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Study the Structure and Dynamics of Antifungal Agent Ketoconazole by CSA and Site-Specific Spin-Lattice Relaxation Time Measurements

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Abstract: An azole antifungal agent, ketoconazole, is widely used in the treatment of mucosal fungal infections related to AIDS immunosuppression, organ transplantation, and cancer chemotherapy. The structure and dynamics of ketoconazole are thoroughly studied by chemical shift anisotropy tensor and site-specific spin-lattice relaxation time measurements. The molecular correlation time at crystallographically different carbon sites is calculated by considering that the spin-lattice relaxation mechanism for the ^{13}C nucleus is mainly governed by chemical shift anisotropy interaction and hetero-nuclear dipole-dipole coupling. The CSA parameters at the crystallographically distinct sites of ketoconazole are determined by two-dimensional phase adjusted spinning sideband (2D PASS) cross-polarization magic angle spinning (CP-MAS) solid-state NMR experiment. The site-specific spin-lattice relaxation time is measured by the Torchia CP experiment. The spin-lattice relaxation rate is slow for all the carbon nuclei sites except C2, C3, C4, C5, and C26 carbon nuclei reside on the piperazine ring and the methyl group. It suggests the close-pack arrangement of the molecule due to π - π stacking interaction. The molecular correlation time of all the carbon atoms reside on the benzene ring, 1,3-dioxolane ring, imidazole ring, and the 2,4-dichlorobenzene ring is of the order of 10^{-4} s, while it is of the order of 10^{-7} s for carbon atoms reside on the piperazine ring. The CSA parameters of the carbon nuclei on the piperazine ring (C2, C3, C4, C5), and the methyl group (C26) are very low compared to other carbon nuclei. The CSA parameters are very high for carbon nuclei reside on the benzene ring, imidazole ring, and the 2,4-

dichlorobenzene ring due to the presence of π -electrons. A huge variation of the spin-lattice relaxation time and the molecular correlation time are observed for numerous carbon nuclei situated on the side-chain of ketoconazole. The spin-lattice relaxation time varies from 500 s to 8 s, and the molecular correlation time varies in the range of 10^{-4} s to 10^{-7} s. These types of investigations portrayed the correlation between the structure and dynamics of the antifungal drug ketoconazole, which will help to develop the advanced antifungal drugs. Additionally, the CSA information of the drug molecules will be immensely useful for NMR crystallography.

1. Introduction:

The azole class compounds are the most potent antifungal drug for its lower toxicity, higher efficacy, and a broad spectrum of activity. Ketoconazole is an azole class compound and a synthetic derivative of phenylpiperazine with antifungal and antineoplastic activity. It is also considered as an antimitotic and a bacteriostatic agent for gram-positive bacteria. Ketoconazole has been used extensively in the treatment of superficial mycoses such as dermatophytosis and candidiasis.¹⁻⁴ This antifungal imidazole agent has also been used for the treatment of histoplasmosis, human systemic fungal infections, against *Candida* species, *Cryptococcus neoformans*, tinea corporis, tinea cruris, tinea manuum and tinea pedis, onychomycosis, seborrheic dermatitis.⁵⁻¹⁶ Ketoconazole prohibits the conversion of lanosterol to ergosterol (which is required to maintain the integrity of the organism's cell membrane) by inhibiting the fungal cytochrome P450 (CYP51). Cytochrome P-450 enzyme is necessary to remove the C-14 methyl group of lanosterol.^{17,18} Ketoconazole also can blockage the conversion of lanosterol to cholesterol in mammals. It can block adrenal steroidogenesis by inhibiting the corticoid 11β –hydroxylase. Because of this property, ketoconazole is used for the treatment of prostate cancer and Cushing's syndrome, respectively.¹⁸

