

Research article

Open Access

NMR and molecular modelling studies on the interaction of fluconazole with β -cyclodextrin

Santosh Kumar Upadhyay* and Gyanendra Kumar

Address: National Institute of Immunology, New Delhi-110 067, India

Email: Santosh Kumar Upadhyay* - skupadhyay@msn.com; Gyanendra Kumar - gyan@nii.res.in

* Corresponding author

Published: 10 August 2009

Received: 11 May 2009

Chemistry Central Journal 2009, 3:9 doi:10.1186/1752-153X-3-9

Accepted: 10 August 2009

This article is available from: <http://journal.chemistrycentral.com/content/3/1/9>

© 2009 Upadhyay et al

Abstract

Background: Fluconazole (FLZ) is a synthetic, bistriazole antifungal agent, effective in treating superficial and systemic infections caused by *Candida* species. Major challenges in formulating this drug for clinical applications include solubility enhancement and improving stability in biological systems. Cyclodextrins (CDs) are chiral, truncated cone shaped macrocycles, and can easily encapsulate fluconazole inside their hydrophobic cavity. NMR spectroscopy has been recognized as an important tool for the interaction study of cyclodextrin and pharmaceutical compounds in solution state.

Results: Inclusion complex of fluconazole with β -cyclodextrins (β -CD) were investigated by applying NMR and molecular modelling methods. The 1:1 stoichiometry of FLZ: β -CD complex was determined by continuous variation (Job's plot) method and the overall association constant was determined by using Scott's method. The association constant was determined to be 68.7 M^{-1} which is consistent with efficient FLZ: β -CD complexation. The shielding of cavity protons of β -CD and deshielding of aromatic protons of FLZ in various $^1\text{H-NMR}$ experiments show complexation between β -CD and FLZ. Based on spectral data obtained from 2D ROESY, a reasonable geometry for the complex could be proposed implicating the insertion of the *m*-difluorophenyl ring of FLZ into the wide end of the torus cavity of β -CD. Molecular modelling studies were conducted to further interpret the NMR data. Indeed the best docked complex in terms of binding free energy supports the model proposed from NMR experiments and the *m*-difluorophenyl ring of FLZ is observed to enter into the torus cavity of β -CD from the wider end.

Conclusion: Various NMR spectroscopic studies of FLZ in the presence of β -CD in D_2O at room temperature confirmed the formation of a 1:1 (FLZ: β -CD) inclusion complex in which *m*-difluorophenyl ring acts as guest. The induced shift changes as well as splitting of most of the signals of FLZ in the presence of β -CD suggest some chiral differentiation of guest by β -CD.

Background

Fluconazole (FLZ), diflucan, chemically known as α -(2,4)-difluorophenyl)- α -(1H-1,2, 4-triazol-1-ylmethyl)-1H-1,2,4-triazole-1-ethanol (figure 1), used to treat variety of fungal pathogens that cause systemic mycoses. It is an orally active bistriazole antifungal agent, well absorbed

and has been found to be safe and effective in treating superficial and systemic infections with *Candida* species and maintenance therapy for cryptococcal meningitis, particularly in patients with AIDS [1]. It is also effective in preventing invasive *Candida* infections in patients with acute leukaemia without increasing non-albicans infec-

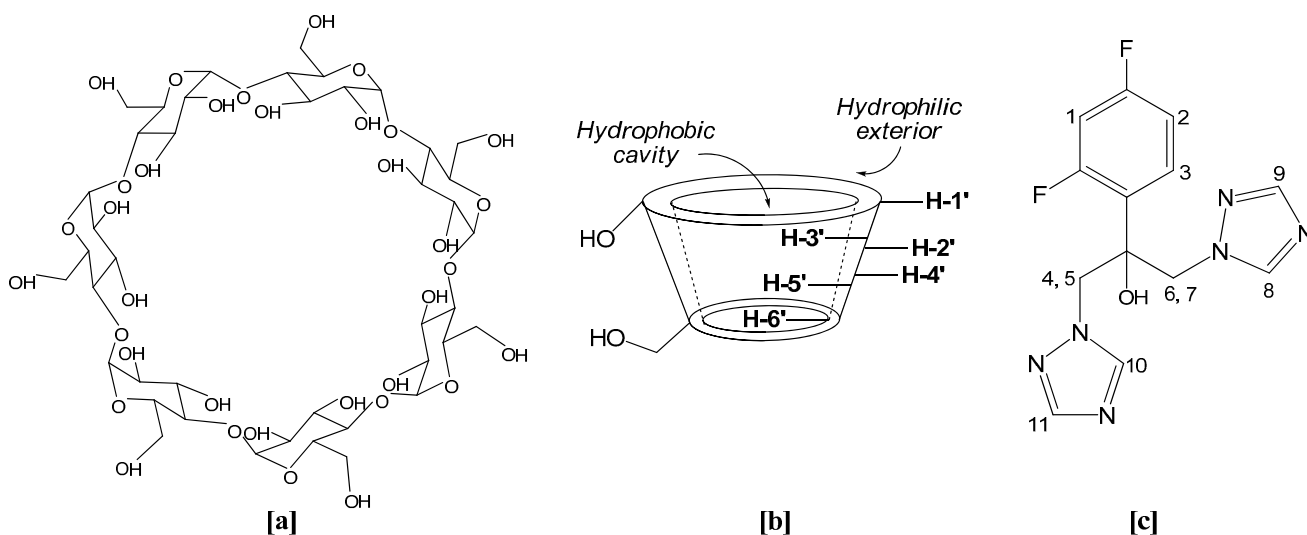


Figure 1
(a) Chemical structure of the host β -cyclodextrin (β -CD); (b) truncated cone shape of β -CD; and (c) guest fluconazole (FLZ).

tions [2]. FLZ is commonly used to prevent yeast infections in patients undergoing bone marrow transplantation [3].

