Modeling of Triazolic Fungicide Inhibitors Binding with Iron-Containing Sterol 14α-Demethylase with the PM6 Semiempirical Method

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Keywords: CYP51, triazole fungicides, PM6 semiempirical model.

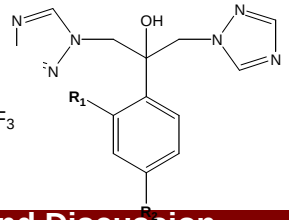
Introduction

Sterol 14α-demethylase (CYP51) catalyses the oxidative removal of the 14α-methyl from lanosterol and eburicol in fungi leading to the $\Delta^{14,15}$-insaturated intermediate in the biosynthesis of ergosterol[1]. CYP51 is an interesting target for the development of compounds with antifungal activity[2]. The most prominent antifungals are members of the triazolic and imidazolic classes of compounds. The enzyme inhibiton is related to the fact that these compounds contain a heterocyclic N atom that is able to coordinate with the heme Fe atom of CYP51.

Molecular modeling studies were implemented with CYP51 models from *Candida* and *Aspergillus*, the fungal pathogens that account for the majority of invasive and opportunistic fungal infections, respectively, occurring in patients with immunodeficiency. The objective of these studies was to identify and quantify the possible interactions between the azole fungicides and the enzyme active site in order to propose more efficient and specific inhibitors [3,4,5]. The previous works were generally based on classic force field models; the objective of the present work is the modeling of CYP51/triazole inhibitors complexes with a new semiempirical molecular orbital method available in Mopac2007, PM6[6], which is expected to better describe the stereoelectronic aspects associated with the complexation of azole fungicides with the Fe atom.

This work was based on the atomic coordinates from the crystallographic structure of *Mycobacterium tuberculosis*[1] CYP51 available in the *Protein Data Bank* (PDB), code 1EA1. A series of five triazole derivatives with IC_{50} values obtained against *C. albicans* CYP51[5] was used in the present work:

1: $R_1 = CF_3$; $R_2 = H$
2: $R_1 = CF_3$; $R_2 = F$
3: $R_1 = OCH_3$; $R_2 = CF_3$
4: $R_1 = H$; $R_2 = CF_3$
5: $R_1 = F$; $R_2 = F$



Results and Discussion

The binding enthalpy between CYP51 and the triazole inhibitors was calculated as the ΔH of the following reaction:

Enzyme-H_2O + Inhibitor ➔ Enzyme-Inhibitor + H_2O,

$$\Delta H_{bin} = \Delta H_{EI} + \Delta H_W - (\Delta H_{EW} + \Delta H_I),$$

where ΔH_{bin} = binding enthalpy between enzyme and inhibitor, ΔH_{EI} = enthalpy of formation of enzyme-inhibitor complex, ΔH_W = enthalpy of formation of water, ΔH_{EW} = enthalpy of formation of enzyme-water complex and ΔH_I = enthalpy of formation of inhibitor. The calculated binding enthalpies together with the available IC_{50} values for *C. albicans* CYP51 are presented on Table 1.

Table 1. Experimental activities and PM6 calculated binding enthalpies of azole fungicides with *C. albicans* CYP51 active site.

Fungicide	IC_{50} (μmol.L^{-1})	ΔH_{bin} (kcal.mol^{-1})
1	103.45	4.60
2	100.88	-1.65
3	28.50	-6.87
4	3.25	-8.67
5	1.97	-10,43

It could be observed that the binding enthalpy is more favorable for compounds with greater potency as a CYP51 inhibitor. In fact, there is a very good correlation between experimental and theoretical data (r = 0.933).

Conclusions

The excellent correlation between theoretical binding enthalpy and the IC_{50} values is indicative that the semiempirical molecular orbital method PM6 is an adequate tool for the modeling of the binding of azole inhibitors to the heme group of CYP51. Together with the fastness of this method as compared to other quantum-chemical methods used in the modeling of metal-containing systems (*e.g.* DFT), this result strongly suggests the use of PM6 in routine evaluation of new CYP51 inhibitors.

Acknowledgements

Faperj, CAPES, CNPq.

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