Antileukemic Activity of Derivatives of 1,2-Dimethyl-3,4-bis(hydroxymethyl)-5-phenylpyrrole Bis(*N*-methylcarbamate)[1]

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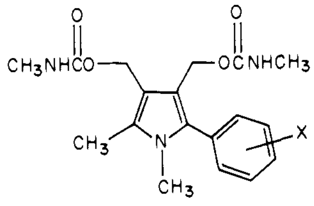
A series of phenyl-substituted derivatives of 1,2-dimethyl-3,4-bis(hydroxymethyl)-5-phenylpyrrole bis(*N*-methylcarbamate) (1) were synthesized and tested for antileukemic activity against P388 lymphocytic leukemia in the mouse. All of the compounds tested, 1a–r, showed significant activity in this assay. Selected derivatives of 1 were tested against several bacteria and were found to have little or no antibacterial activity in the systems examined.

We recently reported the synthesis and antineoplastic activity of 5-substituted 2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine diesters[2] and derivatives of 1-phenyl-2,5-dimethyl-3,4-bis(hydroxymethyl)pyrrole bis(*N*-methylcarbamates).[3] The impressive levels of activity shown by selected agents from these two groups of compounds[4] against a variety of solid tumors in mice prompted us to study this chemical class in some more detail.

A series of 1,2-dimethyl-3,4-bis(hydroxymethyl)-5-phenylpyrrole bis(*N*-methylcarbamate) derivatives, 1, were chosen as the subject of this study. The substituents, X, on the phenyl ring of 1 were chosen on the basis of a heirarchial cluster analysis method.[5] The correlation matrix of the prepared compounds in the parameters π, $\mathcal{F}$, $\mathcal{R}$, and MR shows no significant interparameter correlation.



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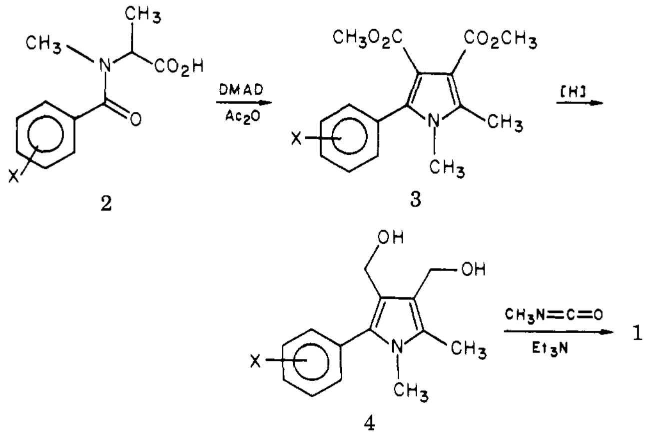
Chemistry. The synthesis of compounds **1a**–**q** is outlined in Scheme I; yields, recrystallization solvents, melting points, and, where appropriate, reaction condition variables are given in Table I. The *N*-aroyl-*N*-methylalanines **2a**–**q** were prepared by N-acylation of *N*-methylalanine (obtained from the reaction of 2-chloropropionic acid and methylamine)[7] with the appropriate benzoyl chloride; the benzoyl chlorides which were not commercially available were prepared by standard methods. The benzoylation reactions were conducted in aqueous sodium hydroxide solution; acid chloride hydrolysis was minimized by the use of cold temperatures and slow addition of the acid chloride.

Treatment of crude **2** with excess dimethyl acetylenedicarboxylate (DMAD) in acetic anhydride gave the 1,3-dipolar cycloaddition products, pyrroles **3a**–**q**. Lithium aluminum hydride reduction of **3a**–**p** afforded the diols **4a**–**p**; **4q** was prepared from **3q** by reduction with aluminum hydride. The bis(*N*-methylcarbamates) **1a**–**q** were prepared from the corresponding diol by treatment with methyl isocyanate in dichloromethane containing a catalytic amount of triethylamine. The bis(*N*-methylcarbamate) **1r** (X = 4′-NH_2) was prepared by the catalytic hydrogenation (PtO_2; 50 psi of hydrogen) of **1q** (X = 4′-NO_2).

We had initially selected several derivatives of **1r** to be prepared; however, **1r** was quite unstable, and this property was exacerbated by reaction conditions which were used in attempts to derivatize the free amino group.

