COMPARATIVE STUDY OF THE INTERACTIONS OF AMPHOTERICIN B WITH CHOLESTERYL / STIGMASTERYL TRIFLUOROMETHYLPHENYL-CARBAMATE

Loredana Elena Vîjan
University of Pitesti, Targu din Vale No. 1, Pitesti, 110040, Romania
E-mail: loredana.vijan@upit.ro

Abstract

The binding of polyene antibiotic amphotericin B to cholesteryl trifluoromethylphenyl-carbamate, respectively stigmasteryl trifluoromethylphenyl-carbamate was studied using UV-Vis absorption spectroscopy. It is supposed a 1:1 binding ratio and does not take into account explicitly for either the dimerization of the drug or cooperativity effects on the binding. The binding constants have rationalized in terms of methods Benesi-Hildebrand, Scott and Scatchard.

Keywords: amphotericin B (AmB), cholesteryl trifluoromethylphenyl-carbamate (Ch-CF3), stigmasteryl trifluoromethylphenyl-carbamate (Stg-CF3), UV-Vis absorption spectroscopy

INTRODUCTION

Amphotericin B (fungilin, fungizone, abelcet, fungisome, amphocil, amphotec) is a polyene antifungal drug, often used intravenously for systemic fungal infections. It was originally extracted from *Streptomyces nodosus*, a filamentous bacterium, in 1955 at the Squibb Institute for Medical Research from cultures of an streptomycete isolated from the soil collected in the Orinoco River region of Venezuela [1]. Its name originates from the chemical's amphoteric properties. Amphotericin B is available as cholesteryl sulfate complex, as lipid complex and as liposomal formulation.
As with other polyene antifungals, amphotericin B associates with ergosterol from the membrane of fungi, forming a pore that leads to K^+ leakage and fungal cell death. However, researchers have found evidence that pore formation is not necessarily linked to cell death and that the mechanism of action is more complex. Amphotericin B is believed to interact with membrane sterols (ergosterol) to produce an aggregate that forms a transmembrane channel. Intermolecular hydrogen bonding interactions among hydroxyl, carboxyl and amino groups stabilize the channel in its open form, destroying activity and allowing the cytoplasmic contents to leak out [2,3].
The purpose of the present work is to analyse the interaction between amphotericin B (AmB) and two compounds derived from cholesterol/ stigmasterol with a bulky substituent at C-3 sterolic and trifluoromethylphenyl moiety. The chemical structures of the compounds used are shown in Figure 1. In AmB structure (Figure 1a) one may distinguish: (I) polar part containing polar head and polyol chain and (II) hydrophobic part containing chromophore region. Cholesteryl trifluoromethylphenyl-carbamate (Ch-CF_3, Figure 1b) and stigmasteryl trifluoromethylphenyl-carbamate (Stg-CF_3, Figure 1c) were synthesized at University of Pitesti. These two compounds present a bulky polarizable substituent on *metha* position of aromatic substituent at C-3 of steroidic ring.

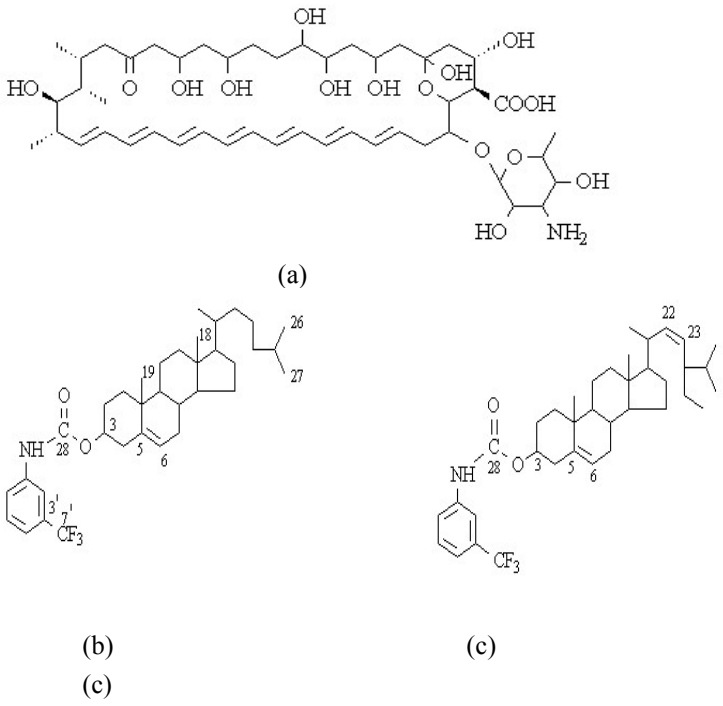


Figure 1. The structure of AmB (a), Ch-CF_3 (b) and Stg-CF_3 (c).