The principle objective of the work embodied in this article was to study the complexation of fluconazole with β -cyclodextrin in aqueous solution. Complexation of pharmaceutical compounds with cyclodextrins (CDs), to form a host-guest complex in solution as well as solid states, results in altered physicochemical properties of the guest, like solubility, stability, dispersibility, volatility, masking of undesirable properties and so on, which are desirable for their use as pharmaceuticals [4]. Moreover, these host-guest complexes are considered as new entities and are required to be characterized for their approval as drugs. CDs are chiral saccharides that exhibit chiral recognition i.e. they form diastereomeric complexes, usually of different stability, with enantiomeric species [5]. The separation of enantiomers of a racemic drug is of great importance to the pharmaceutical industry because in a racemic drug, only one enantiomer is usually desired. CDs are all-purpose molecular containers for organic, inorganic, organometallic, and metal organic compounds that may be neutral, cationic, anionic or even radical.

The CDs belongs to the family of cyclic oligosaccharides, and have been studied extensively as a host in supramolecular chemistry. The three major types of CDs are crystalline, homogeneous, nonhygroscopic, consisting of six (α -), seven (β -), and eight (γ -) D-glucose units, respectively, attached by α -(1 \rightarrow 4) glycosidic linkages (figure 1)

[4,5]. Each of the chiral glucose units is in the rigid 4C_1 -chair conformation, giving the macrocycle shape of a hollow truncated cone with all the secondary hydroxyl groups located on the wider rim, while all the primary hydroxyl groups on the narrower rim (figure 1). The primary and secondary hydroxyls on the outside of the CDs make it water-soluble. The non-bonding electron pairs of the glycosidic oxygen bridges are directed toward the inside of the cavity, producing a high electron density and lending it a Lewis base character. These features suggest that the CD cavity is relatively hydrophobic compared to the exterior faces which are hydrophilic.

Structure determination is of particular importance for supramolecular host-guest complexes, which are the basis of most cyclodextrin applications in medicine, catalysis, separation and sensor technology and also food chemistry. NMR spectroscopy has been recognized as an important tool for the structural elucidation of organic compounds, particularly in solution state in view of its application in drug discovery. This technique also gives information on the topology of the interaction between the guest and β -CD; furnishing information not only on the structure of inclusion complexes but also deriving the stoichiometry and association or binding constant of guest: β -CD complexes [6]. 2D COSY and ROESY experiments are important in cyclodextrin related studies, as they complement each other, COSY provides information on coupling of protons while 2D ROESY gives same information through space, i.e., the two nuclei are at 3–4 Å from each other (intermolecular distance) [5,7].

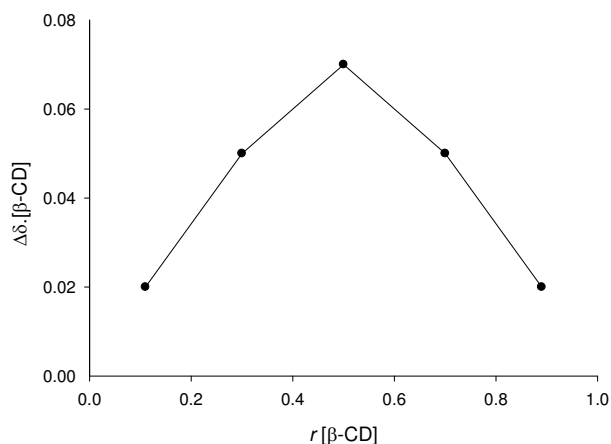


Figure 2
Job's plot (continuous variation method) of FLZ:β-CD inclusion complex showing 1:1 stoichiometry. $\Delta\delta = \delta_{(\text{complex})} - \delta_{(\text{free})}$.

Here we present a study highlighting the interaction between antifungal drug fluconazole and β-cyclodextrin, using NMR as a spectroscopic tool. In order to better understand the structure of the inclusion complex between the two chemical species, we have complemented these studies with molecular modelling simulations.

Experimental

Materials

Fluconazole (FLZ) was obtained from Dr. Reddy's Ltd., India while β-cyclodextrin was a generous gift from DKSH India Pvt. Ltd. and these were used as received. All other reagents were of analytical reagent grade.

