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Original Research Article

Complexation of Ketoconazole by Native and Modified Cyclodextrins

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Abstract

Complexation of ketoconazole (KET), a broad-spectrum antifungal drug, with β- and γ-cyclodextrins (CDs), heptakis (2,6-di-O-methyl)-β-CD (2,6-DM-β-CD), heptakis (2,3,6-tri-O-methyl)-β-CD (TM-β-CD), 2-hydroxypropyl-β-CD (2HP-β-CD) and carboxymethyl-β-CD (CM-β-CD) was studied. The stability constants were determined by the solubility method at pH = 6 and for 2,6-DM-β-CD and CM-β-CD at pH = 5. At pH = 6, the stability constants increased in the order: TM-β-D < γ-CD < 2HP-β-CD < β-CD < CM-β-CD < 2,6-DM-β-CD. At pH = 5, due to the increased ionization of KET, the stability constant with CM-β-CD increased and with 2,6-DM-β-CD decreased. For complexes of KET with 2HP-β-CD and 2,6-DM-β-CD, the thermodynamic parameters of complexation were determined from the temperature dependence of the corresponding stability constants. For β–γ and TM-β-CD complexes, calculations using HyperChem 6 software by the Amber force field were carried out to gain some insight into the host–guest geometry.

Introduction

Ketoconazole (KET) is a product of substituted imidiazole derivatives (Figure 1) used as an active, broad-spectrum antifungal agent [1–3] possessing also antithyroid activity [4]. It is marketed as a (±)-*cis* racemic mixture. The drug is a weak base with pK value of 6.95 [5], very slightly soluble in water. As it was demonstrated by Carlson *et al.* [6] and Esclusa-Diaz *et al.* [7], the solubility of KET rapidly increases at pH values lower than 4.5.

Cyclodextrins (CDs) are truncated cone-shaped cyclic oligosaccharides built up from six (α-CD), seven (β-CD) and eight (γ-CD) D-glucopyranose units linked by α-glycosidic bonds [8]. The most important property of these molecules is the ability to form host–guest inclusion compounds with a variety of substances [8, 9]. Through the formation of inclusion complexes the physical, chemical and biological properties of guest molecules can be altered. The most common pharmaceutical application of CDs is to enhance the solubility, stability and bioavailability of drug molecules [10–12]. The effect of CDs on drug absorption is largely dependent on magnitude of the stability constant and the dissolution rate of inclusion compound. Besides native CDs, various kinds of CD derivatives have been prepared so as to extend the inclusion capacity as novel drug carriers [12–14]. Among the chemically modified CDs, methylated and hydroxyalkylated CDs have received considerable attention because their physicochemical properties and aqueous solubility are significantly changed and the inclusion behavior is largely magnified, depending on the degree of substitution [15].

The aim of this work was to study the influence of native and modified CDs on complexation of KET by the determination of the corresponding stability constants in the hope to improve some pharmaceutical properties of KET. To gain some insight into the intermolecular interactions accompanying the complexation, we estimated the thermodynamic parameters of complexation and performed the molecular modeling studies using the HyperChem 6 program. Studies were carried out in aqueous solutions containing phosphate buffer at pH = 6 with β-, γ-CD and modified CDs – heptakis (2,6-di-O-methyl)-β-CD (2,6-DM-β-CD), heptakis (2,3,6-tri-O-methyl)-β-CD (TM-β-CD), 2-hydroxypropyl-β-CD (2HP-β-CD), and carboxymethyl-β-CD (CM-β-CD) and at pH = 5 with 2,6-DM-β-CD and CM-β-CD. The stability constants were determined by the solubility method.

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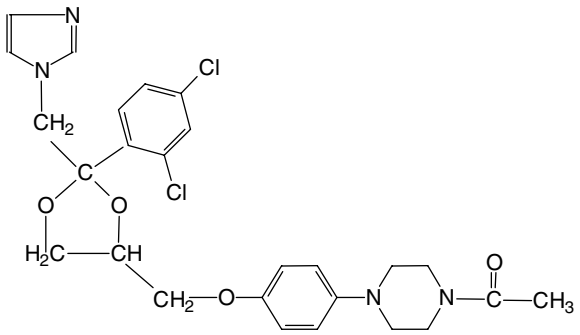

Figure 1. Structure of ketoconazole.