The present study has based of the results obtained from UV-Vis absorption spectroscopy and it has followed the determination of the binding constants from Benesi-Hildebrand, Scott and Scatchard methods, on the assumption of the binding is in 1:1 system.

EXPERIMENTAL

Amphotericin B (AmB) was obtained from Sigma-Aldrich, Germany. The stock solutions of AmB in ethanol were stored at -20^0C in the dark before being used. Their concentrations were determined by absorption spectroscopy using a molar absorption coefficient of $160000 M^{-1}cm^{-1}$ at 407nm [4].

Cholesteryl trifluoromethylphenyl-carbamate (Ch-CF_3) and stigmasteryl trifluoromethylphenyl-carbamate (Stg-CF_3) were obtained from cholesterol (Ch), stigmasterol (Stg) and *α,α,α*–trifluoro-*m*-tolyl isocyanate by method described in literature [5]. All solvents used in synthesis and recrystallizations Ch-CF_3 and Stg-CF_3 were purified by distillation before use. The purity of Ch-CF_3 and Stg-CF_3 was checked by TLC silica gel plates 0.25 mm (Merck) using petroleum ether: ethyl ether 9:1 as eluent mixture. The stock solutions of Ch-CF_3 and Stg-CF_3 were also prepared in ethanol.
The absorption spectra were recorded at the range of 300-500nm, in a Lambda 25 PerkinElmer spectrometer, with quartz cells, at room temperature.

RESULTS AND DISCUSSION

Figure 2 presents the absorption spectra of the AmB solutions. In the range of 300-500nm, one observes five distinct maxima centred at 428, 408, 383, 364 and 310nm. It may be noted that the spectra change gradually with increasing antibiotic concentration.

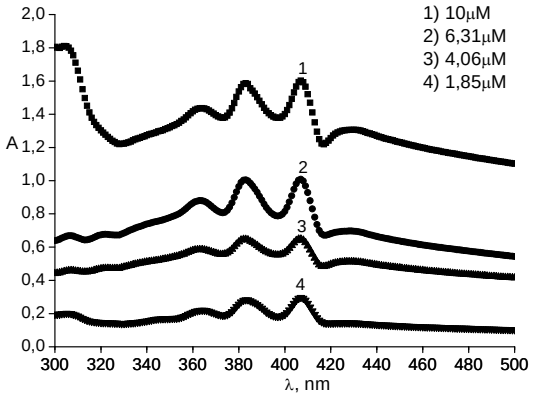


Figure 2. Absorption spectra of AmB solutions, at different concentrations of AmB.

By two methods, the molar absorption coefficient of the monomer $\varepsilon_{408nm}=160000M^{-1}cm^{-1}$ and the dimerization constant $K_d=5000M^{-1}$ were been determined [4].
A family of curves obtained at the titration of AmB solutions with Ch-CF_3 is presented in Figure 3. It may be observed that the AmB – Ch-CF_3 complex is characterized by the decrease of the major bands, at small and medium Ch-CF_3 to AmB concentration ratios (noted *p*). The binding of AmB to Ch-CF_3/ Stg-CF_3 induced a hypochromic effect in the bands at 408, 383 and 364nm. Similar behaviour was observed in the case of the AmB – Stg-CF_3 interaction.