NMR studies

Samples were prepared in 99.96% D₂O for NMR analysis. All the ¹H NMR spectra were recorded with a Bruker Avance 400 MHz spectrometer operating at 300 K and were acquired with a spectral width of 5995.204 Hz, 128 scans and 65536 data-points. Both 2D COSY and ROESY experiments were acquired on a Bruker DRX 500 MHz using 5 mm BBI ¹H-BB probe or a Varian Inova 500 MHz, equipped with a triple resonance, Z pulsed field gradient probe. 2D COSY spectra displaying ¹H-¹H cross correlation for free FLZ, free β-CD and FLZ:β-CD mixture were acquired using 2048 data-points with 128 increments and 18 scans for each increment. 2D ROESY spectrum of FLZ:β-CD was acquired using 2048 data-points, 256 increments, 8 scans for each increment and 500 ms mixing time. ¹H NMR spectra of five samples containing mixtures of β-CD and FLZ with FLZ/β-CD molar ratios ranging from 0.2 to 1.2 was recorded. As there was no separate

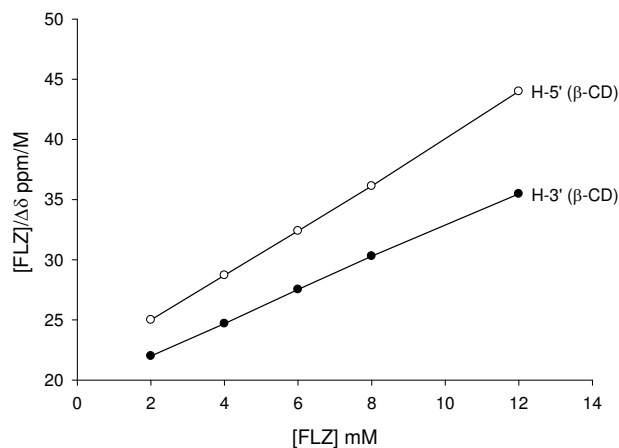


Figure 3
A typical Scott's plot for FLZ:β-CD inclusion complex showing overall association constant (K_a) = 68.7 M⁻¹.

peak for free as well as complexed form of FLZ, we presume that it undergoes rapid exchange between free and bound state on the NMR time scale. The resonance at 4.7 ppm due to residual solvents (H₂O and HDO) was used as internal reference. Chemical shift (δ) reported in ppm and chemical shift changes ($\Delta\delta$) was calculated by using the formula: $\Delta\delta = \delta_{(\text{complex})} - \delta_{(\text{free})}$.

Molecular modelling studies

Molecular modelling studies were done using the software package MOE 2005.06 (Molecular Operating Environment, Version 2005.06). 3D coordinates for FLZ were generated using builder module of MOE and the molecule was energy-minimized using MMFF94x force field. The 3d coordinates of β-CD were taken from the Protein Data Bank file with PDB id: 1DMB. Since the positions of hydrogen atoms are not included in the PDB files, these were added using MOE, and energy-minimized with MMFF94x force field. The dock application built in MOE was used to dock FLZ with β-CD. This application is divided into three stages: 1. *Conformational analysis* during which ligands are treated in a flexible manner by rotating rotatable bonds; ring conformations are not searched. 2. *Placement* during which a collection of orientation is generated from the pool of ligand conformations. In this case, the alpha triangle placement method was used, it generates orientations by superposition of ligand atom triplets and triplets points in the receptor site. The receptor site points are *alpha sphere* centers which represent locations of tight packing. At each iteration a random conformation is selected, a random triplet of ligand atoms and a random triplet of alpha sphere centers are used to determine the orientation. 3. *Scoring*, during which each orientation generated by the placement methodology is subjected to scoring in an effort to identify the most favorable orientations.

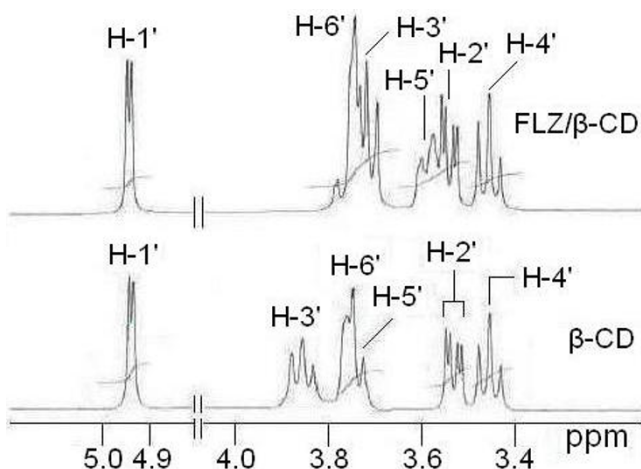


Figure 4
Expansion of a part of ^1H NMR data (500 MHz), showing $\beta\text{-CD}$ protons in the presence as well as in the absence of FLZ.

The dock application provides a framework for the integration of multiple scoring methodologies; each such scoring methodology will have different properties. Typically, scoring functions emphasize favorable hydrophobic, ionic and hydrogen bond contacts. The default method uses "affinity dG" scoring function to assess candidate orientations. This function estimates the enthalpic contribution to the free energy of binding using a linear function:

$$G = C_{\text{hb}}f_{\text{hb}} + C_{\text{ion}}f_{\text{ion}} + C_{\text{mlig}}f_{\text{mlig}} + C_{\text{hh}}f_{\text{hh}} + C_{\text{hp}}f_{\text{hp}} + C_{\text{aa}}f_{\text{aa}}$$

where the f terms fractionally count atomic contacts of specific types and the C 's are coefficients that weight the term contributions to affinity estimate. The individual terms are: hb, interactions between hydrogen bond donor-acceptor pairs; ion, ionic interactions; mlig, metal ligation; hh, hydrophobic interactions; hp, interactions between hydrophobic and polar atoms; aa, an interaction between any two atoms. The docked complex was finally energy-minimized keeping both the ligand and the receptor flexible.