Solid state NMR spectroscopy is an indispensable tool to determine the structure and dynamics of a compound. The chemical shift anisotropy (CSA) tensor is one of the most important parameter which can be measured by applying sophisticated SSNMR techniques. It can provide information about the electronic distribution, molecular orientation, and molecular dynamics surrounding a nucleus. The Larmor precession frequency of the nucleus depends on the orientation of the molecular moiety with respect to the external magnetic field and the electronic distribution around it. The shift of the precession frequency of the nucleus inside the matter is expressed as $\omega(\theta, \varphi) - \omega_0 = \omega_{iso} + (\Delta\delta/2)(3\cos^2\theta - 1 - \eta\sin^2\theta\cos 2\varphi)$, where the anisotropy parameter $\Delta\delta$ measures the deviation of the electron cloud from the spherically symmetric charge distribution, and the asymmetry parameter η measures the deviation of electronic charge distribution from axially symmetric shape. In principal axis system (PAS), θ , and φ are respectively the polar and azimuthal angles with respect to the direction of the applied magnetic field($\mathbf{B}_0$).¹⁹ The effective magnetic field experienced by the nucleus is $\mathbf{B}_{eff} = (1 \pm \delta)\mathbf{B}_0$. The dimensionless quantity δ is the chemical shift anisotropy (CSA) tensor. It is a second rank tensor with nine components.¹⁹⁻²⁶ The diagonal components of this tensor in the principal axis system (PAS) are^{27,28}

$$\delta_{33} = \frac{e^2}{2m} \langle 0 \left| \frac{x^2 + y^2}{r^3} \right| 0 \rangle - \left(\frac{e\hbar}{2m} \right)^2 \sum_n \left\{ \frac{\langle 0 | L_z | n \rangle \langle n | \frac{2L_z}{r^3} | 0 \rangle}{(E_n - E_0)} + \frac{\langle 0 | \frac{2L_z}{r^3} | n \rangle \langle n | L_z | 0 \rangle}{(E_n - E_0)} \right\} \quad (1)$$

$$\delta_{11} = \frac{e^2}{2m} \langle 0 \left| \frac{y^2 + z^2}{r^3} \right| 0 \rangle - \left(\frac{e\hbar}{2m} \right)^2 \sum_n \left\{ \frac{\langle 0 | L_x | n \rangle \langle n | \frac{2L_x}{r^3} | 0 \rangle}{(E_n - E_0)} + \frac{\langle 0 | \frac{2L_x}{r^3} | n \rangle \langle n | L_x | 0 \rangle}{(E_n - E_0)} \right\} \quad (2)$$

$$\delta_{22} = \frac{e^2}{2m} \langle 0 | \frac{x^2 + z^2}{r^3} | 0 \rangle - \left(\frac{e\hbar}{2m} \right)^2 \sum_n \left\{ \frac{\langle 0 | L_y | n \rangle \langle n | \frac{2L_y}{r^3} | 0 \rangle}{(E_n - E_0)} + \frac{\langle 0 | \frac{2L_y}{r^3} | n \rangle \langle n | L_y | 0 \rangle}{(E_n - E_0)} \right\} \quad (3)$$

Where L_x , L_y and L_z are the components of angular momentum along the x , y and z axes respectively. The first part of these three equations originates from the spherical distribution of electronic charges when the electrons are in s orbital state. The second term comes from the distortion of spherical charge distribution when electrons are in p orbital state. In liquid-state NMR spectroscopy, due to the random motion of the molecule, the off-diagonal term of CSA tensor is averaged out and only the diagonal components survive.²⁹

The CSA tensor can be determined by solid-state NMR techniques like two dimensional MAS/CSA NMR experiment;³⁰ SUPER (separation of undistorted powder patterns by effortless recoupling);³¹ ROCSA (recoupling of chemical shift anisotropy);³² RNCSEA (γ -encoded RN_n^V -symmetry based chemical shift anisotropy);³³ 2DMAF (Two-dimensional magic angle flipping) experiment;³⁴⁻³⁶ 2DMAT (two-dimensional magic angle turning) experiment.³⁷ 2DPASS CP-MAS (two-dimensional phase adjusted spinning sideband cross-polarization magic angle spinning) SSNMR experiment^{38,39} was developed to separate anisotropic and isotropic chemical shift interactions under low magic angle spinning (MAS) frequency using sequences of five π pulses. The correlation between the structure and dynamics of biopolymer and drugs molecules⁴⁰⁻⁵¹ were studied by measuring principal components of CSA parameters and site specific spin-lattice relaxation time by applying 2DPASS CP-MAS SSNMR experiments^{38,39} and Torchia CP experiment⁵² respectively. The main focus of the present work is to study the structure and dynamics of antifungal agent ketoconazole by the 2DPASS CP-MAS SSNMR experiments^{38,39} and the Torchia CP