Scheme I



X (at the 4′ position unless otherwise specified) = a, H; b, CH_3; c, $C(CH_3)_3$; d, Ph; e, F; f, Cl; g, Br; h, OCH_3; i, O-*n*-C_4H_9; j, SCH_3; k, SO_2CH_3; m, SPh; n, SO_2Ph; p, 3′,4′-Cl_2; q, NO_2; r, NH_2; s, $NHCONHCH_3$; t, $NHSO_2Ph$; u, $NHCOCH_3$; v, NHC_2H_5

The stable amino derivative **3r** was prepared from the nitro compound **3q** by catalytic hydrogenation; **3r** was subsequently converted to several amino derivatives, including **3s** (X = 4′-$NHCONHCH_3$), **3t** (X = 4′-$NHSO_2Ph$), and **3u** (X = 4′-$NHCOCH_3$). Lithium aluminum hydride reduction of **3s** gave an inseparable mixture of products, while LAH reduction of **3t** and **3u** gave the overreduced 1,2,3,4-tetramethyl derivatives. Calcium or aluminum borohydride reductions afforded complex mixtures. Lithium di-*tert*-butoxyaluminum dihydride affected hydrolysis of the sulfonamide moiety in **3t** with concomitant reduction to the unstable amino diol **4r**; reduction of **3u** with this agent gave the unstable secondary amino compound **4v** (X = 4′-NHC_2H_5). The diol **4r** was very unstable and all attempts to purify this intermediate or to convert it to the bis(*N*-methylcarbamate) derivative **1r** were frustrated by excessive decomposition.

Biological Activity and Discussion. The activity of **1a**–**r** against P388 lymphocytic leukemia (PS) is given in Table II. All of the compounds tested showed significant reproducible activity in the PS assay. A plot of dose–response relationships (log dose vs. percent T/C) for **1a**–**r** shows most of the curves in a cluster, with the amino derivative **1r** standing out as the least active compound. The differences in activity between most of the compounds in the series were not sufficiently large to allow meaningful quantitative structure–activity relationship studies to be conducted. Furthermore, the variation in the data (often more than 10% in T/C) from test to test makes some of the differences between compounds insignificant.

Five selected compounds were also tested for antibacterial activity by means of the disk sensitivity assay.

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Table I

no.	anal.	% yield	recrystn solv	mp, °C	reaction time; temp
2a		76	CH_2Cl_2-Et_2O	131-133[a]	1 h
2b		86	EtOAc-Et_2O	152-154	1 h
2c		83	EtOAc	170-171	1 h
2d		86	EtOAc	167-169	1 h
2e		85	EtOAc-pet. ether	80-82	1.5 h
2f		67	Et_2O	87-88	1.5 h
2g		96	EtOAc-pet. ether	110-112	1.5 h
2h		81	CH_2Cl_2-Et_2O	117-118	1 h
2i		99	CH_2Cl_2-pet. ether	129-131	1 h
2j		87	EtOAc	142-144	2.5 h
2k		97	EtOAc	171-173	2.5 h
2m		82	EtOAc	128-130	3 h
2n		85	EtOAc-pet. ether	156-158	2 h
2p		52	CH_2Cl_2-Et_2O	137-138	1.5 h
2q		65	EtOAc	139-141	4 h
3a	C, H, N	85	MeOH	93-94[b]	90 °C
3b	C, H, N	85	MeOH	108-109	60 °C
3c	C, H, N	93	MeOH	150-151	50 °C
3d	C, H, N	96	95% EtOH	140-141	70 °C
3e	C, H, N	96	MeOH	115-116	50 °C
3f	C, H, N	100	MeOH	117-118	55 °C
3g	C, H, N	93	MeOH	132-133	60 °C
3h	C, H, N	91	MeOH	112-113	80 °C
3i	C, H, N	89	MeOH-H_2O	93.5-94.5	60 °C
3j	C, H, N	94	MeOH	144-145	70 °C
3k	C, H, N	75	MeOH	168-169	80 °C
3m	C, H, N	90	MeOH	100-101	60 °C
3n	C, H, N	91	95% EtOH	217-218	70 °C
3p	C, H, N	82	MeOH	104-105	90 °C
3q	C, H, N	99	MeOH	159-160	40 °C
4a	C, H, N	90	CH_2Cl_2-pet. ether	91-92[c]	
4b	C, H, N	96	EtOAc-pet. ether	109-110[c]	
4c	C, H, N	93	EtOAc-pet. ether	177-178[c]	
4d	C, H, N	76	$CHCl_3$-EtOAc	182-183[c]	
4e	C, H, N	91	EtOAc-pet. ether	121-122[c]	
4f	C, H, N	67	CH_2Cl_2-pet. ether	143-144[c]	
4g	C, H, N	88	EtOAc-pet. ether	155-156[c]	
4h	C, H, N	97	CH_2Cl_2-pet. ether	109-110[c]	
4i	C, H, N	90	EtOAc-pet. ether	117-118[c]	
4j	C, H, N	78	EtOAc-pet. ether	146-147[c]	
4k	C, H, N	85	$CHCl_3$-pet. ether	165-166[c]	
4m	C, H, N	96	EtOAc-pet. ether	115-116[c]	
4n	C, H, N	91	EtOAc-pet. ether	153-154[c]	
4p	C, H, N	91	CH_2Cl_2-pet ether	140-141[c]	
4q	C, H, N	91[d]	EtOAc	129.5-130.5[c]	
1a	C, H, N	73	EtOAc-(*i*-$Pr)_2O$	144-145[c]	
1b	C, H, N	74	EtOAc	169-170[c]	
1c	C, H, N	78	EtOAc-(*i*-$Pr)_2O$	192-193[c]	
1d	C, H, N	89	EtOAc	175-176[c]	
1e	C, H N	78	EtOAc	166-167[c]	
1f	C, H, N	87	EtOAc	181-182[c]	
1g	C, H, N	91	EtOAc	196-197[c]	
1h	C, H, N	80	EtOAc-(*i*-$Pr)_2O$	164-165[c]	
1i	C, H, N	82	EtOAc-(*i*-$Pr)_2O$	163-164[c]	
1j	C, H, N	89	$CHCl_3$-EtOAc	192-193[c]	
1k	C, H; N[f,h]	88	CH_2Cl_2-EtOAc	171-172[c]	
1m	C, H, N	92	EtOAc-(*i*-$Pr)_2O$	181-182[c]	
1n	C, H, N	91	CH_2Cl_2-EtOAc	190-191[c]	
1p	C, H, N	88	EtOAc-(*i*-$Pr)_2O$	125-126[c]	
1q	C, H, N	95	CH_2Cl_2-*i*-PrOH	188-189[c]	
1r	H; C, N[g,h]	89[e]	EtOAc-(*i*-$Pr)_2O$	114-115[c]	

