paper chromatography[2] showed the material to be homogeneous it was reduced to the amine without further purification. Reduction with Fe dust by the previously described method[30] gave the amine which separated from H_2O containing sodium *p*-toluenesulfonate as pale yellow prisms (1.19 g, 67%), mp 261–262°. *Anal.* ($C_{19}H_{20}N_2O_3S$) C, H, S.

TABLE II

Compd	R	Mp, °C	Formula	Analyses
XXIX	*a*	>360	$C_{34}H_{28}N_6O_4$	C, H, N
XXIX	CH_3[b]	326–327	$C_{50}H_{48}N_6O_{10}S_2 \cdot 1.5H_2O$	C, H, S
XXX	*a*	>360	$C_{34}H_{28}N_6O_4$	C, H, N
XXX	CH_3[b]	329–330	$C_{50}H_{48}N_6O_{10}S_2 \cdot 1.5H_2O$	C, H, S
XXXI	*a*	>360	$C_{36}H_{26}N_6O_4$	C, H, N
XLII	CH_3[b]	61–62	$C_{61}H_{51}N_5O_9S_3$	C, H, N, S
XLII	CH_3[a]	306–307	$C_{30}H_{30}I_3N_5$	C, H, N, I
XLII	C_2H_5[c]	304–305	$C_{33}H_{35}I_3N_5$	C, H, N, I
XLIII	*a*	324–325	$C_{27}H_{21}N_5O$	C, H, N
XLIII	CH_3[c]	214–216	$C_{29}H_{27}I_2N_5O$	C, H, I
XLIII	C_2H_5[c]	202–204	$C_{31}H_{31}I_2N_5O$	C, H, I
XLIII	$CH_3(CH_2)_2$[c]	189–190	$C_{33}H_{35}N_5I_2O \cdot 0.5H_2O$	C, H, I

[a] Free base. [b] Anion *p*-toluenesulfonate. [c] Anion iodide.

4-[*p*-(*p*-Nitrobenzamido)phenyl]pyridine was prepared by interaction of *p*-nitrobenzoyl chloride and 4-(*p*-aminophenyl)-pyridine in pyridine solution. The free base separated from solution in DMF–H_2O as yellow needles, mp 286–287°. *Anal.* ($C_{18}H_{13}N_3O_2$) C, H, N.

1-Methyl-4-[*p*-(*p*-nitrobenzamido)phenyl]pyridinium *p*-Toluenesulfonate.—A solution of the corresponding base (1.55 g) in 1-methyl-2-pyrrolidone (5 ml) was heated to 160° and methyl *p*-toluenesulfonate (2 ml) was added. After 10 min at 160° the solution was cooled. The crystalline quaternary salt was washed well (Et_2O), dried, and then crystallized from MeOH–H_2O; yellow prisms (2.32 g, 89%), mp 328–330°. *Anal.* ($C_{26}H_{23}N_3O_6S \cdot 0.5H_2O$) C, H, S.

1-Methyl-4-[*p*-(*p*-aminobenzamido)phenyl]pyridinium *p*-toluenesulfonate was prepared by Fe dust reduction of the corresponding nitro compound by the method described earlier.[30] The amine separated from MeOH–H_2O, containing sodium *p*-toluenesulfonate as pale yellow prisms, mp 298–299°. *Anal.* ($C_{45}H_{42}N_6O_7S_2$) C, H, S.

XVIII.—A suspension of the preceding amino-substituted quaternary salt (1.894 g) in H_2O (25 ml) and HCl (1.6 ml) was diazotized by slow addition of a solution of $NaNO_2$ (0.39 g) in H_2O (2 ml), the temperature being maintained below 2°. The solution was stirred for 0.5 hr, saturated aqueous sulfamic acid (1 ml) was added, and the solution was stirred for a further 5 min. An ice-cold solution of 1-methyl-4-(*p*-aminophenyl)-pyridinium *p*-toluenesulfonate (1.56 g) in H_2O (10 ml) was then stirred in followed immediately by cold saturated NaOAc solution (21 ml). The reaction was stirred in the ice bath for a further 0.5 hr, then crude product precipitated by the addition of sodium *p*-toluenesulfonate (15 g). The precipitated salt was collected, washed well with cold aqueous sodium *p*-toluenesulfonate (10%), and crystallized from MeOH–H_2O containing sodium *p*-toluenesulfonate, the temperature not being raised above 60°. Several further recrystallizations gave material homogeneous when examined by paper chromatography.[2] Pure compound separated from MeOH–H_2O as orange needles (3.03 g), mp 196° dec. *Anal.* ($C_{45}H_{42}N_6O_7S_2$) C, H, S.