experiments.⁵² The molecular correlation time at crystallographically different carbon sites of ketoconazole is also calculated by considering that the spin-lattice relaxation mechanism for ^{13}C carbon is mainly governed by hetero-nuclear dipole-dipole coupling and CSA-interaction. These types of investigations will provide a vivid picture about the structure and dynamics of ketoconazole, which will help to develop advanced antifungal drugs.

2. Experimental

2.1 NMR- Measurements

The active pharmaceutical ingredient of ketoconazole was purchased from Sigma Aldrich. ^{13}C CP-MAS solid-state NMR experiments were performed on a JEOL ECX 500 NMR spectrometer. The resonance frequency for the ^{13}C nucleus was 125.721 MHz. All the experiments were performed by using a 3.2 mm JEOL double resonance MAS probe. The MAS frequency for the ^{13}C CP-MAS NMR experiment was 10 kHz. Contact time for Cross-Polarization (CP) was 2 ms, with a repetition interval of 30 s, and SPINAL-64 ^1H decoupling at 3072 accumulations time. ^{13}C spin-lattice relaxation experiment was performed by using the Torchia CP method with contact time 2ms.⁵²

2.2 CSA-Measurements

The analysis of static ^{13}C solid-state NMR spectrum encounters considerable difficulties because anisotropic interactions give rise to a broad powder-pattern and a considerable loss of sensitivity compared to ^{13}C liquid state NMR spectrum. Besides, the spin-lattice relaxation time of the ^{13}C nucleus of some organic compounds is extremely large, which makes the experiments unreasonably long. ^{13}C CP-MAS SSNMR experiment removes anisotropic interactions of the second rank and the spin-lattice relaxation time of proton is considerably fast. Hence, the ^{13}C CP-MAS SSNMR experiment enhanced the sensitivity, decrease the duration of the experiment, and make to spectrum simple. But the information about the three-dimensional molecular conformation and local electronic distribution surrounding a

nucleus is lost, because all these informations are encoded in the anisotropic interactions. The chemical shift anisotropy (CSA) parameters can be restored by reducing the magic angle spinning (MAS) frequency less than the span of the chemical shift anisotropy. In that condition, the solid-state NMR spectra contain sets of sidebands spaced in MAS frequency.^{53,54} ¹³C 2DPASS CP-MAS SSNMR experiments^{38,39} can extract the CSA parameters by manipulating spinning sidebands. Chemical shift anisotropy (CSA) tensor can be extracted by using the intensities of these spinning sidebands by incorporating Herzfeld and Berger method.⁵⁵ This experiment is preferable compared to two-dimensional magic angle flipping (2DMAF) experiment,^{35,36} and two-dimensional magic angle turning (2DMAT) experiment³⁷ due to the following reasons. To achieve the flipping of the magic angle during one experiment in 2DMAF experiment, it is required a complicated probe design. On the other hand, 2DMAT is not a constant time experiment, so it would be difficult to extract CSA information from the two-dimensional spectrum.

The pulse sequence of the 2DPASS experiment with five π pulses was introduced by Antzutkin et al.³⁸ The time duration of the PASS sequence is constant and the time gap among five π pulses varied according to the PASS equations. The 2DPASS CP MAS NMR experiments were carried out at two MAS frequencies 600 Hz and 2000 Hz. The contact time was 2ms to maintain CP condition. The 90 degree pulse length for ¹³C nucleus was 3 μ s.