Experimental

Materials

β-CD, 2,6 DM-β-CD (purity > 98%), TM-β-CD, CM-β-CD (average degree of substitution DS $\approx$ 3) and 2HP-β-CD (DS = 2.7) were purchased from Cyclolab (Budapest, Hungary). They were used without additional purification. KET was supplied by Anpharm (Warsaw, Poland) and was used as received. KH_2PO_4 and Na_2HPO_4 salts used for preparation of buffer solutions were of analytical grade (POCh, Gliwice, Poland). Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) distilled additionally from a quartz still was used for solution preparation.

Solubility measurements

Solubility studies were carried out according to the method of Higuchi and Connors [16]. An excess amount of KET was added to 5 cm^{-3} of an aqueous phosphate buffer solutions, pH 6 or 5, containing various concentrations of the studied CDs. The suspensions were shaken in screw-capped vials placed in a water bath at the temperature of the experiment. After the equilibrium had been reached 6–8 days, the contents of each vial were filtered through a Waters Corporation Nylon 0.2 μm pore size filter and the concentration of KET in the filtered solution was measured by UV spectrophotometry at 225 nm using Cary 1 spectrophotometer (Varian) equipped with a thermostatically controlled cell compartment. In the reference cuvette was always the same concentration of CD as in the studied solution. All the data were the average of at least three independent determinations.

Results and discussion

Solubility studies

The phase solubility diagrams of KET in aqueous solutions of all studied CDs obtained at 25 °C at pH = 6 are shown in Figure 2 and in solutions of CM-β-CD and 2,6-DM-β-CD at pH = 5 in Figure 3.

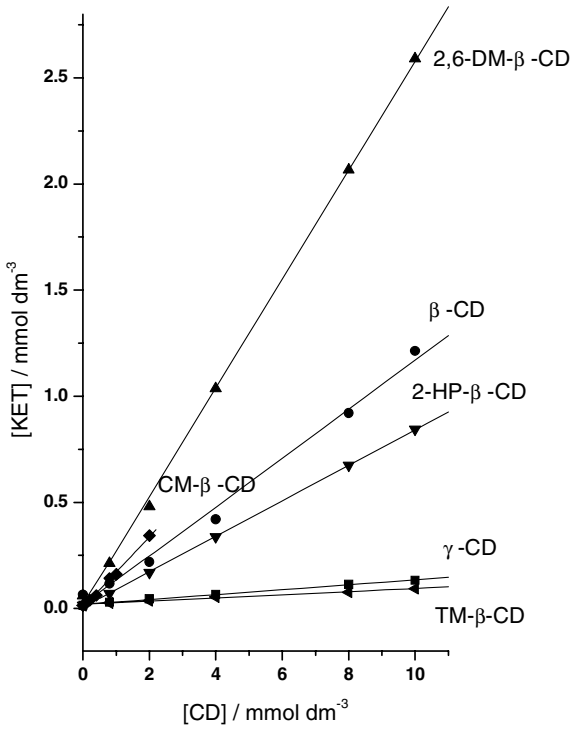

Figure 2. Phase-solubility diagrams of ketoconazole in the presence of increasing concentrations of the studied cyclodextrins at 25 °C and pH = 6: (◀) TM-β-CD; (■) γ-CD; (▼) 2HP-β-CD; (●) β-CD; (◆) CM-β-CD; (▲) 2,6-DM-β-CD.

