^{1}H NMR Spectroscopy Study of the Interaction between Pyrimethamine Hydrochloride and Bovine Serum Albumin

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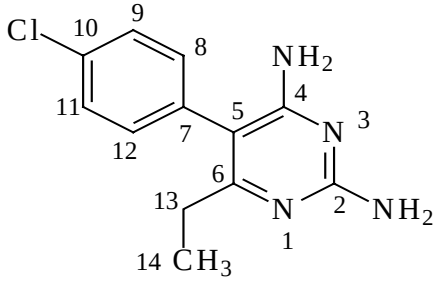
***Abstract**: The effects of the changes in sample concentration on the NMR chemical shifts and on the spin-lattice relaxation times (T_1), were measured in DMSO/D_2O (10% v/v) for pyrimethamine hydrochloride solutions, both free and in the presence of Bovine Serum Albumin (BSA). The results were used to determine the topology and degree of interaction of this anti-malarial drug with BSA.*

***Resumo**: Os efeitos da variação de concentração da amostra, nos deslocamentos químicos e tempos de relaxação spin-rede (T_1), foram medidos em solução de D_2O contendo 10% v/v de DMSO-d_6 para o cloridrato de pirimetamina, livre e em presença da Albumina do Soro bovino (BSA). Os resultados foram usados para determinar a topologia e grau de interação desta droga antimalarial com BSA.*

Introduction

Malaria is one of the major public health problems in Brazil, with 450,000 cases and approximately 10,000 deaths reported annually. The Amazon region, in which migrant populations, great distances, and poor access to diagnosis and treatment are the major obstacles to malaria control, accounts for more than 95% of the cases in Brazil.[1] The malaria problem is much worsen by the appearance of resistant strains of this illness causative agent, the protozoa *Plasmodium falciparum*. The resistance problem is most intense in dihydrofolate reductase (DHFR) inhibitors, such as pyrimethamine.[2] This drug, which have been most commonly used in combination with structure that can be modified to obtain new analogues without worsening desired transport and permeation properties. An important protein is the Bovine Serum Albumin (BSA), which has as one of its functions the transport of substances in the blood. This work is a study of the interaction between pyrimethamine hydrochloride (**1**),[5] and BSA. The objective of this study was to determine the probable sites for structural modifications of **1** to project analogues with the same ability to be transported in the blood.

Drug-protein systems are well studied through ^{1}H spin-lattice nuclear relaxation times (T_1), a methodology that have been applied to study the sulfadoxime in Brazil[1] and Africa,[3] is now ineffective against most *P. falciparum* infections in South America. Accordingly, the development of new inhibitors for the mutant-type *P. falciparum* DHFR is of outmost importance to fight back the actual world malaria epidemic. Along this line, we are working on the development of a model for the active site of the native and mutant DHFR, using molecular modeling,[4] in order to design and synthesize new antimalarial drugs. Clearly, in the search of new antimalarials, the knowledge of the nature of the interaction between the known drugs used as templates, such as pyrimethamine, with some important proteins is fundamental to determine the sites on the template interactions between small molecules and macromolecules or micelles.[6-8]



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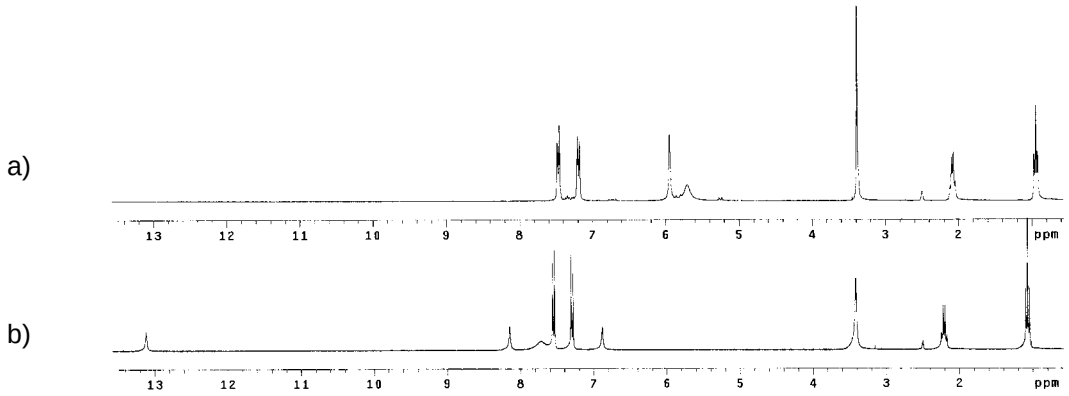

Figure 1. a) ^{1}H NMR spectrum of Pyrimethamine (**1**); b) ^{1}H NMR spectrum of Pyrimethamine Hidrochloride (**2**)

Experimental

In this work, pure pyrimethamine (Roche) was used. The original neutral form of the drug was converted to its hydrochloride by treatment of a water suspension of **1** with conc. HCl, followed by evaporation of the solvent and recrystalization of the crude salt from 95% ethanol. The solutions for the interaction studies were prepared by dissolving the pure salt in D_2O containing 10% of the DMSO-d_6 until the desired concentration (25,50,75 mM) in a 7,25 x 10^{-6} M stock solution of BSA (98% *Aldrich Chemical Company*) was obtained.