Table 1: ^1H NMR (500 MHz) chemical shift (δ) data of pure $\beta\text{-CD}$ and $\beta\text{-CD}:\text{FLZ}$ mixtures

Sample	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'
Pure $\beta\text{-CD}$	4.948	3.556	3.882	3.486	3.754	3.782
$\beta\text{-CD}/\text{FLZ} = 0.2$	4.947	3.554	3.791	3.482	3.676	3.784
$\beta\text{-CD}/\text{FLZ} = 0.4$	4.948	3.554	3.718	3.479	3.615	3.787
$\beta\text{-CD}/\text{FLZ} = 0.6$	4.946	3.555	3.659	3.485	3.566	3.789
$\beta\text{-CD}/\text{FLZ} = 0.8$	4.948	3.553	3.609	3.480	3.530	3.787
$\beta\text{-CD}/\text{FLZ} = 1.2$	4.947	3.551	3.529	3.485	3.471	3.784

Results and discussion

Determination of the FLZ: $\beta\text{-CD}$ inclusion complex stoichiometry by continuous variation method (Job's Plot)

The stoichiometry of host-guest complex was determined by the continuous variation (Job's plot) method [8]. It involves preparing a series of solutions containing both the host and the guest in varying proportions so that a complete range of mole ratios is sampled ($0 > [\text{H}]/[\text{H}] + [\text{G}] < 1$) and where the total concentration $[\text{H}] + [\text{G}]$ is kept constant for each solution. The experimentally observed parameter is a host or guest chemical shift that is sensitive to complex formation. The plot of $\Delta\delta \times [\beta\text{-CD}]$ against the mole fraction of $\beta\text{-CD}$, ($r = m/[m/n]$), where m and n represent the stoichiometric ratios of $\beta\text{-CD}$ and FLZ, respectively, for the H-6' protons of $\beta\text{-CD}$ is presented in figure 2. As presented in figure 2, the plot shows a maximum value at $r = 0.5$ and a highly symmetrical shape, which demonstrates the existence of a FLZ: $\beta\text{-CD}$ complex with a 1:1 stoichiometry.

Determination of association constant (K_a)

The association constant (K_a) of the FLZ: $\beta\text{-CD}$ complex were determined by using well known Scott's method [9] which is a modification of Benesi-Hildebrand equation [10]. Equation (1) refers the Scott's equation:

$$[\text{FLZ}] / \Delta\delta_{\text{obs}} = [\text{FLZ}] / \Delta\delta_{\text{max}} + \Delta\delta_{\text{max}} / K_a \quad (1)$$

where $[\text{FLZ}]$ is the molar concentration of the guest, $\Delta\delta_{\text{obs}}$ the observed chemical shift change for a given $[\text{FLZ}]$ concentration, $\Delta\delta_{\text{max}}$ the chemical shift change between a pure sample of complex and the free component at saturation.

In this procedure, the plot of chemical shift changes ($\Delta\delta$) for the $\beta\text{-CD}$ protons against $[\text{FLZ}]$ in the form of $[\text{FLZ}] / \Delta\delta_{\text{obs}}$ versus $[\text{FLZ}]$ (referred to as y-reciprocal plot) should be linear for 1:1 inclusion complex. The slope of the plot thus equals to $1/\Delta\delta_{\text{max}}$ and the intercept with the vertical axis to $\Delta\delta_{\text{max}}/K_a$, allowing the estimation of association constant (K_a). A typical Scott's plot for FLZ: $\beta\text{-CD}$ inclusion complex is shown in figure 3 and the association constant (K_a) was calculated to be 68.7 M^{-1} .

NMR studies

The assignment of the $\beta\text{-CD}$ protons was made on the basis of their splitting pattern, ^1H NMR and 2D $^1\text{H}-^1\text{H}$ COSY and $^1\text{H}-^1\text{H}$ ROESY spectral data. Comparison the ^1H NMR spectra of pure $\beta\text{-CD}$ and FLZ: $\beta\text{-CD}$ mixture, suggests that the chemical shift of $\beta\text{-CD}$ proton is changes upon bonding to FLZ. $\beta\text{-CD}$ cavity protons H-3' and H-5' show maximum shielding compared to H-1', H-2' and H-4' in presence of varying amounts of FLZ. However, H-6' proton of $\beta\text{-CD}$ shows a deshielding of a similar magnitude, confirming that guest FLZ only interacts with the