[a] Lit. mp 129-129.5 °C; ref 8. [b] Lit. mp 94-95 °C; ref 9. [c] The sample slowly decomposed over a wide temperature range, the melting point values were obtained by introducing the sample tube into a heated oil bath and recording the temperature required to produce melting in 5 s. [d] Obtained from an AlH_3 reduction. [e] Obtained from a catalytic (PtO_2) hydrogenation. [f] N: calcd, 9.92; found, 9.10. [g] Calcd: C, 59.49; N, 15.55. Found: C, 58.63; N, 14.16. [h] The analytical samples contained quantities of ethyl acetate which could not be removed even under prolonged evacuation.

Compounds 1e (X = 4′-F) and 1h (X = 4′-OCH_3) exhibited marginal activity against *Staphylococcus aureus* and *S. epidermidis*; **1p** (X = 3′,4′-Cl_2) showed marginal activity against *S. epidermidis*, while **1c** [X = 4′-$C(CH_3)_3$] and **1q** (X = 4′-NO_2) showed no activity against the panel of eight microorganisms used in this assay. The paucity of antimicrobial activity shown by 1 is surprising in view of the potent antibacterial activity exhibited by mitomycin C[10] and by other alkylating agents[11] as part of their general cytotoxic profile.

From the preceding discussions it is clear that the electronic and lipophilic character of the substituent X in 1 can be varied rather extensively without loss of significant antileukemic activity. Whether this is due to reduced

Table II. Antileukemic Activity (P388 Lymphocytic Leukemia) of Derivatives of 1[a,b]