NMR measurements were carried out on a Varian Unity-300 (300 MHz) NMR spectrometer. The variable temperature experiments were carried out from 27.0 ^{o}C to 67.0 ^{o}C using temperature increments of 10 ^{o}C. Spin-lattice relaxation rates were measured in triplicate with the inversion-recovery pulse sequence.[6,7] In all the reported relaxation rate results, the relative uncertainly was less than 30%, with most results having uncertainties lower than 10%. The chemical shifts were measured using TMS as internal standard.

Results and Discussion

The ^{1}H NMR spectra of **1** and its salt **2** are given in Figures 1a and 1b, respectively. The assignment of the chemical shifts (δ) with respect to TMS of the 75mM solution in DMSO-d_6 , are presented in Table 1.

It can be observed that the spectral pattern for the amine hydrogens is very different for the free base (**1**) and for its hydrochloride (**2**). The signal at 13.12 ppm indicates that one of those hydrogens suffered a strong deshielding through protonation. The fact that the salt spectrum shows five signals for the amine hydrogens is indicative that the protonation has taken place at one of the pyrimidine ring nitrogens. If protonation takes place at one of the two NH_2 nitrogens, it would be expected that the NH_3^+ group formed would present free rotation through the N-C bond, thus leading to equivalence of the three hydrogens. In this case three N-H signals would be observed, at the most. On the other hand, if the protonation takes place at one of the pyrimidine ring nitrogens, the existence of resonance forms would explain the presence of four signals for the five hydrogens, as shown in Scheme 1.

The appearance of only five labile hydrogens in four different signals can be explained only if the protonation occurs at one of the pyrimidine nitrogens and the charge delocalization involves only one of the two NH_2 groups, thus suggesting that such protonation occurs at N-1. Interestingly, this suggestion have been previously done for pyrimethamine based on molecular modeling studies.[9]

Scheme 1

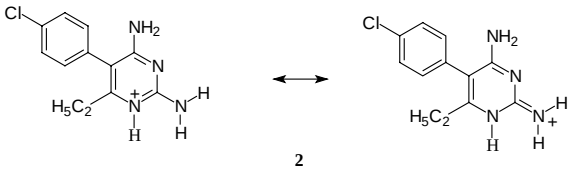

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Table 1

^{1}H and ^{13}C NMR Assignment for Pyrimetamine and its Hydrochloride

Atom Number	Pyrimethamine (**1**)		Pyrimetamine Hydrochloride (**2**)	
	δ ^{1}H (ppm)	δ ^{13}C (ppm)	δ ^{1}H (ppm)	δ ^{13}C (ppm)
2	5.59*	162.1	6.88 8.15	155.0
3	-	-	13.12	-
4	5.87*	166.5	7.70	163.92
5	-	105.3	-	107.0
6	-	135.0	-	133.5
7	-	108.6	-	108.5
8/12	7.20	132.2	7.56	132.0
9/11	7.47	130.0	7.29	129.5
10	-	162.0	-	153.9
13	2.09	27.5	2.21	23.6
14	0.96	13.2	1.04	12.3

*The values can be interchanged

In the study with the free hydrochloride it was determined that variations in sample concentration and temperature did not affect the values of δ ^{1}H, as shown in Figures 2a and 2b.

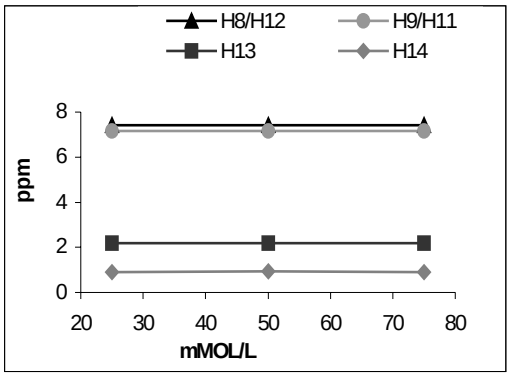

Figure 2a. Effect of concentration on the ^{1}H chemical shifts of free **2**.

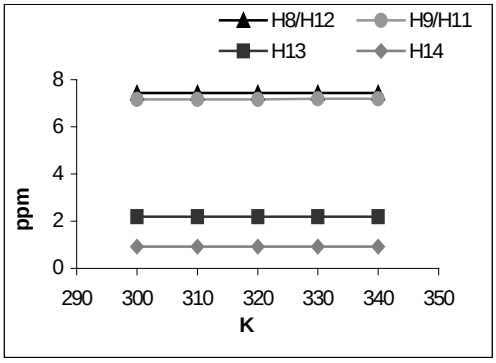

Figure 2b. Effect of temperature on the ^{1}H chemical shifts of free **2**.