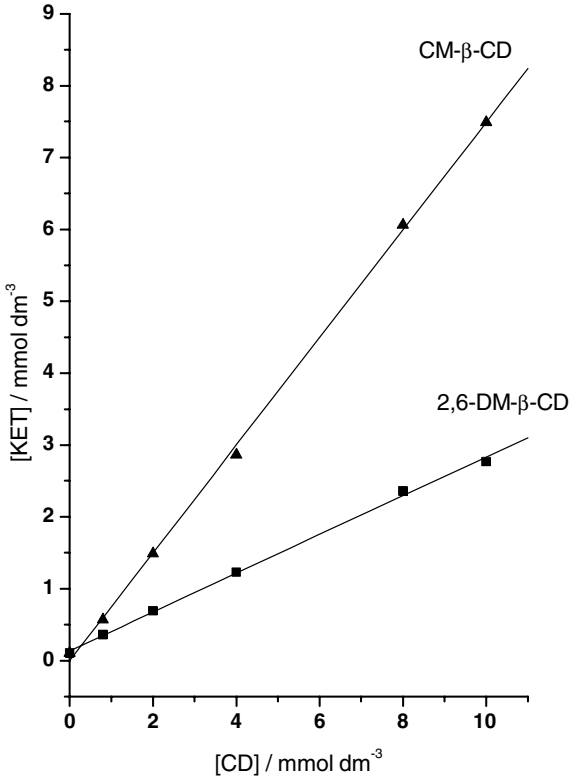

Figure 3. Phase-solubility diagrams of ketoconazole in the presence of increasing concentrations of 2,6-DM-β-CD (■) and CM-β-CD (▲) at 25 °C and pH = 5.

The solubility of KET increased linearly as a function of CDs concentration and over the range of the concentrations studied showed the features of an A_L type following Higuchi and Connors classification [16]. The increase in solubility can be attributed to the formation of inclusion complexes between KET and the studied CDs characterized by greater solubilities than that of KET alone. As the slope of solubility curves is less than unity, it can be assumed that the stoichiometry of inclusion complexes is 1:1. The stability constants of the inclusion complexes were calculated from the straight line diagrams according to the equation

$$K = s/s_0(1 - s) \quad (1)$$

where s_0 is the solubility of KET in the absence of CD (the intercept) and s denotes the slope of the straight line.

The aqueous solubility of KET at pH = 6 in function of temperature and at pH = 5 at 25 °C as well as the enhancement of the solubility at 25 °C in the presence of 10^{-2} mol dm^{-3} of studied CDs in solution is presented in Table 1.

In solutions with pH = 6 containing higher concentrations of CM-β-CD than 2×10^{-3} mol dm^{-3} the solubility of KET significantly increased and the solubility diagram showed the feature of A_p type. Such a significant increase in solubility (Table 1), it is difficult to explain the formation of the higher order complex with respect to CM-β-CD (i.e. 1:2 drug–CD complex) [16]. As it has been demonstrated by Loftsson *et al.* [17], the increased solubility of drugs in a solution containing cyclodextrin can be explained not only by the inclusion complex formation but also by association of the uncomplexed drug with the complex. We assume that such a phenomena may explain a significant increase of KET solubility at higher CM-CD concentrations at pH = 6.

The solubility of KET in solutions of 2,6-DM-β-CD and 2HP-β-CD at pH = 6 was also measured in function of temperature. The corresponding phase solubility diagrams are presented in Figures 4 and 5.

