Synthesis and Antiprotozoal Activity of 2,5-Bis(4-guanylphenyl)thiophenes and -pyrroles

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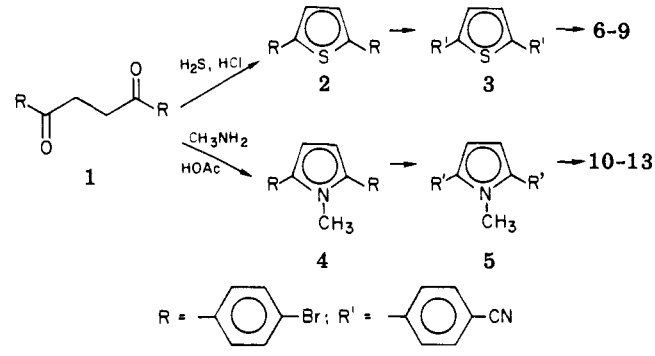
2,5-Bis(4-guanylphenyl)thiophene and 2,5-bis(4-guanylphenyl)-*N*-methylpyrroles and several of their "cyclic amidine" analogues have been synthesized and their antimalarial and antitrypanosomal activity has been assessed. None of these compounds showed significant antimalarial activity; however, all displayed good levels of activity against *Trypanosoma rhodesiense* in mice. 2,5-Bis(4-guanylphenyl)thiophene and 2,5-bis(4-guanylphenyl)-*N*-methylpyrrole produced cures in mice at the ~1 mg/kg dosage level. These two compounds are of comparable activity to stilbamidine, hydroxystilbamidine, and pentamidine in this test. The "cyclic amidines" generally exhibited lower antitrypanosomal activity than their guanyl counterparts. The thiophenes and pyrroles were synthesized by treatment of 1,4-bis(*p*-bromophenyl)-1,4-butanedione with H_2S–HCl or CH_3NH_2–HOAc, respectively, to produce the corresponding 2,5-bis(4-bromophenyl)thiophene and -*N*-methylpyrrole. The dibromophenyl compounds were converted into the corresponding bis-nitriles by reaction with $Cu_2(CN)_2$. The latter compounds were converted by way of imidate esters to the guanyl and "cyclic guanyl" targets.

In a recent publication we reported potent antitrypanosomal activity for a series of substituted 2,5-bis(4-guanylphenyl)furans and related "cyclic amidines".[1] These furan compounds were envisioned[1] as potential antiprotozoan agents based upon structural analogy with biologically active aryldiamidines[2-4] and upon their potential to interact with DNA as their bioreceptor.[1] In addition to expected antitrypanosomal activity, it was hoped that these compounds might also exhibit antimalarial activity in view of the reported activity of diminazene and pentamidine against *Plasmodium vinckei*.[5] In view of the potent activity observed for the 2,5-bis(4-guanylphenyl)furans, we have prepared and evaluated the analogous thiophenes and *N*-methylpyrroles and the results of these efforts constitute this report.

Chemistry. The synthesis of guanyl and "cyclic guanyl" compounds shown in Table I was prepared by a method very similar to that which has been reported for the preparation of the related furans.[1] Our approach is outlined in Scheme I.

Scheme I



Biological Activity. Compounds **6–13** were screened for antimalarial activity by testing against *Plasmodium berghei* in mice[6] and none of them exhibited any significant activity. These same compounds were tested against *Trypanosoma rhodesiense* in mice by the method of Rane[7] and the results are shown in Table II. Included in Table II are test results for stilbamidine (**14**), hydroxystilbamidine (**15**), pentamidine (**16**), and 2,5-bis(4-guanylphenyl)furan (**17**), a very active compound from our previously described furan series.[1] In light of the results reported here and our previous results,[1] we conclude that "cyclic amidines" exhibit lower orders of antitrypanosomal activity than their true guanyl analogues and that acute toxicity is generally encountered with the cyclic guanyl compounds, particularly at higher dosage levels. The two most active compounds, **6** and **10**, exhibit comparable levels of activity to the standard diamidines, **14–16**, and they are approximately as effective trypanocides as their furan counterparts. The fact that the thiophene, *N*-methylpyrrole, and furan systems show comparable activity suggests that the role of the five-numbered ring heterocycle in these compounds could be nothing more than a relatively inert spacer for the guanylphenyl functions. However, our earlier work on the furan system shows that enhanced activity is found by substitution on the furan ring. These observations were attributed to distribution differences and/or differences in binding to the bioreceptor. A study to determine if binding to DNA (the possible bioreceptor) can be related to the structural variations in these substituted furan, thiophene, and *N*-methylpyrrole systems is underway. Because the activity of the thiophene and pyrrole derivatives reported here did not surpass that of their furan analogues, a study of the effect of substitution on these heterocyclic ring systems was not undertaken.