no.	dose, mg/kg[c]	toxicity day survivors[d]	animal wt loss (T − C)	% T/C	no.	dose, mg/kg[c]	toxicity day survivors[d]	animal wt loss (T − C)	% T/C
1a[e]	100	5/6	−6.0	<70	1i[g]	120	3/6	−3.4	
	50	6/6	−4.6	90		60	5/6	−4.1	90
	25	6/6	−3.9	145		30	6/6	−2.8	144
	12.5	6/6	−3.3	130		15	6/6	−2.1	146
	6.25	6/6	−2.5	135		7.5	6/6	−1.3	137
1b[f]	100	0/6	−1.8		1j[g]	50	5/6	−2.9	150
	50	3/6	−4.5			25	6/6	−3.1	144
	25	6/6	−3.4	144		12.5	6/6	−4.3	144
	12.5	6/6	−3.2	144		6.25	6/6	−1.4	143
	6.25	6/6	−1.4	138		3.12	6/6	−2.1	187
1c[e]	100	1/6	−4.4		1k[g]	200	6/6	−3.7	198
	50	5/6	−5.1	97		100	6/6	−2.1	180
	25	6/6	−4.0	158		50	6/6	−1.1	162
	12.5	6/6	−3.8	155		25	6/6	−0.8	144
	6.25	6/6	−2.0	152		12.5	6/6	−0.4	136
1d[g]	30	6/6	−3.6	144	1m[g]	200	6/6	−3.0	108
	15	6/6	−3.1	153		100	6/6	−3.3	171
	7.5	6/6	−2.2	144		50	6/6	−2.1	162
	3.75	6/6	−1.3	144		25	6/6	−2.1	146
	1.88	6/6	−0.6	135		12.5	6/6	−0.9	142
1e[f]	100	0/6	−1.8		1n[g]	80	6/6	−3.6	153
	50	6/6	−5.1	132		40	6/6	−3.1	153
	25	6/6	−3.9	150		20	6/6	−1.9	146
	12.5	6/6	−4.3	130		10	6/6	−0.9	136
	6.25	6/6	−2.4	134		5	6/6	−1.3	137
1f[e]	100	2/6	−7.5		1p[e]	100	2/6	−5.9	
	50	6/6	−5.7	<80		50	6/6	−3.2	133
	25	6/6	−4.4	165		25	6/6	−2.4	163
	12.5	6/6	−3.2	153		12.5	6/6	−2.4	148
	6.25	6/6	−3.4	155		6.25	6/6	−1.7	145
1g[g]	40	5/6	−5.0	<75	1q[f]	100	1/6	−4.4	
	20	6/6	−2.8	155		50	2/6	−6.6	<75
	10	6/6	−2.8	146		25	6/6	−5.5	<90
	5	6/6	−1.8	144		12.5	6/6	−4.1	169
	2.5	6/6	−1.2	138		6.25	6/6	−2.9	153
1h[e]	100	4/6	−5.5	<70	1r[g]	100	5/6	−3.5	135
	50	6/6	−4.4	152		50	6/6	−2.5	119
	25	6/6	−3.4	145		25	5/6	−0.4	100
	12.5	6/6	−3.2	138		12.5	6/6	−0.1	98
	6.25	6/6	−2.4	135		6.25	6/6	−0.7	92

[a] Determined under the auspices of the National Cancer Institute, National Institutes of Health. For general screening procedures and data interpretation, see R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3(2), 1 (1972). [b] Ascitic fluid containing ca. 6 × 10^6 cells was inoculated (ip route) into male CD$_2$F$_1$ mice; in this assay, median survival times of % T/C $\geq$ 120 are considered significant. [c] The compounds were administered by the ip route in a distilled water–Tween 80 suspension. A total of nine daily doses were given starting 24 h after tumor inoculation. [d] Recorded on the 5th day (i.e., 4 days after the first injection of compound). [e] Control group 1; control animals average body-weight change was +1.9 g. [f] Control group 2; control animals average body-weight change was +1.8 g. [g] Control group 3; control animals average body-weight change was +1.6 g. Control group 4; control animals average body-weight change was +1.3 g.

coplanarity in the biaryl system or to some other factors is the subject of current investigations.

Experimental Section

Melting points (uncorrected) were determined in an open capillary with a Thomas-Hoover Unimelt apparatus. Infrared spectra were determined for KBr wafers (unless otherwise specified) with a Perkin-Elmer 237 or 227B spectrophotometer. NMR spectra (^{1}H and ^{13}C) were determined for CDCl$_3$ solutions (unless otherwise specified) containing 1% (v/v) tetramethylsilane as an internal standard with a Varian T-60 or FT-80 spectrometer. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, Ga.

General Procedure for the Synthesis of *N*-(Substituted benzoyl)-*N*-methylalanine (2). A solution of the benzoyl chloride (0.21 mol) in dichloromethane (the minimum volume necessary to effect solution) was added dropwise to a vigorously stirred, cold (ice-bath temperature) solution of *N*-methylalanine (20.62 g, 0.20 mol) and sodium hydroxide (16.00 g, 0.40 mol) in water (80 mL). The mixture was stirred at ice-bath temperature for 1 h, the ice bath was removed, and stirring was continued for an additional 1–4 h (the less reactive benzoyl chlorides required more time). The mixture was extracted with dichloromethane, and the aqueous phase was acidified to pH 1 with concentrated aqueous HCl and extracted with ethyl acetate. The ethyl acetate solution was dried (Na$_2$SO$_4$) and concentrated in vacuo. The solid residue was crystallized once. The yields, melting points, crystallization solvents, and reaction times are reported in Table I.