A sample of the compound on warming with Cu_2Cl_2 3 *N* HCl readily evolved N_2, confirming the correctness of formulation as a diazoamino compound.[50]

XX.—A suspension of 1-methyl-3-{*p*-[*p*-(*p*-aminophenylcarbamoyl)benzamido]benzamido}pyridinium *p*-toluenesulfonate (3.18 g)[27] in H_2O–MeOH (30:20 ml) containing HCl (4 ml) was diazotized by dropwise addition of a solution of $NaNO_2$ (0.45 g) in H_2O (3 ml) to the vigorously stirred suspension, the temperature being maintained below 2°. The mixture was stirred for a further 15 min after complete dissolution. Saturated aqueous sulfamic acid (1 ml) was added, the solution was stirred for a further 5 min, and then *p*-aminobenzamidine monohydrochloride (1 g) in H_2O (5 ml) was added followed immediately by cold saturated NaOAc (25 ml). The viscous mixture was stirred for a further 0.5 hr when sodium *p*-toluenesulfonate (20 g) was added. After 0.5 hr the precipitated salt was collected, washed well with ice-water and crystallized repeatedly from MeOH–H_2O containing sodium *p*-toluenesulfonate at below 60°. When homogeneous by paper chromatography[2] the yellow *p*-

(48) It has been postulated that a small proportion of pseudobase in homidium (XXXV, R = C_2H_5) may facilitate distribution of this type of drug: T. I. Watkins, *J. Chem. Soc.*, 3059 (1952). However, such arguments can not be extended to the discharged variants XXXVII and XXXVIII, and the amount of neutral species in these would be vanishingly small.

(49) There is an apparent familial relationship between two new antitumor agents uncovered by the CCNSC screening program (XXXIX and XL) and trypaflavin (XXXVI) and homidium (XXXV, R = C_2H_5). It may be profitable to examine XXXIX and XL for (a) intercalation into DNA and (b) trypanosomal activity. These molecules could then serve as the basis for preparation of a family of drugs related to prothidium (XXXVIII) and isometamidium (XXXVII).

(50) S. S. Berg, *J. Chem. Soc.*, 3635 (1963).

Table III

Compd	Dose, mg/kg/day	Survivors	Wt change	Av survival days Treated	Control	T/C, %
XVIII	7.5	6	−1.4	11.9	10.8	
	5.0	6	+0.2	16.9	10.8	156
	3.3	6	+1.8	23.2	10.8	214
	2.2	6	+3.7	23.8	10.8	222
	1.5	6	+6.3	17.6	10.8	163
	1.0	6	+5.7	11.2	10.8	
XX	37.5	5	−1.3	11.2	10.4	
	25	6	−0.9	14.3	10.4	137
	17	6	−0.6	21.4	10.4	206
	11	6	+0.3	25.8	10.4	248
	7.4	6	+1.2	16.9	10.4	163
	4.9	6	+2.8	11.0	11.2	
XXIX	25	4	−1.7	10.0	10.3	
	17	6	−1.2	14.6	10.3	142
	11	6	−0.4	18.1	10.3	176
	7.4	6	+1.2	16.2	10.3	158
	4.9	6	+4.3	12.2	10.3	
XXXI	250	0				
	170	6	−1.8	13.9	10.6	131
	110	6	−0.6	16.6	10.6	156
XXXI	74	6	+1.2	15.7	10.6	148
	49	6	+2.3	13.8	10.6	130
XLII	25	1				
	17	6	−1.4	12.6	9.8	129
	11	6	+0.8	15.9	9.8	162
	7.4	6	+2.0	17.6	9.8	180
	4.9	6	+2.5	15.3	9.8	156
	3.3	6	+3.3	11.6	9.8	
XLIII, R =	20	4		6.9	10.2	
CH_3	13	6	−1.8	19.0	10.2	186
	8.7	6	+1.2	25.3	10.2	248
	5.8	6	+0.8	23.8	10.2	234
	3.8	6	+2.4	19.0	10.2	186
	2.6	6	+3.2	17.6	10.2	173
	1.7	6	+2.8	12.8	10.6	121
XLIII, R =	20	3		4.2	10.2	
C_2H_5	13	6	−3.0	19.8	10.2	19.4
	8.7	6	−0.6	21.6	10.2	212
	5.8	6	−0.2	18.2	10.2	178
XLIII, R =	3.8	6	+1.5	13.5	10.2	132
C_2H_5	2.6	6	+2.1	11.0	10.2	
XLIII, R =	20	4		5.6	9.7	
$CH_3(CH_2)_2$	13	6	−1.5	15.2	9.7	156
	8.7	6	−0.6	13.8	9.7	142
	5.8	6	+2.3	11.6	9.7	

toluenesulfonate salt had mp 189° dec. *Anal.* ($C_{48}H_{45}N_5O_6S_2 \cdot 0.5H_2O$) C, H, N, S. A sample on warming with Cu_2Cl_2 3 *N* HCl evolved N_2.[50]