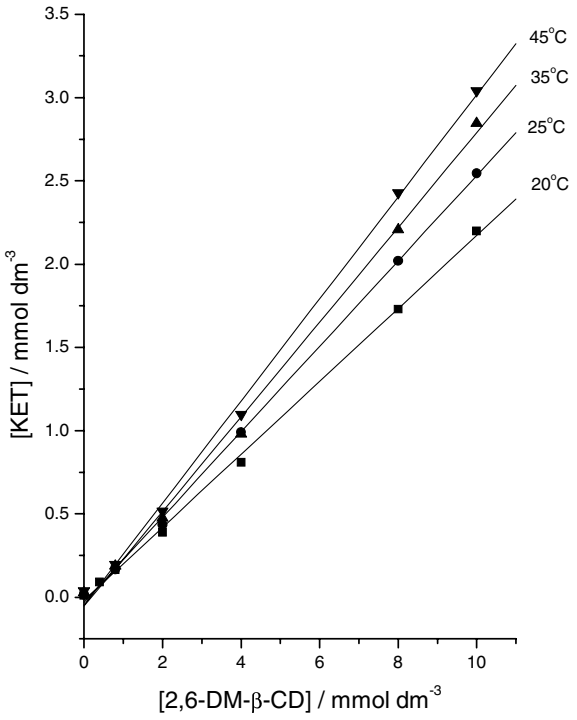

Figure 4. Phase-solubility diagrams of ketoconazole in the presence of increasing concentrations of 2,6-DM-β-CD at pH = 6 and various temperatures: (■) 20 °C; (●) 25 °C; (▲) 35 °C; (▼) 45 °C.

Stability constants and thermodynamic parameters

The apparent stability constants of KET at 25 °C with native and modified CDs determined by the solubility method are given in Table 2 together with the literature data of Esclusa-Diaz *et al.* [7] and Taneri *et al.* [18] As can be seen from Table 2 at pH = 6, the magnitude of stability constant increases in the order TM-β-CD < γ-CD < 2HP-β-CD < β-CD < CM-β-CD < 2,6-DM-β-CD. A possible reason of a big discrepancy between the values of the stability constant of KET with β-CD and 2HP-β-CD obtained by us and by Esclusa-Diaz *et al.* [7], determined by the solubility method, may be due to the fact that the above mentioned authors did not take into account the interference of CDs in the spectrophotometric assay at 225 nm. We found that all studied CDs absorb at this wave length, and therefore in our mea-

Table 1. The aqueous solubility of ketoconazole in function of temperature and pH and the enhancement of solubility (s/s_0) in the presence of 10^{-2} mol dm^{-3} CDs in solution

KET solubility (mol dm^{-3})	pH 6				pH 5
	20 °C, 0.97×10^{-5}	25 °C, 1.41×10^{-5}	35 °C, 2.76×10^{-5}	45 °C, 3.95×10^{-5}	25 °C, 1.07×10^{-4}
Cyclodextrin	Solubility enhancement				
β-CD	–	82	–	–	–
γ-CD	–	9.3	–	–	–
2-HP-β-CD	80	59	34	24	–
CM-β-CD	–	430	–	–	70
2,6-DM-β-CD	239	179	98	85	26
TM-β-CD	–	6.6	–	–	–

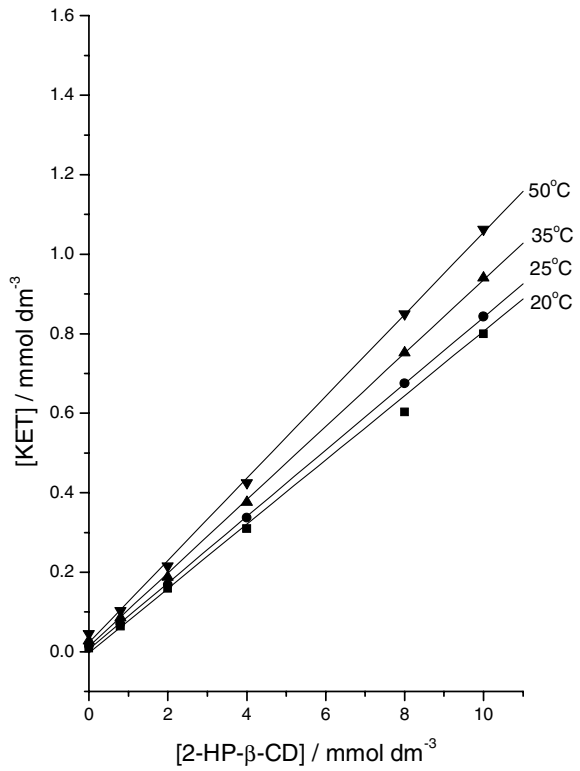

Figure 5. Phase-solubility diagrams of ketoconazole in the presence of increasing concentrations of 2HP-β-CD at pH = 6 and various temperatures: (■) 20 °C; (●) 25 °C; (▲) 35 °C; (▼) 50 °C.

surements in the reference cuvette was always the same concentration of CD as in the studied solution. In the case of 2HP-β-CD a different average degree of substitution is also important because increasing degree of substitution impairs complexing due to the steric hindrance of the host molecule [12].