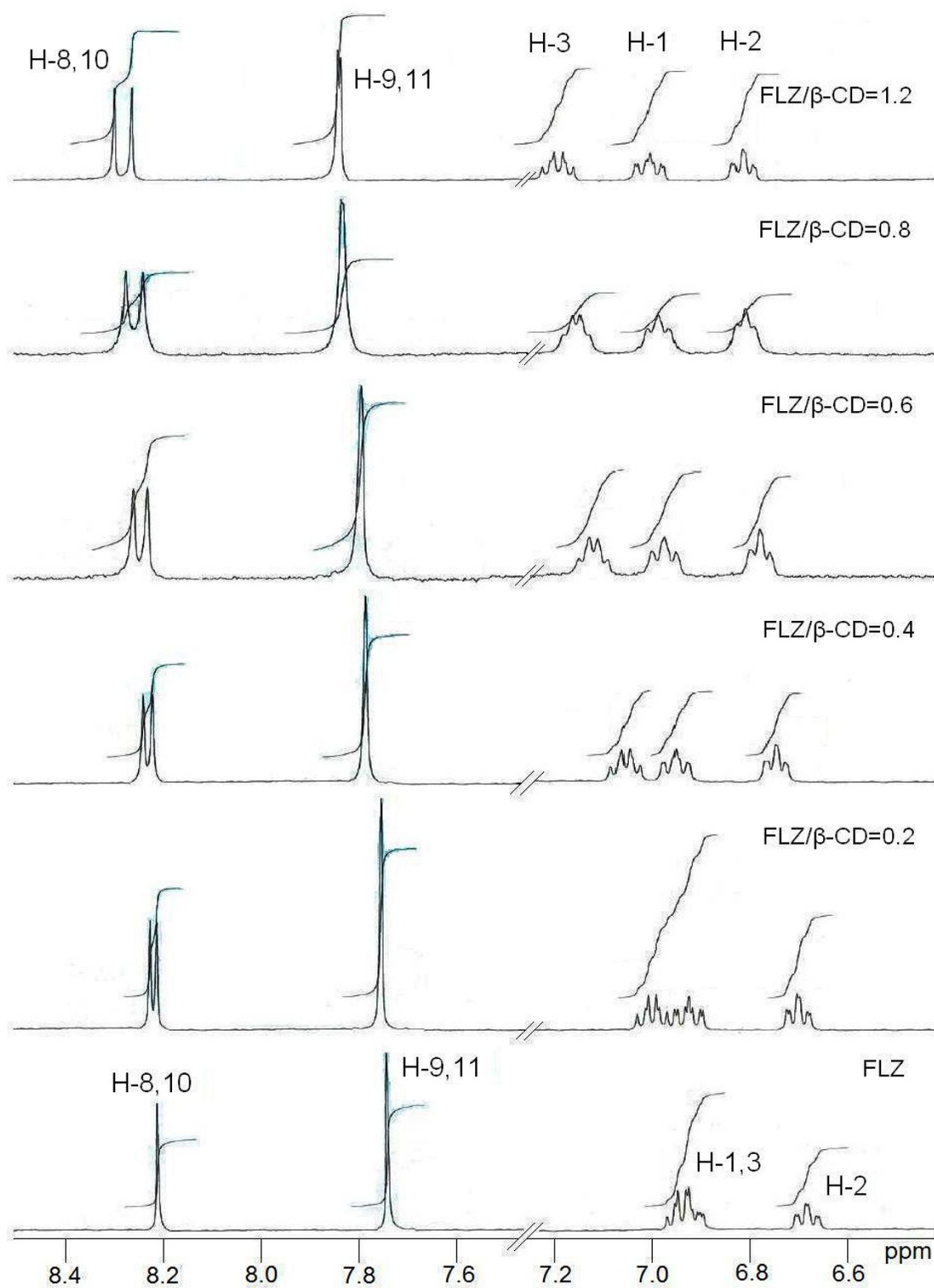


Figure 5
Expansion of a part of ^1H NMR data (500 MHz), of FLZ protons showing deshielding in the presence of varying amount of β -CD.

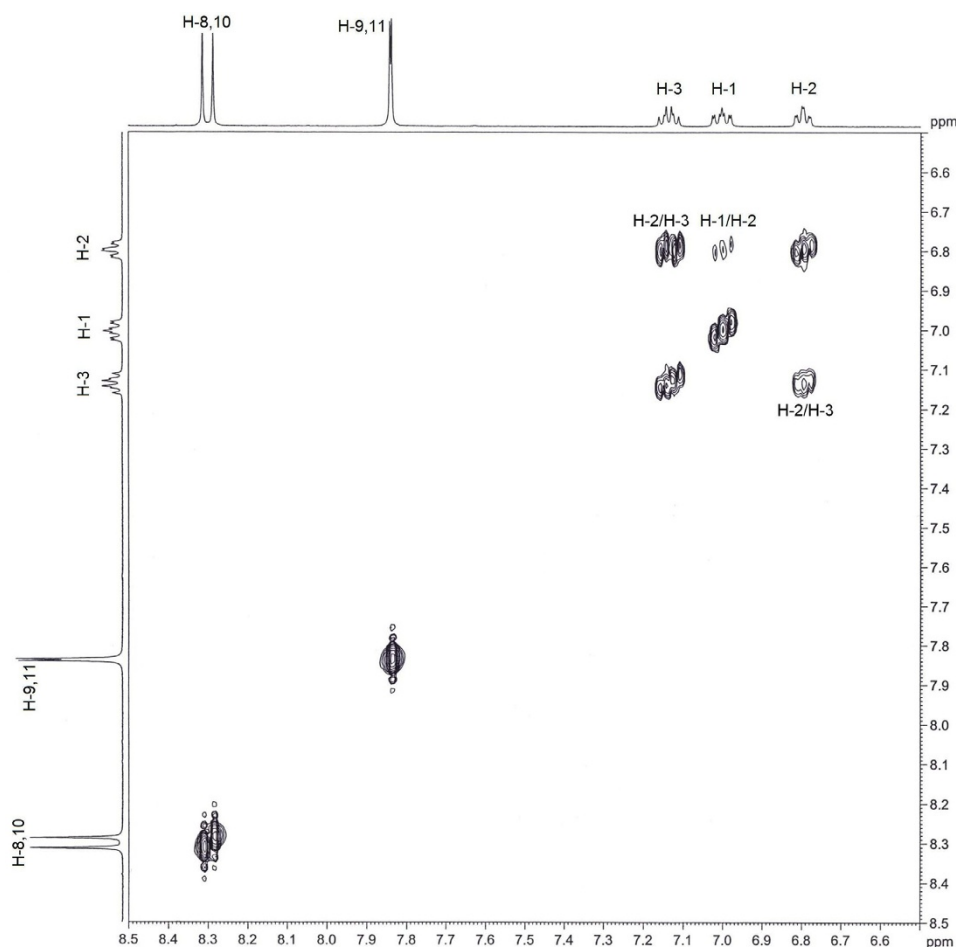


Figure 6
2D COSY (500 MHz) spectra of FLZ:β-CD mixture (1:1) showing the cross peak between aromatic protons of FLZ.