In each instance, the IR and NMR spectra were consistent with the assigned structure. Elemental analyses were not carried out for 2.

General Procedure for the Synthesis of Dimethyl 1,2-Dimethyl-5-(substituted phenyl)pyrrole-3,4-dicarboxylate (3). A solution of 2 (0.10 mol) in acetic anhydride (100 mL) and dimethyl acetylenedicarboxylate (25 mL, 0.20 mol) was stirred in a flask equipped with a reflux condenser and a gas bubbler to monitor CO$_2$ evolution; the mixture was heated to the minimum bath temperature necessary to initiate CO$_2$, evolution and this temperature was maintained for 1 h after gas evolution had stopped. The reaction mixture was concentrated in vacuo and the residue was crystallized twice. The yields, melting points, crystallization solvents, and bath temperature needed to initiate CO$_2$ evolution are reported in Table I.

Dimethyl 1,2-Dimethyl-5-(4′-aminophenyl)pyrrole-3,4-dicarboxylate (3r). A solution of **3q** (25.00 g, of 0.075 mol) in reagent grade acetone (210 mL) containing PtO_2 catalyst (100 mg) was shaken in a Burgess–Parr hydrogenation apparatus under 50 psi of hydrogen for 2.5 h. The mixture was filtered through a Celite bed and the filtrate was concentrated to dryness in vacuo. The solid residue was crystallized twice from methanol to give 22.01 g (97%) of **3r** as small yellow prisms: mp 157–158 °C; IR 3435, 3344, 2953, 1718, 1692, 1539, 1504, 1453, 1295, 1190, 1163, and 1076 cm^{-1}; ^1H NMR δ 2.45 (s, 3 H), 3.28 (s, 3 H), 3.62 (s, 3 H), 3.77 (s, 3 H), 3.82 (s, 2 H; $-NH_2$), 6.61 (d, $|J_{AB}|$ = 8 Hz, 2 H), 7.02 (d, $|J_{AB}|$ = 8 Hz, 2 H). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

Dimethyl 1,2-Dimethyl-5-[4′-(3-methylureido)phenyl]-pyrrole-3,4-dicarboxylate (3s). A solution of **3r** (1.000 g, 0.0033 mol) and triethylamine (0.5 mL) in dichloromethane (50 mL) was treated with methyl isocyanate (1.0 mL, 0.017 mol) and heated at reflux for 4 h. The mixture was concentrated to dryness in vacuo and the solid residue was crystallized twice from methanol to give 0.932 g (78%) of yellow crystals, mp 223–224 °C. Anal. ($C_{18}H_{21}N_3O_5$) C, H, N.

Dimethyl 1,2-Dimethyl-5-[4′-(benzenesulfonamido)-phenyl]pyrrole-3,4-dicarboxylate (3t). A solution of **3r** (20.00 g, 0.066 mol) in glacial acetic acid (50 mL) and benzenesulfonyl chloride (11 mL, 0.086 mol) was heated at reflux for 1 h. Anhydrous sodium acetate (6.0 g, 0.073 mol) was added as 2.00-g portions in 20-min intervals. The hot reaction mixture was filtered and the residue was washed with 95% ethanol. The product, which crystallized from the cooled filtrate, was recrystallized from 95% ethanol to give 21.46 g (73%) of **3t** as yellow crystals: mp 209–211 °C. Anal. ($C_{22}H_{22}N_2O_6S$) C, H, N.

Dimethyl 1,2-Dimethyl-5-(4′-acetamidophenyl)-3,4-dicarboxylate (3u). A solution of **3r** (10.00 g, 0.033 mol) in glacial acetic acid (50 mL) and acetic anhydride (10 mL) was heated at reflux for 2 h. The reaction mixture was concentrated in vacuo and the solid residue was crystallized twice from methanol to give 9.98 g (88%) of yellow crystals, mp 196–197 °C. Anal. ($C_{18}H_{20}N_2O_5$) C, H, N.