The significant differences in the stability constants for β-CD, 2,6-DM-β-CD and 2HP-β-CD at various pH values are connected with a different drug ionization degree, which increases with lowering of pH (at pH = 5 KET is almost fully ionized), and with a consequent less affinity for neutral CD cavity. A similar effect of pH on the stability constants of other imidiazole derivatives – econazole and miconazole was observed by Pedersen *et al*. [19]. Much lower value of K_s for KET with 2,6-DM-β-CD at pH = 5, obtained by us, is in agreement with the general observation that ionized species are weak forming agents with neutral cyclodextrins [10]. In the case of CM-β-CD, the increased ionization of KET at pH = 5 is the reason of a significant increase in the stability constant due to the additional electrostatic interaction between negatively charged CM-β-CD (at pH = 5, the carboxylic groups are deprotonated, $pK_a < 4$), and positively charged KET. Zhang *et al*. [20] studied the influence of pH on the complexation of methylene blue, which is an ionic species with positive electric charge, by β-CD, HP-β-CD and CM-β-CD. The stability constants with HP-β-CD were higher than with β-CD, but in both cases they did not change much with pH in the range 2–11. Significant changes in the stability constants with pH were observed for complex with CM-β-CD. At pH = 2, the stability constant considerably decreased due to the protonation of the carboxylic groups.

The results presented indicate that the size and the hydrophobicity of the cyclodextrin cavity play an important role in the complex formation. Methylation extends the hydrophobic depth of the cavity of β-CD since most of the methyl groups attached to O-2 and O-6 point away from the center of the cavity [21]. This may be one of the reasons of high value of the stability constant of KET with 2,6-DM-β-CD. A significant increase in the stability constants of many drugs in the presence of 2,6-DM-β-CD in comparison with β-CD has been reported [22, 23]. However, the 3-O-methyl groups in TM-β-CD apparently reduce the necessary interaction between KET and the host molecule what may be one of the reasons of low value of the stability constant. Decrease of the stability constants of complexes with TM-β-CD in comparison with β-CD has been described in literature [24, 25].

The stability constants of KET with 2,6-DM-β-CD and 2HP-β-CD determined as a function of temperature and the thermodynamic parameters of the inclusion process estimated from the temperature dependence of the stability constants using van't Hoff relation are presented in Table 3. From Table 3, it can be seen that

Table 2. Apparent stability constants, K_s, of inclusion complexes of ketoconazole with CDs at 25 °C

Cyclodextrin	K_s ($dm^3\ mol^{-1}$)			
	pH = 6	pH = 5	pH = 7	In water
β-CD	9336 (±200)	827 [7]		
	1271 [7]			
γ-CD	853 (±30)			
2HP-β-CD (DS; 2.7)	6431 (±300)			
2HP-β-CD (DS; 5.5)	3472 [7]	627 [7]	2456 [18]	2652 [18]
CM-β-CD	14,376 (±350)	26,514 (±1400)		
2,6-DM-β-CD	23,836 (±500)	3428 (±150)		
TM-β-CD	532 (±30)			
M-β-CD			5680 [18]	7605 [18]

Table 3. Apparent stability constants, K_s of inclusion complexes of ketoconzole with CDs determined at different temperatures and thermodynamic parameters

Temperature	KET-2,6-DM-β-CD				KET-2-HP-β-CD			
	20 °C	25 °C	35 °C	45 °C	20 °C	25 °C	35 °C	50 °C
K_s (dm^3 mol^{-1})	30,200 (±1000)	23,836 (±500)	15,000 (±500)	11,400 (±200)	8469 (±400)	6431 (±300)	3675 (±300)	2556 (±300)
ΔH (kJ mol^{-1})	−30.2 (±1.2)				−31.7 (±1.3)			
ΔS (J mol^{-1} K^{-1})	−17.5 (±4.0)				−33.6 (±4.2)			
ΔG (kJ mol^{-1})	−25.0 (±2.4)				−21.6 (±2.5)			