cavity of truncated cone of β-CD and forms an inclusion complex. The shielding β-CD cavity proton arises due to magnetic anisotropic effects in the β-CD cavity, as a result of the inclusion of a π-electron rich group. Such a group in FLZ molecule being a phenyl or triazole ring suggests its inclusion in the β-CD cavity. Furthermore, magnitude of the change in the chemical shift of these β-CD protons increased with increase in concentration of FLZ. H-3' and H-5' protons of β-CD show a greater shift than H-6' (positioned at narrow mouth), indicating the insertion of FLZ molecule most plausibly from wider rim side of β-CD cavity. The chemical shift (δ) data of β-CD protons in the presence as well as in the absence of varying amounts of FLZ is shown in table 1. Expanded region of part of ^1H NMR spectra displaying β-CD protons in the presence, as well as absence, of FLZ is shown in figure 4.

Unambiguous resonance assignments of FLZ protons were made on the basis of ^1H NMR data, 2D COSY and

2D ROESY spectra. Some of the peaks that were completely not distinct in the spectrum of the pure FLZ separated well in the presence of β-CD aiding the assignment. FLZ has one difluoro substituted phenyl ring and two triazole rings and its ^1H NMR chemical shift appeared separately. Here, we are giving the ^1H NMR assignment of pure FLZ. The two singlets at $\delta = 8.23$ and 7.74 were assigned as H-8, 10 and H-9, 11 resonance, consistent with the two protons of the two equivalent triazole rings of FLZ. Two highly coupled resonance patterns (probably multiplet) appeared near $\delta = 6.9$ (H-1, 3) and 6.7 (H-2), integrating with the ratio of 2:1 protons of three consistent aromatic protons of the *m*-difluorophenyl ring of FLZ. An AB pattern appeared at $\delta = 4.9$ (H-4, 5, 6, 7), totally integrating for the four protons consistent for the two sets of β-methylene protons of FLZ.

The ^1H NMR spectrum of FLZ:β-CD mixture is different compared to that of pure FLZ. In the presence of β-CD,

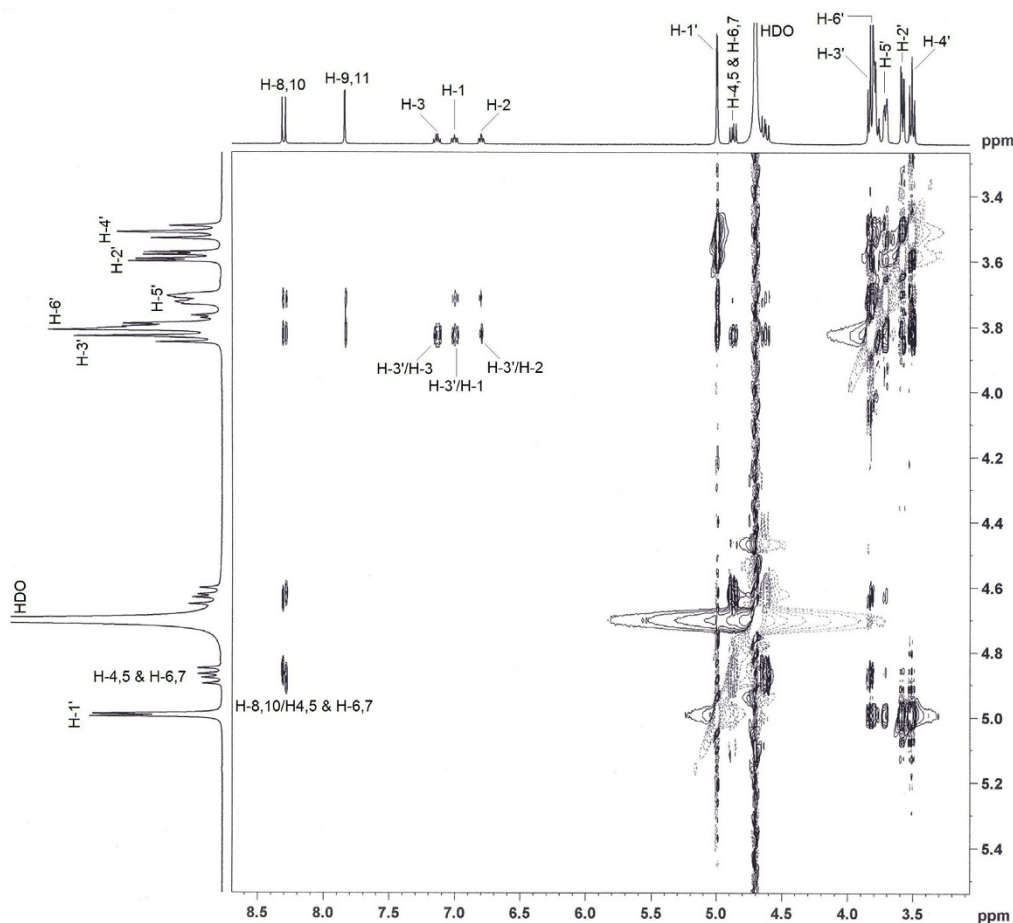
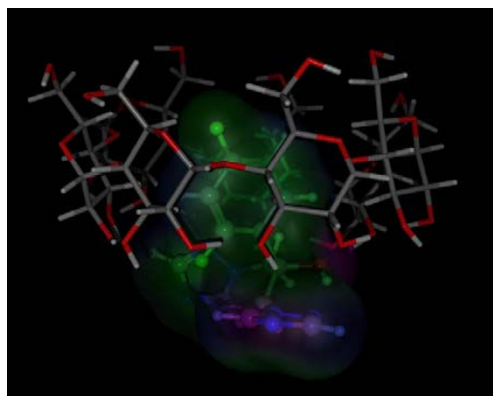