On the other hand, the same variations in concentration and temperature led to observable changes in the hydrogen longitudinal relaxation times of the free pyrimethamine hydrochloride. The increase in sample concentration led to decrease of

T_1 while the increase in temperature led to a significant increase of T_1, as shown in Figures 3a and 3b. Although an aggregative behavior is not completely ruled out, we believe that such variations are due to sample viscosity perturbations.

These results indicate that in D_20 there are not important drug-drug interaction effects. On the other hand, in the study of the intermolecular interaction drug-BSA, the presence of BSA led to a considerable decrease in the values of T_1. This fact could be observed through the results of Table 2.

The data on Table 2 show that the interaction between the salt of pyrimethamine with BSA is relatively weak but strong enough to guarantee its efficient transport in the blood. Still, it can be observed that the interaction occurs preferentially through the benzene-type aromatic ring of **2**. It seems that the participation of the ethyl group hydrogens in the Pyrimethamine-BSA interaction is of secondary importance. These results show the same tendency at the other tested concentrations, as it is possible to observe in Figure 4. Also, a similar trend is observed at other temperatures.

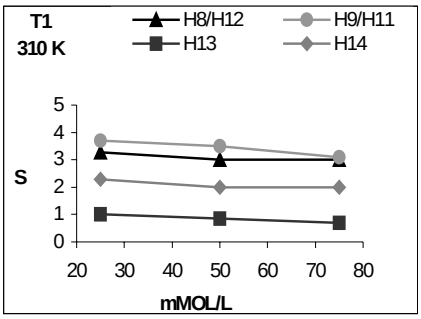

Figure 3a. Effect of concentration on T_1 of free **2**

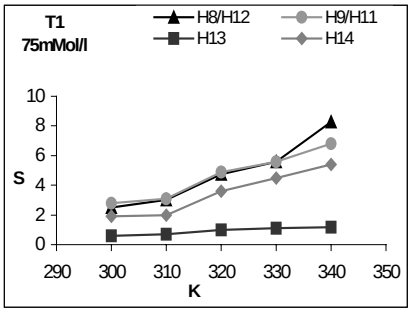

Figure 3b. Effect of temperature on T_1 of free **2**

Table 2

ΔT_1 values induced on the hydrogens of **2** (75mMOL/L) by the presence BSA at 310K*

Hydrogen	T_1 (**2**, s)	T_1 (**2** + BSA, s)	ΔT_1 (s)
H8/H12	3.0	1.6	-1.4
H9/H11	3.1	2.1	-1.0
H13	0.70	0.57	-0.13
H14	2.0	1.51	-0.49

*All values are in seconds.

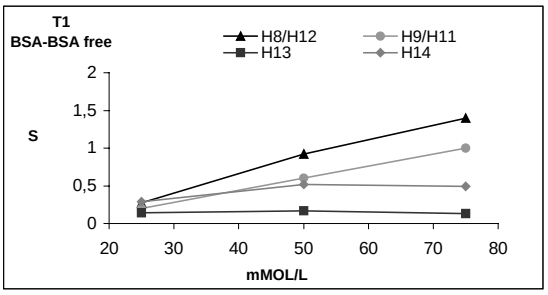

Figure 4. Effect of the changes in concentration of **2** in the variation of T_1in the presence of BSA.

Conclusion

Spin-lattice relaxation rates measurements are sensitive and convenient for the investigation of the topology of binding of small molecules to macromolecules. Our results indicate that pyrimethamine is able to establish intermolecular interactions with BSA, suggesting that other new 2,4-diaminopyrimidines may behave similarly if an aromatic ring is maintained at C-5 of the pyridine ring. Our results suggest that small structural modifications at the ethyl side chain of compound **1** could be carried out without compromising the interaction with albumin.

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References

1. Vasconcellos, K. F.; Plowe, C. V.; Fontes, C. J.; Kyle, D., Wirth, D. F.; Pereira da Silva, L. H.;
2. Zalis, M. G., *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, **2000**, 95(5), 721-728.
3. Sirawaraporn, W.; Sathitkul, T.; Sirawaraporn, R.; Yuthavong, Y.; Santi, D.V., *Biochemistry*, **1997**, 94, 1124-1129.
4. *J. Infections Diseases*, **2000**, 181, 2023.
5. Santos-Filho, O. A.; Alencastro, R. B.; Figueroa-Villar, J. D., *Biophys. Chem.*, **2001**, 91, 305-317.
6. *Prog. Nucl. Magn. Reson. Spectrosc.*, **1998**, 33, 105-151.
7. Tinoco, L. W.; Figueroa-Villar, J. D., *J. Braz. Chem. Soc.*, **1999**, 10(4), 281-28
8. *Spectrosc. Lett.*, **1999**, 32(6), 941-952
9. Borges, M. N.; Figueroa-Villar, J. D., *Biopolymers*, **2001**, 62(1), 9-14.
10. Warhurst, D. C., *Drug Discovery Today*, **1998**, 3(12), 538-546.