for both CDs, the stability constants decrease with increase in temperature and the complexation is enthalpy controlled as for many other drug–cyclodextrin complexes [26]. Negative values of ΔH and ΔS indicate that the van der Waals forces are important in the complexation process. Despite the negative ΔH and ΔS values, the hydrophobic interaction also seems to contribute essentially to the association. This is supported by the experimental data that the expansion of the hydrophobic region of the CD cavity enhances the substrate binding in the case of 2,6-DM-β-CD. The role of hydrogen binding also seems to be important because the stability constant with β-CD is significantly higher than with TM-β-CD. The hydrogen binding may also play a role in the case of other cyclodextrins.

Molecular modeling

Calculations by the Amber force field using HyperChem 6 software were performed in order to obtain some global information about the geometry of the host–guest complexes and to find which residue of the KET molecule interacts with the CD cavity. Simulations were performed for β–γ and TM-β-CD using conjugate algorithm included in the HyperChem 6 software package with the convergence criteria of 0.001 kcal mol^{-1} (1 kcal = 4.184 kJ). Simulations started from the crystallographic host geometries [27–29] which were minimized. The crystallographic geometry of KET [30] was also minimized. Subsequently, the guest was pushed trough the CD cavity for various starting geometries. The resulting energies of the minimum geometries were calculated and compared with the energy for the separated host–guest pair. For three studied CDs, the lowest energies were found when dichlorophenyl ring was inserted into the cavity from the wider rim. It is in agreement with an interaction model of KET with β-CD in the presence of tartaric acid built on the basis of ^{1}H NMR and ^{13}C NMR data by Redenti *et al*. [31]. The lowest energy conformation of the β-CD–KET complex was found at −37 kcal mol^{-1}, of the γ-CD–KET complex at −31 kcal mol^{-1} and of the TM-β-CD–KET complex at −25 kcal mol^{-1}.

The sterically feasible complex geometries are presented in Figure 6 together with the upside perpendicular views. In the case of complex with β-CD, the dichlorophenyl ring is buried in the cavity and the imidiazole as well as dixolan rings are partly protruding from it. The phenoxy fragment is located near the wider rim of β-CD in agreement with the data of Redenti *et al*. [31]. The benzene ring as well as the piperazine moiety are outside of the cavity [Figure 6 (a) and (a')]. In the case of complex with γ-CD, part of the dichlorophenyl ring with two chlorine substituents is protruding from the cavity. Imidiazole and dixolane rings are hidden. The phenoxy fragment is buried only partly with part of the benzene ring protruding. The piperazine moiety is not interacting with CD cavity [Figure 6 (b) and (b')]. In the case of complex with TM-β-CD, the dichlorophenyl ring is only partly buried in the cavity with one chlorine substituent protruding. Other parts of the KET molecule are outside of the cavity [Figure 6 (c) and (c')].

Taking into account the possibility that the imidiazole ring and the phenoxy fragment may form the hydrogen bonds with the OH groups at the wider rim of β-CD the lowest energy conformation of β-CD-KET complex was at −61 kcal mol^{-1}. In the case of γ-CD–KET complex assumption of hydrogen bond formation between phenoxy fragment and hydroxyl groups of CD gave the lowest energy conformation of γ-CD–KET complex at −37 kcal mol^{-1}. The calculated binding energies indicate that the formation of the hydrogen bonds may be also responsible for the complex formation and stability. The role of the hydrogen bonding in determining the stability constants has been discussed in literature [32, 33].

Conclusion

The KET molecule forms inclusion complexes with all the CDs studied. The stoichiometry of the complexes is 1:1. At pH = 6, the magnitude of stability constant increases in the order TM-β-D < γ-CD < 2HP-β-CD < β-CD < CM- β-CD < 2,6-DM-β-CD. The significant differences in stability constants at various pH values are connected with different drug ionization degree and a consequent various affinity for CD cavity. For 2,6-DM-β-CD and 2HP-β-CD stability constants decreased with increase in temperature. The size and the hydrophobicity of the cyclodextrin cavity as well as the van der Waals forces and the hydrogen bonds play an important role in the complex formation.