General Procedure for the Synthesis of 1,2-Dimethyl-3,4-bis(hydroxymethyl)-5-(substituted phenyl)pyrrole (4). A solution of **3** (0.05 mol) in dichloromethane (125 mL) was added dropwise to a stirred mixture of lithium aluminum hydride (4.00 g, 0.105 mol) in anhydrous ether (200 mL) at 0 °C. The reaction mixture was heated at reflux for 20 min and then cooled in an ice bath. The excess hydride was carefully decomposed by the sequential addition of water (4.0 mL), 15% aqueous NaOH (4.0 mL), and water (12.0 mL). The mixture was filtered and the inorganic residue was washed with boiling ethyl acetate. The filtrate was concentrated in vacuo and the solid residue was crystallized. The yields, melting points, and crystallization solvents are given in Table I.

1,2-Dimethyl-3,4-bis(hydroxymethyl)-5-(4′-nitrophenyl)pyrrole (4q). Concentrated sulfuric acid (6.463 g, 0.066 mol) was added dropwise to a stirred mixture of lithium aluminum hydride (5.000 g, 0.132 mol) in dry tetrahydrofuran (450 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and **3q** (14.00 g, 0.042 mol) was added as a solid in small portions over a 10-min period. The mixture was stirred at 0 °C for 15 min and at room temperature for 1 h. Water (25 mL) was added, the mixture was filtered, and the inorganic residue was washed with boiling ethyl acetate. The filtrate was concentrated in vacuo and the solid residue was crystallized from ethyl acetate to give 10.54 g (91%) of orange crystals: IR 3279, 2915, 2857, 1608, 1520, 1346, 1117, 1014, and 988 cm^{-1}; ^1H NMR δ 2.28 (s, 3 H), 3.18 (s, 2 H; –OH), 3.43 (s, 3 H), 4.43 (s, 2 H), 4.58 (s, 2 H), 7.47 (d, $|J_{AB}|$ = 9 Hz, 2 H), 8.20 (d, $|J_{AB}|$ = 9 Hz, 2 H).

General Procedure for the Synthesis of 1,2-Dimethyl-3,4-bis(hydroxymethyl)-5-(substituted phenyl)pyrrole Bis(*N*-methylcarbamate) (1). A solution of diol **4** (0.02 mol) and triethylamine (0.5 mL) in dichloromethane (65 mL) was treated with methyl isocyanate (8.0 mL, 0.14 mol) and heated at reflux for 12 h. The mixture was concentrated to dryness in vacuo and the solid residue was crystallized twice. Prolonged heating of these compounds, either as solids or in solution, must be avoided. The crystalline product was collected by suction filtration under a nitrogen atmosphere. The yields, melting points, and crystallization solvents are reported in Table I.

1,2-Dimethyl-3,4-bis(hydroxymethyl)-5-(4′-aminophenyl)pyrrole Bis(*N*-methylcarbamate) (1r). A solution of **1q** (2.400 g, 0.006 mol) in reagent grade acetone (75 mL) containing PtO_2 catalyst (0.120 g) was shaken in a Burgess–Parr hydrogenation apparatus for 1 h under 50 psi of hydrogen. The mixture was filtered through a Celite bed and the filtrate was concentrated to dryness in vacuo. The residue was recrystallized twice from ethyl acetate–isopropyl ether to give 1.98 g (89%) of yellow crystals: IR 3335, 2944, 1683, 1538, 1258, 1134, and 958 cm^{-1}; ^1H NMR δ 2.28 (s, 3 H), 2.70 (s, 3 H), 2.78 (s, 3 H), 3.33 (s, 3 H), 3.65 (br s, 2 H, $-NH_2$), 4.65 (br s, 2 H, –NH–), 4.95 (s, 2 H), 5.12 (s, 2 H), 6.69 (d, $|J_{AB}|$ = 9 Hz, 2 H), 7.04 (d, $|J_{AB}|$ = 9 Hz, 2 H).

The antibacterial disk sensitivity assay used eight microorganisms: *Enterobacter cloacae* (ATCC 23355), *Klebsiella pneumoniae* (ATCC 23357), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 14028), *Serratia marcescens* (ATCC 8100), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATC 25922), and *Proteus vulgaris* (ATCC 6380). A lawn was prepared on tripticase soy agar plates using 1 mL of a 24-h growth of the test organisms in tripticase soy broth. Sterile paper disks (6 mm), impregnated with 1 mg of the test compound, were placed on the agar and incubated for 24 h at 37 °C. After incubation, the zone of inhibition around each disk was measured; compounds exhibiting a zone of inhibition less than 7 mm in diameter were considered inactive. Furoxone was used as a positive standard.

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