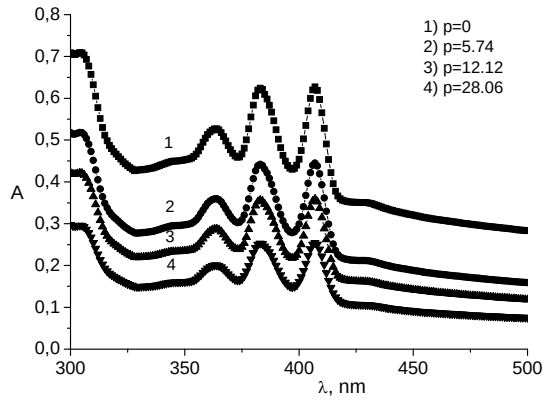
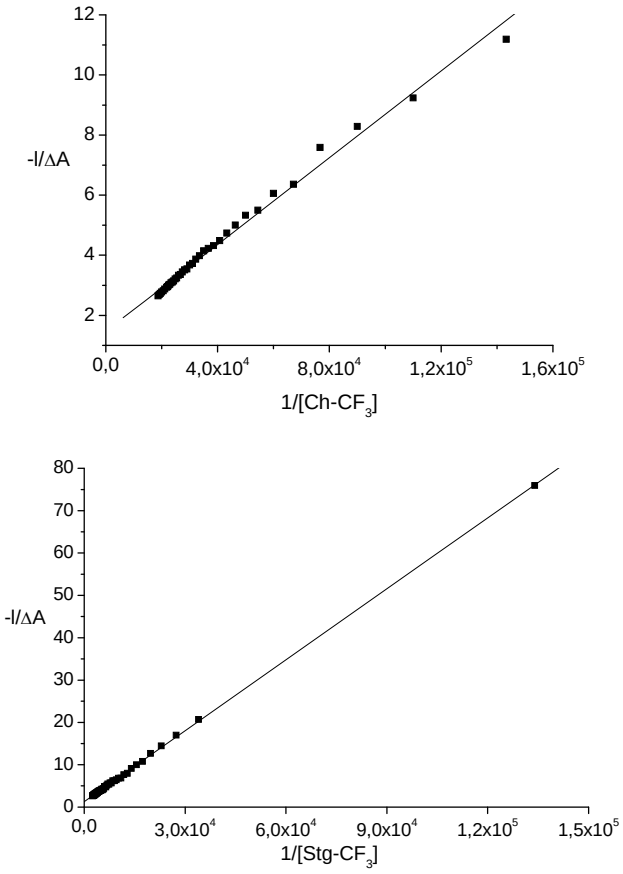


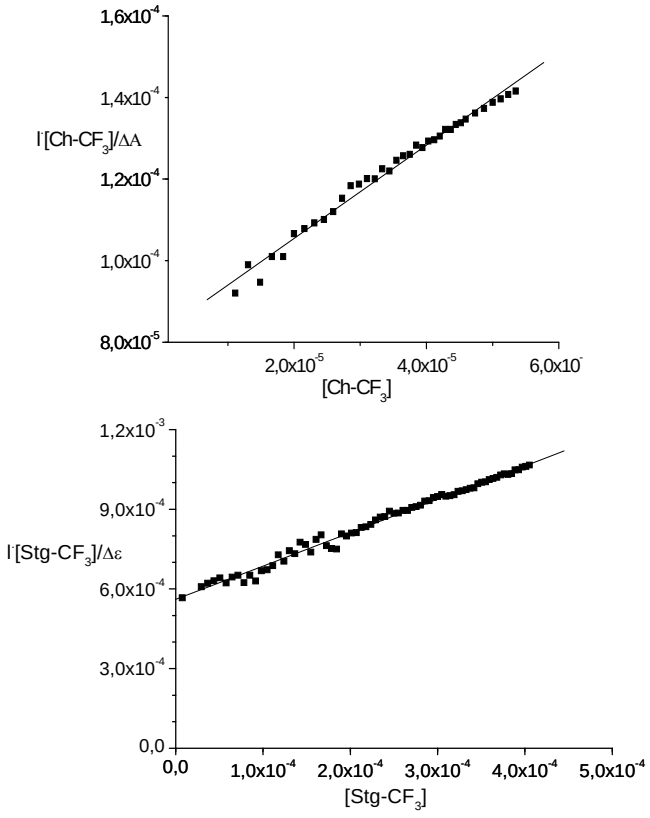
Figure 3. Absorption spectra of AmB in the presence of varying amounts of Ch-CF_3.

In the assumption of the binding AmB to Ch-CF_3/ Stg-CF_3 is in 1:1 system, the binding constants have determined from the methods proposed by Benesi-Hildebrand [6], Scott [7] and Scatchard [8]. In figures 4-6 are presented these plots. The equations utilized and the results obtained are summarized in Table 1.



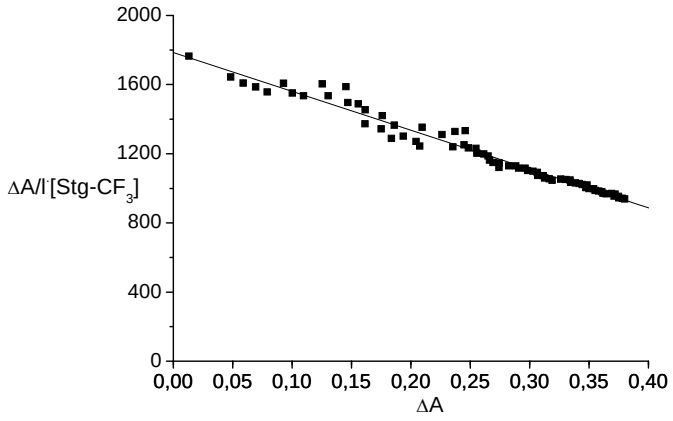
(a) (b)

Figure 4. Benesi-Hildebrand plots for the interaction of AmB with Ch-CF_3 (a), respectively Stg-CF_3 (b).