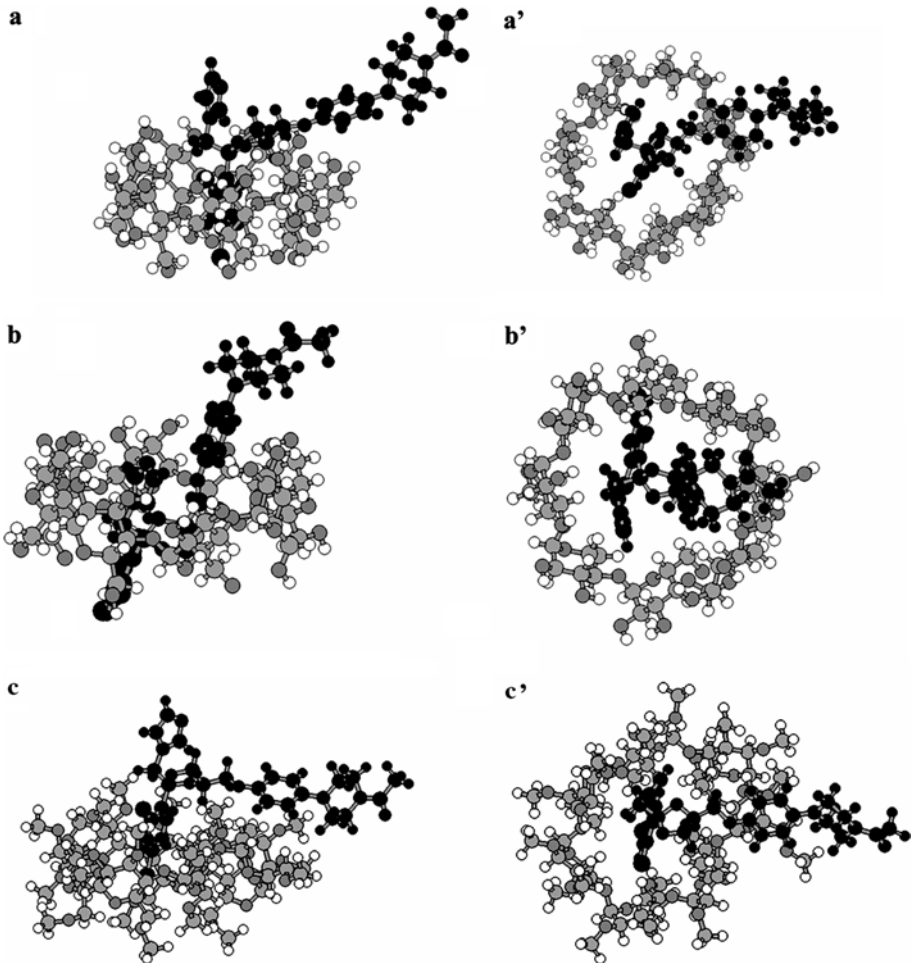

Figure 6. Minimized geometries of the inclusion complexes of ketoconazole with (a) β-CD; (b) γ-CD; (c) TM-β-CD, and the corresponding upside perpendicular views [(a')–(c')]. Ketoconazole molecule is shown by black circles.