Experimental Section

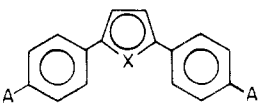
Melting points reported under 300 °C were taken on a Thomas-Hoover melting point apparatus; the melting points of compounds melting above 300 °C were obtained on a Mel-Temp apparatus and all melting points are uncorrected. IR spectra were recorded on all new compounds with a Perkin-Elmer Model 337 spectrometer, ^1H NMR spectra were recorded on selected compounds with a Varian A-60A instrument, and ^{13}C NMR spectra on selected compounds were obtained with a JEOL FX-60 instrument. All spectra were in accord with the structures assigned. Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga.

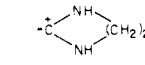
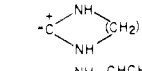
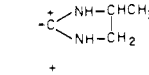
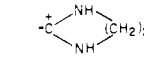
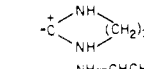
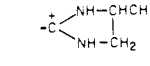
Compounds **6** and **10** were prepared from the appropriate bis-nitrile by the method outlined for preparation of **10**. The "cyclic amidines" **7–9** and **11–13** were prepared by the method given for **11**.

2,5-Bis(4-cyanophenyl)thiophene (3). Dry HCl gas was passed into a suspension of 4.0 g (0.01 mol) of 1,2-bis(4-bromobenzoyl)ethane[1] (**1**) in 160 mL of $CHCl_3$ and 6.0 g (0.02 mol) of $SnCl_4$ for 1 min. Then HCl and H_2S were bubbled through the mixture at approximate rates of 1 and 2 mL per second, respectively, for 1.5 h. The mixture was filtered and the filtrate on evaporation gave (2.0 g, 50%) 2,5-bis(4-bromophenyl)thiophene (**2**); recrystallization from ethanol gave mp 198–199 °C (lit.[8] mp 198–199 °C).

A mixture of 2.0 g (0.005 mol) of **2**, 5.6 g (0.03 mol) of $Cu_2(CN)_2$, and 15 mL of quinoline was refluxed for 2 h. The reaction mixture was cooled and extracted with hot $CHCl_3$, the solvent layer was removed, washed with dilute HCl and with water, and dried, and the solvent was evaporated. The residue was dissolved in a small volume of acetone and passed through a short alumina column to remove traces of copper salts.[1] Evaporation of the eluent and

Table I. 2,5-Bis(4-substituted phenyl)thiophenes and -*N*-methylpyrroles



Compd no.	X	A	Mp, °C[e]	% yield[f]	Mol formula
3[a]	S	CN	288-290	83	$C_{18}H_{10}N_2S$
5[b]	NCH_3	CN	195-198	77	$C_{19}H_{13}N_3$
6[c]	S	$-\overset{+}{C}(NH_2)_2Cl^-$	398-400	40	$C_{18}H_{18}Cl_2N_4S$
7[a]	S		438-440	55	$C_{22}H_{22}Cl_2N_4S$
8[d]	S		440-442	60	$C_{24}H_{26}Cl_2N_4S\cdot 3H_2O$
9[d]	S		394-396	75	$C_{24}H_{26}Cl_2N_4S$
10[d]	NCH_3	$-\overset{+}{C}(NH_2)_2Cl^-$	370-372	62	$C_{19}H_{21}Cl_2N_5\cdot H_2O$
11[d]	NCH_3		405-407	60	$C_{23}H_{25}Cl_2N_5$
12[d]	NCH_3		426-428	70	$C_{25}H_{29}Cl_2N_5$
13[d]	NCH_3		393-395	50	$C_{25}H_{29}Cl_2N_5$

[a] Recrystallized from dioxane; analyzed for C and H and the results were within ±0.4% of the calculated values. [b] Recrystallized from EtOH-CH_2Cl_2; analyzed for C, H, and N and the results were within ±0.4% of the calculated values. [c] Recrystallized from absolute ethanol; analyzed for C, H, and S and the results were within ±0.4% of the calculated values. [d] Recrystallized from absolute ethanol; analyzed for C, H, and N and the results were within ±0.4% of the calculated values. [e] All melting points are uncorrected; compounds **6-13** melted with decomposition. [f] The yields for **6-13** are based upon the imidate ester hydrochloride.

