Notes

Potential Antitumor Agents. 18. Bisquaternary Ammonium Heterocycles

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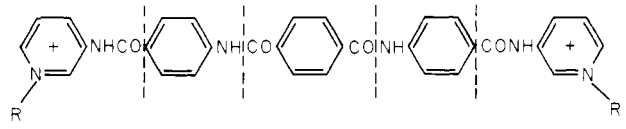
It was earlier proposed that a close approach to overall planarity was a structural prerequisite for antileukemic activity (L1210) in bisquaternary ammonium heterocycles. The preparation of L1210 active 3,3′-[bicyclo[2.2.2]octane-1,4-dicarbonylbis(imino-*p*-phenylenecarbonylimino)]bis(1-alkylpyridinium) salts, containing a nonplanar bridged ring system, negates this view. A replacement proposal is that a relatively rigid molecular framework is necessary to maintain the spacing and positioning of the quaternary functions, thereby ensuring correct site selection. Replacement of a terephthaloyl drug component by a bicyclo[2.2.2]octane-1,4-dicarbonyl residue lowers DNA binding. A terephthaloyl unit confers necessary molecular rigidity, greater DNA binding, and higher L1210 activity.

In earlier publications[1-3] we presented an analysis of the structure–activity relationships for series of experimental antileukemic (L1210) bisquaternary salts. Most readily prepared members of this series are centrosymmetric and can be considered as assembled from three subunits (A, B, C) as denoted in example **1**. The dominant factor



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influencing antileukemic effectiveness was shown to be the lipophilic–hydrophilic balance of the agents.[3,4] R_m values from chromatographic data have again been used as a convenient measure of lipophilic–hydrophilic balance.[5] As seen with examples **2–5** (Table I) convenient control of lipophilic–hydrophilic balance is achieved by lengthening the quaternizing alkyl group R. L1210 activity has been found to change in parabolic fashion with altering R_m values.[3-5] From our earlier studies[3] we had concluded that, overall, subunits A–C should be close to coplanar in the assembled molecules. One supporting line of evidence for this conclusion results from attempts to replace unit A by a flexible aliphatic component. Such alteration inevitably provided inactive congeners.[2,4] Further examples of inactive variants containing an aliphatic A unit are provided in Table I (**9–12**). These utilize the 4-anilinopyridine C unit which has formerly provided very active agents (cf. **6–8**).[6] The R_m values of **9–12** are in the range where L1210 activity could be reasonably expected (cf. **2–8**). It also appears unlikely that the inactivity of **9–12** results from excessive intercharge separation since, once a necessary minimum charge separation of 18 Å has been exceeded, active examples have progressively spanned the interval to 27 Å.[2,3]

Scrutiny of the animals being dosed with aliphatic congeners **9–12**, and similar earlier described examples,[2,4] suggests that the toxicity providing dose limitation may be different from that encountered with the L1210 active variants. The usual toxicity encountered with the latter (e.g., **2–8**) is chronic[7] and there are no early animal deaths (1–8 days) until the administered dose is several fold higher than usual therapeutic doses; toxic deaths at the LD_{50} dose result from slow wasting and inanition of the animals. In contrast, the aliphatic congeners **9–12** prove acutely toxic; animals succumbing at the LD_{50} dose first suffer convulsions and expire shortly afterward.

Those structural units (A–C) which combine to yield a close to planar molecule also provide a relatively rigid assembly. Such rigidity could prevent drug approach to certain host sites providing dose-limiting toxicity. The more flexible aliphatic congeners (e.g., **9–12**) might successfully accommodate to these sites with consequent dose limitation. Unit A, on this hypothesis, might then be successfully replaced by an aliphatic component provided this was sufficiently constraining to maintain the overall placement of the B and C subunits as located in the L1210 active examples. Replacement of A by a bridged ring diacid would ensure nonplanarity, limit molecular flexing, and maintain the desired orientation of the cationic functions. A bicyclo[2.2.2]octane-1,4-dicarboxylic acid component would meet these requirements but, having greater lipophilic character than the replaced terephthaloyl A unit, would require the use of the more hydrophilic quaternary pyridine amide C unit (cf. **2–5**) to obtain overall lipophilic–hydrophilic balance in the region where high L1210 activity could be expected. Variants **13–15** utilizing the bicyclooctane system are convincingly L1210 active. The relatively high R_m value for the methyl quaternary salt **13** suggests that even greater L1210 activity could result if a somewhat more hydrophilic congener was prepared. These new active variants (**13–15**) are not acutely toxic as are earlier prepared aliphatic analogues; the dose-limiting toxicity is chronic as observed with most L1210 active variants.[7]