Acknowledgements

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References

1. J. Heeres, L.J.J. Backx, J.H. Mostmans and J. Van Cutsem: *J. Med. Chem.* **22**, 1003 (1979).
2. R.C. Heel, R.N. Brogden, A. Carmine, P.A. Morley, T.M. Speight and G.S. Avery: *Drugs* **23**, 1 (1982).
3. J.D. Cartledge, J. Midgley, M. Petron, D. Shanson and B.G. Gazzard: *J. Antimicrob. Chemother.* **40**, 517 (1997).
4. F. Comby, J.F. Lagorce, J. Buxeraud and C. Raby: *J. Pharm. Pharmacol.* **46**, 50 (1994).
5. M. Shamsipur and K. Farhadi: *Analyst* **125**, 1639 (2000).
6. J.A. Carlson, H.J. Marn and D.M. Canafax: *Am. J. Hosp. Pharm.* **40**, 1334 (1983).
7. M.T. Esclusa-Diaz, M. Guimaraenes-Méndez, M.B. Pérez-Marcos, J.L. Vila-Jato and J.J. Torres-Labandeira: *Intern. J. Pharmac.* **143**, 203 (1996).
8. J. Szejtli: *Cyclodextrins and Their Inclusion Complexes*, Akadémiai Kiadó, Budapest (1982).
9. J. Szejtli: In J. Szejtli and T. Osa (eds.), *Comprehensive Supramolecular Chemistry*, Vol. 3, Elsevier, New York, (1996), pp. 189–204.
10. J. Szejtli: *Cyclodextrin Technology*, Kluwer, Dordrecht (1988).
11. D. Duchêne and J. Wouessdjewe: *J. Coord. Chem.* **27**, 223 (1992).
12. K. Uekama, F. Fumitoshi and T. Irie: *Chem. Rev.* **98**, 2045 (1998).
13. J. Szejtli: *J. Incl. Phenom.* **14**, 25 (1992).
14. B. Sébille: In D. Duchêne (ed.), *Cyclodextrins and Their Industrial Uses*, Editions de Santé Paris (1987), pp. 351–392.
15. K. Uekama and T. Irie: In D. Duchêne (ed.), *Cyclodextrins and Their Industrial Uses*, Editions de Santé, Paris (1987), p. 395.
16. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instrum.* **4**, 117 (1965).
17. A. Magnusdottir, M. Másson and T. Loftsson: *J. Incl. Phenom.* **44**, 213 (2002).
18. F. Taneri, T. Güneri, Z. Aigner and M. Kata: *J. Incl. Phenom.* **47**, 15 (2003).
19. M. Pedersen, M. Edelsten, V.F. Nielsen, A. Scarpellini, S. Skytte and C. Slot: *Intern. J. Pharmac.* **90**, 247 (1993).
20. G. Zhang, S. Shuang, Ch. Dong and J. Pan: *Spectrochim. Acta* Part A **59**, 2935 (2003).
21. K. Harata: In J.L. Atwood, J.E.D. Davies and D.D. MacNickol (eds.), *Inclusion Compounds*, Vol. 3, Oxford University Press, Oxford (1991), p. 311.
22. J. Taraszewska, K. Migut and M. Koźbiał: *J. Phys. Org. Chem.* **16**, 121 (2003).
23. A.R. Green, E.S. Miller and J.K. Guillory: *J. Pharm. Sci.* **80**, 186 (1991).
24. T. Imai, T. Iria, M. Otagiri and K. Uekama: *J. Incl. Phenom.* **2**, 597 (1984).
25. R.J. Gelb, S. Raso and S.J. Alper: *Supramol. Chem.* **4**, 279 (1995).
26. T. Loftsson and M.E. Brewster: *J. Pharm. Sci.* **85**, 1017 (1996).
27. C. Betzel, W. Saenger, B.E. Hingerty and G.M. Brown: *J. Am. Chem. Soc.* **106**, 7545 (1984).
28. K. Harata: *Bull. Chem. Soc. Jpn.* **60**, 2763 (1987).
29. Steiner T. and Saenger W.: *Angew. Chem. Int. Ed. Engl.* **37**, 3404 (1998).

30. O.M. Peeters, N.M. Blaton and C.J. De Ranter: *Acta Cryst*. **B35**, 2461 (1979).
31. E. Redenti, P. Ventura, G. Fronza, A. Selva, S. Rivara, P.V. Plazzi and M. Mor: *J. Pharm. Sci*. **88**, 599 (1999).
32. P.L. Meo, F. D′Anna, S. Riela, M. Gruttadauria and R. Noto: *Tetrahedron* **58**, 6039 (2002).
33. I. Bea, C. Jaime, J. Redondo, P. Bonnet, A. Torrens and J. Frigola: *Supramol. Chem*. **14**, 33 (2002).