XLII.—A mixture of 2-(*p*-aminostyryl)-6-aminoquinoline (2.61 g), anhydrous *p*-toluenesulfonic acid (5.2 g), N-pyridyl-4-pyridinium chloride hydrochloride (5.7 g), and dry phenol (15 g) was heated under reflux for 1 hr. After removal of phenol by steam distillation crude base was precipitated with excess NaOH. It was suspended in H_2O (75 ml) and HBr was added to the boiling suspension until solution was complete. A generous quantity of decolorizing charcoal was added to the solution and boiling continued for 5 min. Addition of NaBr (20 g) to the hot filtered solution, followed by slow cooling, afforded the trihydrobromide as brilliant red needles. The salt was repeatedly crystallized from H_2O–NaBr–HBr until a persistent impurity (faster running on paper chromatograms using *n*-BuOH–HOAc–H_2O (4:1:5)) was removed. The pure trihydrobromide (4.2 g, 64%) had mp >360°. *Anal.* ($C_{27}H_{24}Br_3N_5$) C, H, N, Br.

The free base, from the salt with excess NaOH, avidly removed CO_2 from the air on storage or on attempted recrystallization. Accordingly, samples of the free base were prepared immediately before use and after drying *in vacuo* at 100° were used for preparation of the quaternary salts XLII, R = CH_3 and R = C_2H_5. See Tables I and II.

4-{*p*-(*p*-Nitrobenzamido)anilino}pyridine was prepared by interaction of *p*-nitrobenzoyl chloride and 4-(*p*-aminoanilino)-pyridine in pyridine solution. The pure base separated from large volumes of EtOH as yellow needles, mp 276–277°. *Anal.* ($C_{25}H_{19}N_5O_3$) C, H, N.

4-{*p*-(*p*-Aminobenzamido)anilino}pyridine, from Fe dust reduction[30] of the preceding nitro compound, separated from EtOH–H_2O as colorless needles, mp 225–226°. *Anal.* ($C_{25}H_{21}N_5O$) C, H, N.

4-{*p*-[*p*-(4-Quinolylamino)benzamido]anilino}pyridine (XLIII).—A solution of 4-{*p*-(*p*-aminobenzamido)anilino}pyridine (3.04 g) in 60% EtOH–H_2O (50 ml) containing HCl (1.7 ml) was prepared. After addition of 4-chloroquinoline (1.72 g) the solution was refluxed for 0.5 hr. Hot saturated aqueous NaCl was added until crystals appeared; on cooling, the dihydrochloride separated as yellow needles. The salt was dissolved in boiling H_2O and filtered from a trace of insoluble material and the solution was strongly basified with NaOH. The base obtained separated from DMF–H_2O as pale yellow needles (3.51 g, 82%), mp 324–325°. *Anal.* ($C_{25}H_{21}N_5O$) C, H, N.

Biological Testing.—The routine test consists of intraperitoneal inoculation of 10^5 L1210 cells into 18.5–22.5-g CaH_2/DBA$_2$ F_1 hybrids on day 1; drug treatment was initiated 24 hr later and continued for 5 days. Average survivals are calculated in the usual way. An attempt was made to test all drugs from a level which is frankly toxic, giving either toxic deaths before control deaths or marked weight loss; serial twofold dilutions were then tested until an obviously nontoxic dose was reached; this usually required a total of three tests. Compounds which under these test conditions were not given T/C values greater than 125% have been classified as negative and this is recorded in the requisite column in Table I. Full test data for negative compounds has not been given. On retesting positives a two-thirds dosage schedule was used, the levels ensuring tests from toxic levels to those which give less than 40% increase in life span.

Table III shows the data obtained and is virtually self-explanatory. All dosage has been in 0.2 ml of H_2O. Groups of six animals per dose level were used and one control group for every 5 tests. The weight-change column records the difference between initial weight and that at day 8 for survivors. The number of animals surviving as long as or longer than controls is listed under survivors. Doses have been rounded off to two significant figures.

Acknowledgments.—We are deeply indebted to Dr. J. L. Hartwell, CCNSC, for helping us obtain samples of IX, X, XI, and XXXIII. Samples of XXXIX and XL were prepared by Dr. D. W. Cheeseman, University of Auckland, Chemistry Department, using methods detailed in the chemical literature. We are grateful to Dr. H. Hirt, Department of Chemistry, Research Institute, and Dr. A. Wander, S. A. Berne, for generous samples of XXXII (n = 0, 3, 8). May and Baker Ltd., Dagenham, U. K., supplied samples of the trypanocidal agents XII, XIII, XV–XXXVII. We are greatly indebted to Miss L. Armiger and her capable assistants for performance of the many biological tests. This work was supported by the Auckland Division, Cancer Society of New Zealand (Inc.).