Table II. 2,5-Bis(4-guanylphenyl)thiophenes and -*N*-methylpyrroles[a]

	Cures[b] or ΔMST[c] at dosage[d] (mg/kg)									
No.	1.25	2.5	5	10	20	40	80	160	320	640
6	2	3	4	5	5	5	5	5		
7								1.6 D	2	3
8				2	3	4	4	4	5	5
9					1.0 D	1.2 D	3.0 D	2	4	4
10	1	2	4	5	5	5	5	5	5	
11				2	3	4	4	5		
12				2	3	5	5	3	T[e]	T
13					3	4	5	5	5	5
14[f]	2	5	5	5	5	5	5	5	5	5
15[g]	2	5	5	5	5	5	5	5	5	5
16[h]	1	4	5	5	5	5	5	5	5	5
17[i]	4	4	5	5	5	5	5	5	5	5

[a] See ref 7. [b] A cure is defined as a 30-day increase in survival time of the treated animals over the controls. Five mice were used per dosage level; hence, five is the maximum number of cures. [c] ΔMST is the increase in mean survival time of test animals vs. controls in days. Δ MST is differentiated from cures by the use of D, i.e., 1.6 D = 1.6 days. [d] Dosage is in milligrams of compound per kilogram of body weight of the test animal. [e] T = toxic death. [f] Stilbamidine. [g] Hydroxystilbamidine. [h] Pentamidine. [i] 2,5-Bis(guanylphenyl)furan.

recrystallization of the solid from dioxane gave 1.2 g (83%) melting at 288–290 °C.

1-Methyl-2,5-bis(4-cyanophenyl)pyrrole (5). A 36% methylamine solution (70 mL) was extracted with ether; the ether layer was added to 200 mL of acetic acid and the mixture was warmed on a steam bath to remove the ether. 1,2-Bis(4-bromobenzoyl)ethane[1] (12.6 g, 0.032 mol) was added to the CH_3NH_2–HOAc solution and the mixture was refluxed 12 h. On cooling to room temperature a solid appeared which was filtered, washed with H_2O, and dried to yield 9.6 g (62%), mp 197–198 °C. This material, whose ^1H NMR was consistent with 1-methyl-2,5-bis(4-bromophenyl)pyrrole (4), was used directly in the next step. A mixture of 7.8 g (0.02 mol) of **4**, 4.0 g (0.022 mol) of $Cu_2(CN)_2$, and 25 mL of quinoline was refluxed for 2 h. The reaction mixture was worked up as described above for **3**, and 4.4 g (77%) of **5**, mp of 195–198 °C after recrystallization from EtOH–CH_2Cl_2, was obtained.

1-Methyl-2,5-bis(4-guanylphenyl)pyrrole (10). A solution of 5.0 g (0.018 mol) of **5**, 100 mL of dioxane, and 25 mL of absolute EtOH was saturated with dry HCl gas at 5 °C. The solution was shaken in a pressure bottle at room temperature for 3 days. The imidate ester hydrochloride (7.5 g, 93%) was obtained by reducing the volume of solvent and allowing the solid to crystallize. That the nitrile group had reacted was confirmed by the IR spectra and the imidate ester was dried in vacuo at room temperature overnight. The dry imidate ester hydrochloride (2.0 g, 0.0044 mol) was suspended in 100 mL of absolute EtOH in a pressure bottle and the mixture was saturated with anhydrous NH_3 gas at 5 °C. The ammonical mixture was shaken for 3 days at room temperature and the solvent volume was reduced under vacuum. The solid obtained by filtration was washed with ether and dried in vacuo to yield 1.3 g (62%). Recrystallization from absolute ethanol acidified with anhydrous HCl gas gave yellow crystals, mp 370–372 °C dec.

2,2′-[1-Methyl-2,5-pyrrolediyl)-*p*-phenylene]di-2-imidazoline (11). A mixture of the imidate ester hydrochloride (2.2 g, 0.049 mol), ethylene diamine (0.6 g, 0.01 mol), and 25 mL of absolute ethanol was refluxed for 18 h. The solid which formed

was filtered and recrystallized from absolute ethanol acidified with anhydrous HCl gas to give 1.3 g (60%), mp 405–407 °C dec.