3. Determination of Chemical Shift Anisotropy Parameter:

The chiral drug ketoconazole is a racemic (1:1) mixture of enantiomers of the cis configuration.^{3,4} The chemical formula of this imidazole derivative is cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine). It is insoluble in water. It is a weak base with pKa values of 6.51 and 2.94. Figure 1 (a) shows the chemical structure of ketoconazole molecule, which is associated with the rich aromatic molecular moieties like N-acetylpiperazine phenyl group,

1,3 dioxolane ring, imidazole ring, and dichlorophenyl ring. The piperazine rings have chair conformations, and the benzene ring is distorted because three atoms of the ring are split on the basis of their thermal parameters.^{56,57} Phenylpiperazine ring has no contribution to interact with the heme group CYP51, it interact with amino acid residues in the substrate access channel. The conformations of the 1,3-dioxolane rings are halfway between an envelope with flap at the unsubstituted carbon atom.^{56,57} Stereochemical orientation of the dioxolane ring plays a vital role in the inhibition of the hedgehog signalling pathway and it is also responsible for inhibition of CYP3A4 to thwart coordination of the molecular oxygen, essential for oxidation.⁵⁸⁻⁶⁰ The imidazole and the 2,4-dichlorobenzene rings have planar conformation.^{56,57} The antifungal action of the azole group ketoconazole is due to the binding of the imidazole nitrogen with cytochrome P45051.⁵⁸⁻⁶¹ The ketoconazole drug is entrenched in the hydrophobic region. The lipid-drug complex is fabricated by the action of the van der Waals type of interactions and electrostatic interaction.⁶² Figure 1(b) shows the ¹³C CP-MAS SSNMR spectrum of ketoconazole. Isotropic chemical shift is highest for those carbon nuclei which are in sp² hybridization and bonded with electronegative atoms oxygen, nitrogen, and chlorine. It is lowest for those carbon atoms which are in sp³ hybridization like C2, C3, C4, C5 atoms reside on the piperazine ring, and the methyl group carbon C26.

Figure 1: (a) Chemical structure of ketoconazole molecule. It is associated with five rings – piperazine ring, benzene ring, 1,3-dioxolane ring, imidazole ring, and 2,4-dichlorobenzene ring. It forms a close-pack arrangement by $\pi \dots \pi$ stacking interaction. (b) ^{13}C CP-MAS NMR spectrum of ketoconazole.

Table1: Principal components CSA tensor of ketoconazole molecule

CSA parameters of ketoconazole molecule								
Car bon nucl ei	δ_{11} (ppm)	δ_{22} (ppm)	δ_{33} (ppm)	Span (ppm) Ω $= \delta_{11} - \delta_{33}$	Skew k $= \frac{3(\delta_{22} - \delta_{iso})}{\Omega}$	δ_{iso} (ppm)	Anisotr opy (ppm) $\Delta\delta = \frac{\delta_{33} - (\delta_{11} + \delta_{22})}{2}$	Asymmetry η $= \frac{\delta_{22} - \delta_{11}}{\delta_{33} - \delta_{iso}}$
C1	240.6 9	171.3 7	95.83	144.8 5	0.04	169.3	-110.2	0.9
C9	231.3 6	150.1 8	78.05	153.3	-0.06	153.2	117.2	0.9
C6	222.0 7	137.5 7	70.64	151.4 3	-0.1	143.4	117.9	0.85
C17	245.3 3	108.7 7	59.3	185.9 9	-0.46	137.8	161.3	0.46
C23 C25	231.9 7	128.4 6	41.26	190.7	-0.08	133.9	147.1	0.89
C21 C24	222.2 6	130.6 8	43.63	178.6	-0.02	132.2	135.1	0.97
C18 C20	208.2 8	128.5 7	51.64	156.6 4	-0.02	129.5	118.2	0.97
C22	189.2 5	133.0 4	47.19	142.0 5	0.2	123.1 6	-113.9	0.74
C19	189.6 3	130.6 9	46.22	143.4 1	0.18	122.1 8	-113.9	0.77
C8, C10	215.6 1	99.92	35.63	179.9 8	-0.3	117.0 5	147.8	0.65
C7, C11	218.3 5	90.47	33.56	184.7 9	-0.38	114.1 2	156.33	0.5
C15	134.7 4	109.2 7	83.88	50.86	0	109.3	38.17	1
C13	121.4 4	59.53	47.92	73.52	-0.68	76.3	67.7	0.2
C12	108.8 5	58.8	37.84	71.01	-0.41	68.5	60.5	0.5
C14	106.6	52.09	40.18	66.44	-0.64	66.3	60.5	0.3
C16	69.23	52.38	36.33	32.89	-0.02	52.6	24.87	0.97
C3, C4	59.49	48.05	34.96	24.52	0.06	47.5	-18.8	0.9
C2, C5	52.9	42.2	26.9	26	0.18	40.68	-20.7	0.77
C26	40.2	14.58	14.5	25.69	-1	23.1	25.66	0