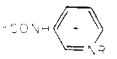
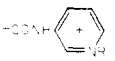
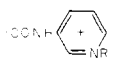
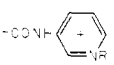
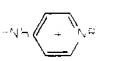
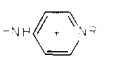
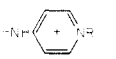
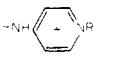
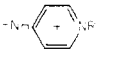
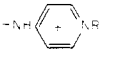
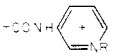
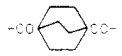
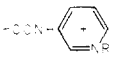
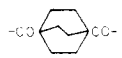
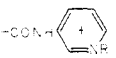
Most structure–activity relationships are generally interpreted in terms of drug fit to the site of action of the targeted cell or organism. In the present drug series certain structural requirements could be dictated by a need to avoid drug residence at susceptible host sites.

Use of a bicyclo[2.2.2]octane ring system, as in **13–15**, increases the depth of the drug profile in comparison with the terephthaloyl congeners (**2–8**). Despite this increase model fitting suggests that lodgement of the bridged ring variants in the minor groove of a DNA helix, as a putative site of action,[3] would not be markedly impeded. Studies of drug binding to calf thymus DNA in vitro, employing an ethidium displacement technique, shows that **13** binds strongly to the nucleic acid but significantly less firmly than does **2** (personal communication, Dr. B. C. Baguley, this laboratory).

Experimental Section

Melting points were determined in open capillaries on an Electrothermal melting point apparatus with the makers' stem

Table I. Agent, Formulas, and L1210 Screening Data

No.	A	B	C	R	Anion	R_m[a]	O.D.[b]	L1210 T/C, %[c]
2	-CO-C_6H_4-*p*-CO-	-HN-C_6H_4-		CH_3	TsO^-[d]	−0.69	27	206
3	-CO-C_6H_4-*p*-CO-	-HN-C_6H_4-		C_2H_5	TsO^-	−0.35	10	265
4	-CO-C_6H_4-*p*-CO-	-HN-C_6H_4-		$(CH_2)_2CH_3$	TsO^-	0.13	20	258
5	-CO-C_6H_4-*p*-CO-	-HN-C_6H_4-		$(CH_2)_3CH_3$	TsO^-	0.51	30	165
6	-CO-C_6H_4-*p*-CO-	-HN-C_6H_4-		CH_3	TsO^-	0.07	2.2	373
7	-CO-C_6H_4-*p*-CO-	-HN-C_6H_4-		C_2H_5	TsO^-	0.46	2.2	342
8	-CO-C_6H_4-*p*-CO-	-HN-C_6H_4-		$(CH_2)_2CH_3$	TsO^-	0.81	3.3	202
9	$-CO(CH_2)_5CO-$	-HN-C_6H_4-		CH_3	Br^-	0.39	0.56	–[e]
10	$-CO(CH_2)_5CO-$	-HN-C_6H_4-		C_2H_5	ClO_4^-	0.70	0.63	–
11	$-CO(CH_2)_6CO-$	-HN-C_6H_4-		CH_3	Br^-	0.58	0.24	–
12	$-CO(CH_2)_6CO-$	-HN-C_6H_4-		C_2H_5	Br^-	0.83	0.43	–
13		-HN-C_6H_4-		CH_3	TsO^-	0.32	27	194
14		-HN-C_6H_4-		C_2H_5	TsO^-	0.59	29	153
15		-HN-C_6H_4-		$(CH_2)_2CH_3$	TsO^-	0.86	30	137

[a] From partition chromatographic data; $R_m = \log(1/R_f - 1)$. [b] O.D. = optimum dose in mg/kg/day; that dose providing maximum T/C in L1210 tests. [c] T/C (%) = ratio of mean life spans of drug-treated leukemic animals to that of controls as a percentage. [d] TsO^- = *p*-toluenesulfonate ion. [e] Significant life extension (T/C >125%) was not observed.

corrected thermometer and are as read. Analyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. The symbol for the requisite element has been used to signify that analytical results were within ±0.4% of the calculated values.

For chromatography of the bisquaternary salts the top phase of a mixture of *i*-BuOH, HOAc, H_2O, and DMF (30:6:24:2.25 v/v) was used with Merck DC cellulose F_{254} cards. Agents were detected by uv scanning and by spraying with Dragendorff's reagent.