(a) (b)

Figure 5. Scott plots for the interaction of AmB with Ch-CF_3 (a), respectively Stg-CF_3 (b).



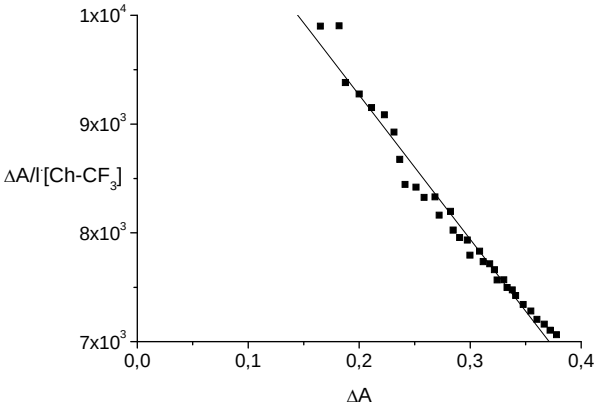
(a) (b)

Figure 6. Scatchard plots for the interaction of AmB with Ch-CF_3 (a), respectively Stg-CF_3 (b).

Table 1. Results of AmB – Ch-CF_3/ Stg-CF_3 interaction

where $\Delta\varepsilon=\varepsilon_B-\varepsilon_F$, ε_F and ε_B are the free and bound drug absorption coefficients, l is path length, ΔA is the observed absorbance change, C^0 is the total concentration of drug and $[Ch\text{-}CF_3]$ is cholesteryl trifluoromethylphenyl-carbamate concentration (concentration in moles per unit volume).

As we can notice, the values for the binding constant of AmB to Ch-CF_3, respectively Stg-CF_3, obtained by the three methods do not differ too much. However, the AmB – Ch-CF_3 system shows higher values for constant binding than the AmB – Stg-CF_3 system.



ACKNOWLEDGEMENTS

It is bring many thanks to Mrs. Carmen Topala for the compounds derived from cholesterol and stigmasterol, which allowed performing spectral analysis.

Method	Equation	K, M^{-1}	
		AmB – Ch-CF_3	AmB – Stg-CF_3
Benesi-Hildebrand	$\frac{1}{\Delta A}=\frac{1}{C^0\cdot K\cdot\Delta\varepsilon}\cdot\frac{1}{[Ch-CF_3]}+\frac{1}{C^0\Delta\varepsilon}$	$1.32\cdot10^4$	$0.49\cdot10^4$
Scott	$\frac{1\cdot[Ch-CF_3]}{\Delta A}=\frac{1}{C^0\cdot\Delta\varepsilon}\cdot[Ch-CF_3]+\frac{1}{C^0\cdot K\cdot\Delta\varepsilon}$	$1.21\cdot10^4$	$0.36\cdot10^4$
Scatchard	$\frac{\Delta A}{1\cdot[Ch-CF_3]}=-\frac{K}{1}\cdot\Delta A+C^0\cdot K\cdot\Delta\varepsilon$	$1.13\cdot10^4$	$0.42\cdot10^4$

REFERENCES

[1] Donovick R., Gold W., Pagano J. F., Stout H.A., Amphotericins A and B, antifungal antibiotics produced by a streptomycete. I. In vitro studies. Antibiot Annu. 1955-1956; 3: 579-586.

[2] Brajtburg J., Powderly W.G., Kobayashi G.S., Medoff G. Amphotericin B, current understanding of mechanism of action. Antimicrob. Agents Chemother. 1990; 34: 183-188.

[3] Bolard J., Legrand P., Heitz F., Cybulska B. One-sided action of amphotericin B on cholesterol-containing membranes is determined by its self-association in the medium. Biochemistry 1991; 30:, 5707-5715.

[4] Vijan L.E., Topala C. The characterizing of the interaction of amphotericin B with cholesteryl trifluoromethylphenyl-carbamate by UV-visible spectroscopy. Rev. Chim. - Bucharest 2008; 59(3): 297-299.

[5] Topala C., Meltzer V., Draghici C. Steryl carbamates mesogens with a trifluoromethylphenyl moiety. Rev. Roum. Chim. 2005; 50: 125-129.

[6] Benesi H., Hildebrand J.H., J. Am. Chem. Soc. 1949; 71: 2703-2707

[7] Scott R.L., Rec. Trav. Chim. 1956; 75: 787-789.

[8] Scatchard G. Ann. N. Y. Acad. Sci. 1949; 51: 660-672.