Figure 7
Expanded region of 2D ROESY (500 MHz) spectra of FLZ:β-CD mixture (1:1) showing the intermolecular NOE's between β-CD protons and aromatic protons of FLZ.

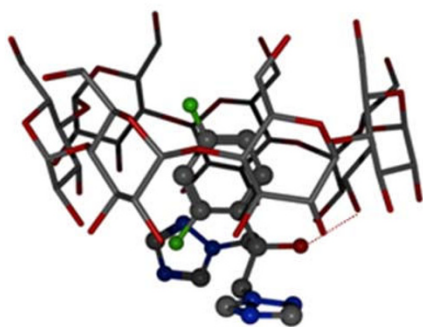
most of the FLZ protons showed splitting. H-8, 10 (s, $\delta = 8.23$) and H-9, 11 (s, $\delta = 7.74$) signal of pure FLZ display a doublet in all the FLZ:β-CD mixtures. Interestingly, H-1, 3 proton (m, $\delta = 6.9$) of pure FLZ split into two multiplets in FLZ:β-CD mixtures. However, the H-2 signal did not show such pattern. The splitting of most of FLZ protons in the presence of β-CD is due to some chiral differentiation of guest FLZ protons. Expansion of part of ^1H NMR spectra showing aromatic protons of FLZ in the presence, as well as in the absence of β-CD is shown in figure 5. The assignment of phenyl ring protons was done using 2D ^1H - ^1H COSY spectral data in which H-2 shows the cross peak with H-1 and H-3 both but H-8, 10 and H-9, 11 protons of FLZ did not show any COSY cross peaks to any signal. The H-2/H-3 coupling was stronger vicinal coupling with the weaker H-1/H-2 coupling. Expansion of part of 2D COSY spectral data showing ^1H - ^1H COSY cross peak of aromatic protons is illustrated in figure 6. The assignment of triazole ring was challenging through it was resolved using NOEs correlation in 2D ^1H - ^1H ROESY spectrum.

Expanded region of a 2D ROESY spectra showing ^1H - ^1H NOEs is shown in figure 7. The H-8, 10 proton of triazole ring is very close to β-methylene proton (H-4, 5 and 6, 7) and therefore we expect see the NOEs between them. On inspecting the ROESY data a cross peak between H-8, 10 and H-4, 5 and 6, 7 was observed confirming that the former is close in space to the latter. No NOEs to triazole ring (H-9, 11) of FLZ was observed to any other protons.

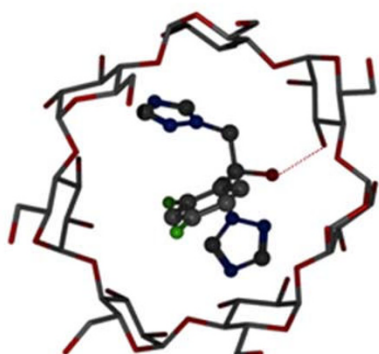
In presence of β-CD, all the aromatic protons deshielded, indicating the proximity of these protons' to an electronegative atom like oxygen. The shielding of β-CD cavity protons and concomitant deshielding of the most of the aromatic protons of FLZ suggest that this ring is inserted in the β-CD cavity [11-13]. More detailed indications concerning the geometry of the inclusion complex can be derived from the evidence of spatial proximities between protons of β-CD and FLZ. Partial contour plot of 2D (^1H - ^1H) ROESY spectra of inclusion complex of β-CD and FLZ is shown in figure 7. Analysis of ROESY data revealed that



(a)



(b)



(c)

Figure 8
FLZ docked with β -CD, FLZ is shown in Ball and Stick, β -CD in sticks (a) an all atom model with Connolly surface representation of FLZ, (b) FLZ and β -CD (side-view) and (c) FLZ and β -CD (top-view).
 Hydrogens are not shown in (b) and (c) for sake of clarity. All other atoms are shown in their elemental colour.

both the cavity protons of β -CD (H-5' and H-3') share weak NOE's with triazole ring protons (H-8, 10 and H-9, 11). H-5' protons of β -CD show NOE's with H-1 (weak), H-2 (weak) and H-3 (very weak). On the other hand H-3' protons of β -CD show NOE's with H-1 (strong), H-3 (strong) and H-2 (weak-to-strong). Collectively, these data suggest that the *m*-difluorophenyl moiety penetrates deep into the β -CD cavity from the wider rim side. The inclusion of *m*-difluorophenyl ring from narrower rim side is not possible based on the weak NOE's with H-5'. The penetration of triazole ring was ruled out on the basis of weak NOE's cross peaks between triazole ring protons and β -CD protons.