***N,N'*-Bis[4-(4-pyridinylamino)phenyl]pentane-1,5-dicarboxamide.** To a well-stirred solution of 4-(*p*-aminoanilino)pyridine[6] (7.56 g, 0.041 mol) and pyridine (3.24 g, 0.082 mol) in dry diglyme (75 ml) maintained at 0 °C was added in dropwise fashion a solution of pentane-1,5-dicarbonyl chloride (3.98 g, 0.02 mol) in dioxane (20 ml). Product started to separate on first addition. When addition was complete the heterogeneous mixture was heated on the water bath for 1 h and cooled well, then product hydrochloride was collected, washed with Et_2O, and dried in vacuo. The salt was stirred with 4 N NH_4OH (200 ml) for 20 min and then the base collected, washed well with water, and dried. Crystallization from EtOH–H_2O (5:1) containing NH_3 provided pure product as colorless prisms of mp 225–226 °C (7.81 g, 78% yield). Anal. ($C_{29}H_{30}N_6O_2$) C, H, N.

***N,N'*-Bis[4-(4-pyridinylamino)phenyl]hexane-1,6-dicarboxamide** was prepared in equivalent fashion (74% yield) and separated from EtOH–H_2O (6:1) as colorless plates of mp 254–255 °C. Anal. ($C_{30}H_{32}N_6O_2$) C, H, N.

Quaternary salts 9–12 were prepared by reaction of the requisite bis-base with 4.0 mol equiv of MeOTs or EtOTs in *N*-methyl-2-pyrrolidone at 135 °C for 10 min. Products were precipitated from the reaction mixtures with Et_2O and washed well with this solvent. Crude products were extracted with the minimum necessary volume of boiling H_2O, the solutions were clarified, and the Na or K salt of the requisite anion was added to the hot solution until product started to separate. After thorough cooling the solid was collected and dried in vacuo. To avoid gel formation the salts were recrystallized from small volumes of EtOH–H_2O containing at least 10 g/100 ml of the Na or K salt of the requisite anion. Purity was best monitored by TLC.

Bicyclo[2.2.2]octane-1,4-dicarboxylic acid was prepared by hydrogenation of dimethyl bicyclo[2.2.2]oct-2-ene-1,4-dicarboxylate with following saponification of ester functions according to a published procedure.[9] The acid was recrystallized from HOAc and had mp >360 °C (lit.[9] mp 422 °C dec).

Bicyclo[2.2.2]octane-1,4-dicarbonyl Chloride. A suspension of 3.5 g of the aforementioned acid (oven dried to constant weight) in $SOCl_2$ (30 ml) containing 1 drop of DMF was heated at reflux for 2 h. Excess $SOCl_2$ was removed in vacuo at 25 °C and the residue crystallized from petroleum ether. A further crystallization provided colorless needles of the acid chloride (3.42 g, 82%) of mp 97–98 °C (lit.[9] mp 97.5–98.5 °C).

***N,N'*-Bis[4-(3-pyridinyliminocarbonyl)phenyl]bicyclo[2.2.2]octane-1,4-dicarboxamide.** A solution of bicyclo[2.2.2]octane-1,4-dicarbonyl chloride (1.72 g, 73 mmol) in dry C_6H_6 (20 ml) was added in one portion to a stirred solution of 4-amino-*N*-(3-pyridinyl)benzamide (3.27 g, 15.3 mmol) and pyridine (1.35 ml, 16.8 mmol) in dry *N*-methyl-2-pyrrolidone (15 ml). The heterogeneous mixture was heated on a steam bath for 1.5 h and then cooled and the product hydrochloride was collected, washed with C_6H_6, and dried. The hydrochloride was suspended in *N*-methyl-2-pyrrolidone (30 ml) at 100 °C and concentrated NH_3 (5 ml) slowly added, a solution resulting. On addition of H_2O to the hot solution product commenced to crystallize and the mixture was then thoroughly cooled. The collected bis-base was washed well with H_2O and EtOH and then dried. One further

Table II. Quaternary Salts

No.	Formula	Mp, °C	Analyses
9	$C_{31}H_{36}Br_2N_6O_2 \cdot H_2O$	174-176	C, H, N, Br
10	$C_{33}H_{40}Cl_2N_6O_{10}$	194-195	C, H, N, Cl
11	$C_{32}H_{38}Br_2N_6O_2 \cdot 2H_2O$	280-281	C, H, N, Br
12	$C_{34}H_{42}Br_2N_6O_2$	214-216	C, H, N, Br
13	$C_{50}H_{52}N_6O_{10}S_2$	323 dec	C, H, N, S
14	$C_{52}H_{56}N_6O_{10}S_2 \cdot H_2O$	329-330	C, H, N, S
15	$C_{54}H_{60}N_6O_{10}S_2$	279-280	C, H, N, S

crystallization from *N*-methyl-2-pyrrolidone–H_2O provided TLC homogeneous product as colorless prisms of mp >360 °C (3.90 g, 91%). Anal. ($C_{34}H_{32}N_6O_4$) C, H, N.