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References and Notes

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Effect of the Solvent-Dependent Conformational System of Hydroxyureas on Predicted vs. Observed Log *P*[1]

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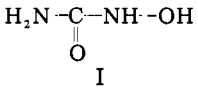
Calculated and observed log *P* values are reported and compared with in vivo and in vitro biological action (L1210 leukemia ILS % and ribonucleotide reductase ID_{50}) for hydroxyurea, the 1-N methyl and ethyl, and the 3-N ethyl, *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, phenyl, and *p*-chlorophenyl analogues. The log *P* values were calculated via the method of Hansch and Leo from literature *f* values and the observed log *P* values were obtained by direct determination after equilibration between octanol and water. Calculations of log *P* for hydroxyurea were found to be appreciably more hydrophilic than the values obtained experimentally. Differences in calculated and observed log *P* (Δ log *P*) for the substituted analogues were lowest with the 1-N and the bulky 3-N substituents and greatest with the 3-N-substituted straight-chain analogues (Δ log *P* = 0.70). Different structural species were observed by infrared spectroscopy in dry octanol vs. octanol after water equilibration and drying, and this is proposed as due to changes in conformational equilibrium in the hydroxyurea systems. Differences between calculated and observed log *P* are explained via the stabilization of internally hydrogen-bonded conformers in the case of 1-N or bulky 3-N analogues or destabilization of various conformers allowing maximal interactions with solvent or water which is the case with straight chain 3-N analogues.

Hydroxyurea (I), a clinically effective antileukemia agent,[2] is a unique drug since molecular modification has not produced an analogue with superior biological action as evidenced by the in vivo L1210 activity of substituted analogues (summarized in Table I).[3]



I

The activity of hydroxyurea has been attributed to its ability to inhibit the enzyme ribonucleoside diphosphate reductase[4a] (RDR), and comparison of the in vitro inhibition of this enzyme from Novikoff hepatoma by selected substituted hydroxyurea[4b] (Table I) with the in vivo data indicates inhibitory ability in some cases at concentrations in the general range observed with hydroxyurea by all of the compounds tested (ID_{50} values). The relative importance of drug transportability, metabolizability, availability, and dynamics at the site of action for this drug class at present is not known. In this paper we wish to report discrepancies between calculated and observed log *P* values for hydroxyurea and some of its substituted analogues and an explanation for these differences due to solvent-dependent conformational preferences of various analogues which may have an influence on biological action in vivo.

The transport of hydroxyurea molecules to their site of action involves passage through membranes and the adsorption and desorption to macromolecules in vivo. The in vivo transportability of drug molecules can be evaluated via the partition coefficient in solvents such as octanol–water[5] (log *P*) which measures a drug's relative affinity toward lipophilic and hydrophilic phases. Log *P* values have been measured for many drugs[6] and methods have been developed by Hansch et al.,[7] whereby the log *P* value for a particular drug can be calculated since log *P* has been shown to be an additive–constitutive property of organic compounds.[8] Log *P* can be calculated by adding the fragment values (*f*) of the component functional groups according to eq 1.[9]

$$\log P = \sum_{1}^{\eta} a_\eta f_\eta \qquad (1)$$

In calculating a log *P* for hydroxyurea, the proximity effect of groups which can hydrogen bond must be taken into consideration and generally the *f* value of the most negative fragment is used. Thus calculated log *P* is −2.71 or −2.18 depending on the fragments used to make hydroxyurea (eq 2 and 3).

$$\underset{-2.71}{f(\text{-CONH})} + \underset{-1.54}{f(\text{-NH}_2)} + \underset{-1.64}{f(\text{-OH})} = \log P = -2.71 \qquad (2)$$

$$\underset{-2.18}{f(\text{-CONH}_2)} + \underset{-2.11}{f(\text{-NH-})} + \underset{-1.64}{f(\text{-OH})} = \log P = -2.18 \qquad (3)$$

When these calculated log *P* values are compared to the experimentally obtained log *P* for hydroxyurea (Table I) it is apparent that the actual log *P* is more lipophilic than that obtained by calculation from the component functional groups. In order to calculate log *P* values for