Figure 2 shows the ^{13}C 2DPASS CP-MAS SSNMR spectrum of ketoconazole. The direct dimension represents the pure isotropic spectrum, and the indirect dimension represents the anisotropic spectrum. Table 1 shows the principal components of CSA parameters at numerous carbon nuclei sites of ketoconazole. Figure 3 and Figure 4 show the spinning CSA sideband pattern of various carbon nuclei reside on the imidazole ring and the side chain of ketoconazole molecule. The centre of gravity of the spinning CSA sideband pattern is the isotropic chemical shift $\left\{ \delta_{iso} = \frac{\delta_{11} + \delta_{22} + \delta_{33}}{3} \right\}$. The resonance frequency of numerous carbon nuclei differs according to the electronic distribution and the orientation of the molecular moiety with respect to the external magnetic field. It is shifted towards the higher frequency side (δ_{11}) and the lower frequency (δ_{33}) side due to the deshielding effect and the shielding effect respectively. The third principal value (δ_{22}) of CSA tensor arises when the orientation of the molecular moiety is perpendicular to the axes of δ_{11} and δ_{33} .²⁹ The left and right edges of the spinning CSA sideband pattern corresponds to the largest (δ_{11}) and smallest (δ_{33}) chemical shift respectively. Whereas δ_{22} corresponds to the position of the maximum intensity of the CSA sideband pattern when $\delta_{11} \geq \delta_{22} \geq \delta_{33}$. The maximum width of the CSA pattern is defined as ‘span’ ($\Omega = \delta_{11} - \delta_{33}$). The asymmetry $\left(\eta = \frac{\delta_{22} - \delta_{11}}{\delta_{33} - \delta_{iso}} \right)$ parameter defines how much the CSA pattern deviates from its axially symmetric shape. If $\delta_{11} = \delta_{22}$ or $\delta_{22} = \delta_{33}$, then the CSA pattern is axially symmetric and the value of the asymmetry parameter is zero. The orientation of the asymmetry of the CSA pattern is represented by the parameter called ‘skew’ $k = \frac{3(\delta_{33} - \delta_{iso})}{\Omega}$. The spinning CSA sideband pattern is asymmetric for C1, C9, C6, C23, C25, C21, C24, C18, C20, C22, C19, C15, C16, C3, C4, C2, and C5 nuclei and it is axially symmetric for C26 nuclei. For C13 and C14 nuclei, the spinning CSA sideband patterns are nearly axially symmetric. The magnitude of the anisotropy $\left[\Delta\delta = \delta_{33} - \frac{(\delta_{11} + \delta_{22})}{2} \right]$ parameter measures the largest separation of spinning CSA sideband pattern from

the center of gravity. The sign of the anisotropy parameters says on which side of the center of gravity, the separation is the largest. Figure 5 shows the bar-diagram of anisotropy and asymmetry parameter at numerous carbon nuclei sites of ketoconazole.