Quaternization, essentially as described above, and crystallization of the quaternary salts from 5% NaOTs–H_2O provided colorless crystals of agents 13–15 (Table II).

Biologic Testing. 10^5 L1210 cells were implanted intraperitoneally into 18.5–22.5-g C3H/DBA2 F_1 hybrid mice on day 0; ip drug treatment was initiated 24 h later and continued daily for 5 days. Drugs were tested from dose levels which were clearly toxic, providing toxic deaths before those of control animals. Twofold dilutions were then screened until a nontoxic or nonactive dose level was reached. All dosage has been intraperitoneal in 0.2-ml volume, H_2O being used as medium. Groups of six animals per dose level have been used with one control group for every six test groups. Compounds that have been tested under these conditions and have given no significant (>25%) increase in life span have been classed as negative.

Acknowledgment. We are indebted to Dr. J. C. Kauer, E. I. du Pont de Nemours and Co., for a generous sample of dimethyl bicyclo[2.2.2]oct-2-ene-1,4-dicarboxylate. The Misses Noeline Price and Carolin West ably assisted with the biologic testing. This work was supported by the Auckland Division of the New Zealand Cancer Society and in part by the Medical Research Council of New Zealand.

References and Notes

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Para-Substituted *N*-Acetyl-L(*S*)- and -D(*R*)-α-amino-*N*-phenylglutarimides. A Structure–Activity Study of Substituent Effects on Stereoselective Anticonvulsant Activity[1]

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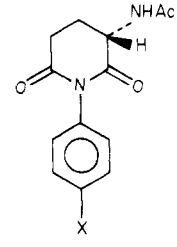
For purposes of carrying out structure–activity studies on a series of pure *R* and *S* enantiomorphs of various para-substituted *N*-acetyl-α-amino-*N*-phenylglutarimides, we synthesized the *p*-acetyl, iodo, cyano, ethyl, and *n*-butyl analogues. These compounds complimented previous *R* and *S* isomers (unsubstituted and the *p*-chloro, methyl, nitro, and methoxyl analogues) synthesized in our laboratories from amino acids of known absolute configuration. The neurotoxic doses (TD_{50}'s), anticonvulsant potencies [maximal electroshock seizures (MES) and subcutaneous metrazole (sc Met) ED_{50}'s], protective indices (PI = TD_{50}/ED_{50}), and effects on minimal seizure threshold (iv Met) were compared with similar values concomitantly determined for clinically employed anticonvulsants. A parallel relationship was shown between neurotoxicity (TD_{50}) and potency (ED_{50}) for the *R* and *S* analogues. In most cases *R* isomers had a more rapid onset of action and possessed greater neurotoxicity and greater anticonvulsant potency.

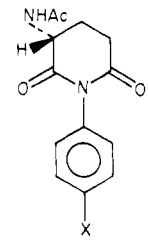
We previously reported a qualitative stereostructure–activity analysis of certain enantiomorphic succinimide and glutarimide (1–5) anticonvulsants of known absolute configuration.[2] Since several analogues compared favorably with well-known clinically employed anticonvulsants, and readily could be synthesized from amino acids of known absolute configuration, we extended the glutarimide series to those having para substituents 6–10 for purposes of analyzing the results by computerized multiparameter regression analysis.[3] However, no acceptable QSAR analysis could be obtained within this extended series wherein substituents show no covariance between parameter values.[4] For these reasons, the results are only discussed from a qualitative point of view.

Pharmacological Results and Qualitative Discussion. The pharmacological data for *R* and *S* isomers 1–5 have been compared with several standard anticonvulsive drugs and reported previously;[2] biological data obtained for *R* and *S* analogues 6–10 are found in Table I. The anticonvulsive activities obtained for standard drugs (diphenylhydantoin, trimethadione, and ethosuximide) in this study were found to be similar to those which we previously reported.[2] Since the pharmacological procedures employed in the evaluation of all drugs were virtually identical, analysis of the data for 1–10 appeared justified.



L(*S*)

1, X = H
2, X = Cl
3, X = CH_3
4, X = NO_2
5, X = CH_3O



D(*R*)

6, X = $COCH_3$
7, X = I
8, X = CN
9, X = CH_2CH_3
10, X = $(CH_2)_3CH_3$

We previously showed[2] that for glutarimides 1–5 the order of decreasing toxicity of these compounds as judged