We performed molecular modelling studies, to rationalize the NMR experiment results described above. The Dock module of MOE was used to dock flexible FLZ into a rigid β -CD (25 runs). From the resulting complex, the one with the best binding energy was then energy-minimized keeping both FLZ and β -CD flexible. The energy-minimized complex of FLZ and β -CD is shown in figure 8. It is interesting to note that starting in a random location and orientation, the docked minimum energy conformation of FLZ with β -CD is in very good agreement with the distances obtained from 2D ROESY spectra. The complex has the *m*-difluorophenyl ring of FLZ nestled in the truncated cone of β -CD entering from the secondary rim (the wider opening), and the triazole rings are oriented towards the primary not secondary rim. A Hydrogen bond is also observed between the OH of FLZ and an OH of β -CD at position 3 that would contribute to the stability of the host-guest complex.

Conclusion

In summary, our NMR experiments confirm that FLZ forms 1:1 complex with β -CD in aqueous medium. Employing experimental and theoretical methods, the present work unambiguously determined the geometrical inclusion parameters of FLZ with β -CD. The ROESY experiments showed that the complex is formed with the *m*-difluorophenyl ring of FLZ inside the β -CD torus cavity. These results were also supported by molecular modelling which also highlight a hydrogen bond formation between the host and the guest providing stability to the complex.

List of abbreviations

FLZ: fluconazole; CD: cyclodextrin; β -CD: β -cyclodextrin; ^1H NMR: proton Nuclear Magnetic Resonance spectroscopy; 2D: two dimensional; COSY: COReLation Spectroscopy; ROESY: Rotating frame Overhauser Effect Spectroscopy; NOE: Nuclear Overhauser Effect; [H]: host; [G]: guest; MOE: Molecular Operating Environment.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SKU conceived of the study, collected the NMR data, processed and analysed all NMR spectra, discussion of the results and wrote the manuscript. GK carried out the molecular docking studies. Both authors read and approved the final manuscript.

Acknowledgements

SKU and GK are recipients of Research Associateship from the centre of excellence, Department of Biotechnology, Government of India, awarded Prof. A. Surolia. SKU is grateful to Prof. A. Surolia and Dr. Monica Sundd for teaching and training me in NMR.

References

1. Meunier F, Aoun M, Gerard M: **Therapy for oropharyngeal candidiasis in the immunocompromised host: a randomized double-blind study of fluconazole vs. ketoconazole.** *Rev Infect Dis* 1990, **12**:S364-S368.
2. Nihtinen A, Anttila VJ, Elonen E, Juvonen E, Volin L, Ruutu T: **Effect of fluconazole prophylaxis on the incidence of invasive candida infections and bacteraemias in patients with acute leukaemia.** *Eur J Haematol* 2008, **80**:391-396.
3. MacMillan ML, Goodman JL, DeFor TE, Weisdorf DJ: **Fluconazole to prevent yeast infections in bone marrow transplantation patients: a randomized trial of high versus reduced dose, and determination of the value of maintenance therapy.** *Am J Med* 2002, **112**:369-379.
4. Szejtli J: **Introduction and general overview of cyclodextrin chemistry.** *Chem Rev* 1998, **98**:1743-1754.
5. Dodziuk H: **Cyclodextrins and Their Complexes. Chemistry, Analytical Methods, Applications.** Wiley-VCH: London; 2006.
6. Fielding L: **Determination of association constant (Ka) from solution NMR data.** *Tetrahedron* 2000, **56**:6151-6170.
7. Neuhaus DL, Williamson MP: **The Nuclear Overhauser Effect in Structural and Conformational Analysis.** 2nd edition. Wiley-VCH, New York; 2000.
8. Job P: **Formation and stability of inorganic complexes in solution.** *Ann Chim* 1925, **9**:113-125.
9. Scott RL: **Some comments on the Benesi-Hildebrand equation.** *Recl Trav Chim Pays-Bas* 1956, **75**:787-789.
10. Benesi HA, Hildebrand JH: **A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons.** *J Am Chem Soc* 1949, **71**:2703-2707.
11. Schneider HJ, Hackett F, Rudiger V, Ikeda H: **NMR studies of cyclodextrins and cyclodextrin complexes.** *Chem Rev* 1998, **98**:1755-1785.
12. Ali SM, Upadhyay SK: **Complexation study of midazolam hydrochloride with β -cyclodextrin: NMR spectroscopic study in solution.** *Magn Reson Chem* 2008, **46**:676-679.
13. Upadhyay SK, Ali SM: **Solution structure of loperamide and β -cyclodextrin inclusion complexes using NMR spectroscopy.** *J Chem Sci* 2009, **121**:521-527.

Publish with **ChemistryCentral** and every scientist can read your work free of charge

"Open access provides opportunities to our colleagues in other parts of the globe, by allowing anyone to view the content free of charge."

W. Jeffery Hurst, The Hershey Company.

- available free of charge to the entire scientific community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
<http://www.chemistrycentral.com/manuscript/>



ChemistryCentral