Ketoconazole molecule is chemically related to miconazole. The bioavailability of this antimycotic agent originates from its capability to penetrate the cell membrane due to the presence of the methyl group at C1 position(carbonyl group carbon).⁶³ The chemical shift is the highest for carbonyl group carbon. One of the reasons of the large value of CSA parameters of carbonyl group carbon is the magnetic anisotropy. There is no symmetry axis in the carbonyl group; naturally, there arise three different magnetic susceptibilities χ_x, χ_y, χ_z along three mutually perpendicular directions of the principal axes system (PAS). There originate two anisotropic susceptibilities -one parallel to the magnetic field ($\Delta\chi_{\parallel} = \chi_z - \chi_x$) and another perpendicular to the magnetic field ($\Delta\chi_{\perp} = \chi_y - \chi_x$).⁶⁴ The McConnell equation⁶⁵ of magnetic anisotropy for non-symmetric carbonyl group is $\delta_{aniso} = \{\Delta\chi_{\parallel}(3\cos^2\theta_1 - 1) + \Delta\chi_{\perp}(3\cos^2\theta_2 - 1)\}/3R^3$, where θ_1 is the angle between the radius vector R and x-axis and θ_2 is the angle between radius vector R and z-axis.⁶⁶ Large amount of directional dependent magnetic field is generated due to this highly anisotropic magnetic susceptibility. This manifests as a large value of anisotropic chemical shift.

Figure 2: ^{13}C 2DPASS CP-MAS NMR spectrum of ketoconazole. Direct dimension represents infinite spinning speed spectrum, and the indirect dimension represents chemical shift anisotropy spectrum.

Among five rings of ketoconazole molecule, CSA parameters are very low for carbon nuclei (C2, C3, C4, C5) reside on the piperazine ring and the 1,3 dioxolane ring. But CSA parameters are very high for carbon nuclei reside on the benzene ring, imidazole ring, and the 2,4-dichlorobenzene ring due to the presence of π -electrons, which induced nonspherical distribution of electronic charge density on three rings. The existence of the π -electron surrounding the nucleus is responsible for the appearance of magnetic shielding and deshielding effect.⁶⁷ A paramagnetic current is generated when the π -electron circulates the nucleus in a clockwise direction and there induced a magnetic field along the direction of the

external magnetic field. As a consequence, the net magnetic field experienced by the nucleus is increased, which is known as the magnetic deshielding effect. On the other hand, when an π –electron revolves around the nucleus along the counter-clockwise direction, a magnetic field is generated along the opposite direction of the external magnetic field. As a consequence, the value of the net magnetic field is decreased, which is known as the magnetic shielding effect. These magnetic shielding and deshielding effects manifest themselves as a large value of chemical shift anisotropy. On the contrary, piperazine and 1,3 dioxolane rings are not associated with π –electrons, hence CSA parameters are not large for carbon nuclei reside on these two rings.

Figure 3: Spinning CSA sideband pattern of crystallographically different carbon sites of ketoconazole.

Figure 4: Spinning CSA sideband pattern of crystallographically different carbon sites of ketoconazole.

Figure 5: The bar-diagram of (a) anisotropy and (b) asymmetry parameter of ketoconazole.

Determination of Spin-lattice Relaxation Time and Molecular Correlation Time:

The dipole-dipole interaction, chemical shift anisotropy (CSA) interaction, spin-rotation interaction, scalar coupling, and the quadrupolar coupling play significant role in the spin-lattice relaxation mechanism. But for large molecules the contribution of spin-rotation interaction and scalar coupling is insignificant. Hence, for spin 1/2 carbon nuclei, the spin-

lattice relaxation mechanism is mainly dominated by chemical shift anisotropy interaction and dipole-dipole coupling interaction. The contribution of chemical shift anisotropy interaction in spin-lattice relaxation mechanism is expressed as⁶⁸⁻⁷¹

$$\frac{1}{T_1^{CSA}} = \frac{2}{15} \gamma^2 B^2 S^2 \left(\frac{\tau_2}{1 + \omega^2 \tau_2^2} \right) \quad (4)$$

Where correlation time $\tau_c = 3 \tau_2$ and B is the applied magnetic field. Where $S^2 = (\Delta\delta)^2(1 + \eta^2/3)$ and $\left[\Delta\delta = \delta_{33} - \frac{(\delta_{22} + \delta_{11})}{2} \right]$, $\left(\eta = \frac{\delta_{22} - \delta_{11}}{\delta_{33} - \delta_{iso}} \right)$.

The role of dipole dipole interaction in spin-lattice relaxation mechanism is expressed as⁷⁰

$$\frac{1}{T_1^{DD}} = \frac{1}{10} \left(\frac{\gamma_C \gamma_X \hbar}{r_{CX}^3} \right)^2 \tau_2 \left[\frac{3}{1 + \omega_C^2 \tau_2^2} + \frac{1}{1 + (\omega_X - \omega_C)^2 \tau_2^2} + \frac{6}{1 + (\omega_X + \omega_C)^2 \tau_2^2} \right] \quad (5)$$

By keeping only the first term,

$$\frac{1}{T_1^{DD}} = \frac{1}{10} \left(\frac{\gamma_C \gamma_X \hbar}{r_{CX}^3} \right)^2 \tau_2 \left[\frac{3}{1 + \omega_C^2 \tau_2^2} \right] \quad (6)$$

Where X represents hydrogen, oxygen, chlorine and nitrogen atoms. r_{CX} is the distance between carbon and the nearest neighbour atoms like hydrogen, oxygen, nitrogen, chlorine.

The bond-distance is calculated by X-ray crystallography study.^{56,57} Larmor precession frequency of carbon nucleus is $\omega_C = 2\pi f = 2 \times 3.14 \times 125.758 \text{ MHz} =$

789.76024 MHz. $B = 11.74 \text{ T}$, $\gamma_C = 10.7084 \text{ MHz/T}$, $\gamma_H = 42.577 \text{ MHz/T}$, $\hbar = 1.054 \times 10^{-34} \text{ Js}$.

The spin-lattice relaxation rate for ^{13}C can be expressed as

$$\begin{aligned}
\frac{1}{T_1} &= \frac{1}{T_1^{CSA}} + \frac{1}{T_1^{DD}} \\
&= \frac{2}{15} \gamma^2 B^2 S^2 \left(\frac{\tau_2}{1 + \omega^2 \tau_2^2} \right) \\
&\quad + \frac{1}{10} \left(\frac{\gamma_C \gamma_X \hbar}{r_{CX}^3} \right)^2 \tau_2 \left[\frac{3}{1 + \omega_c^2 \tau_2^2} \right] \quad (7)
\end{aligned}$$

Molecular correlation time is calculated by this equation.

Table 2: Spin-lattice relaxation time and molecular correlation time of ketoconazole

Carbon Nuclei	Spin-lattice relaxation time (s)	Molecular Correlation time (s)
C1	500 ± 15	6.3×10^{-4}
C9	480 ± 12	6.7×10^{-4}
C6	262 ± 5	3.6×10^{-4}
C17	374 ± 10	8.3×10^{-4}
C23, C25	385 ± 10	8.4×10^{-4}
C21, C24	455 ± 10	8.7×10^{-4}
C18, C20	335 ± 10	4.9×10^{-4}
C22	280 ± 5	3.4×10^{-4}
C19	272 ± 5	3.4×10^{-4}
C8, C10	211 ± 5	4.2×10^{-4}
C7, C11	215 ± 5	4.6×10^{-4}
C15	412 ± 10	6.4×10^{-4}
C13	323 ± 5	1.2×10^{-4}
C12	250 ± 5	7.9×10^{-5}
C14	353 ± 5	1×10^{-4}

C16	213 ± 5	1.4×10^{-5}
C3, C4	8 ± 2	2.9×10^{-7}
C2, C5	12 ± 2	4.9×10^{-7}
C26	9 ± 2	4.7×10^{-7}

Figure 6 shows the longitudinal magnetization decay curves at (a) C1, (b) C9, (c) C6, and (d)

C23, C25 nuclei sites of ketoconazole molecule. Figure 6(e) shows the bar-diagram of the

spin-lattice relaxation time at chemically different carbon nuclei sites. From Table 2, it is

clear that the spin-lattice relaxation rate is slow for the carbon nuclei reside on imidazole

ring, 1,3 dioxolane ring, benzene ring, and 2, 4-dichlorobenzene ring. It suggests the close-

pack arrangement of the molecule due to $\pi - \pi$ stacking interaction.^{61,72} As it is mentioned

before, the CSA parameters of the carbon nuclei reside on these four rings are larger

compared to the carbon nuclei C2, C3, C4, C5 and C26 reside on the piperazine ring and the

methyl group respectively. The spin-lattice relaxation rate is fast for C2, C3, C4, C5 and C26

carbon nuclei. The 1,3-dioxolane ring is responsible for inhibition of CYP3A4 to thwart

coordination of the molecular oxygen, essential for oxidation. The stereochemical orientation

of the 1,3-dioxolane ring plays a significant role in inhibition of the hedgehog signalling

pathway.^{61,72} Spin-lattice relaxation time of C17, C18, and C19 carbon nuclei reside on

dioxolane ring is 374s, 335s and 272s respectively. The molecular correlation time is

8.3×10^{-4} s, 4.9×10^{-4} s and 3.4×10^{-4} s for C17, C18 and C19 respectively. The values

of the CSA parameters are very large. The keto group is located in the polar pocket in

ketoconazole molecule.⁶¹ The spin-lattice relaxation time is longest (500 s) for C1 carbon

reside on keto group. Both the values of isotropic chemical shift and anisotropic chemical

shift are also highest for C1. Table 2 shows that the molecular correlation time of all the

carbon atoms reside on the benzene ring, 1,3-dioxolane ring, imidazole ring, and the 2,4-

dichlorobenzene ring is of the order of 10^{-4} s, while it is of the order of 10^{-7} s for carbon

atoms reside on the piperazine ring and the methyl group. Hence, motional degrees of freedom of the carbon nuclei reside on the piperazine ring are much higher than those who reside on the other four rings of ketoconazole. As it was mentioned before that the piperazine rings have chair conformations, and it has no contribution to interact with the heme group CYP51. It interacts with amino acid residues in the substrate access channel. The antifungal action of theazole group ketoconazole is due to the binding of the imidazole nitrogen with cytochrome P45051. The CSA parameters of those carbon nuclei (C2, C3, C4, C5) reside on the piperazine ring is also very low compared to those carbons reside on the other four rings. The long side-chain of ketoconazole played a vital role in adjusting the physicochemical properties of the molecule to improve the pharmacokinetic and pharmacodynamic behavior. The CSA parameters are very high for carbon nuclei reside on the side-chain of the molecule like benzene ring, imidazole ring, and the 2,4-dichlorobenzene ring due to the presence of π -electrons. **Figure 6(e) and Figure 7** show a substantial variation of the motional degrees of freedom of side-chain carbon nuclei. The spin-lattice relaxation time varies from 500 s to 8 s; and the molecular correlation time varies in the range of 10^{-4} s to 10^{-7} s.

Figure 6: The longitudinal magnetization decay curves at (a) C1, (b) C9, (c) C6, and (d) C23, C25 nuclei sites of ketoconazole. (e) Bar-diagram of spin-lattice relaxation time at chemically different carbon nuclei sites of ketoconazole.

Figure 7: Bar-diagram of molecular correlation time at various carbon nuclei sites of ketoconazole.

4. Conclusion:

A lipophilic imidazole derivative, ketoconazole, is used for the treatment of mucosal fungal infections associated with AIDS immunosuppression, organ transplantation, and cancer chemotherapy. The structure and dynamics of ketoconazole have been explored by measuring chemical shift anisotropy tensor, site-specific spin-lattice relaxation time, and by calculating the molecular correlating time at the crystallographically different carbon sites. The CSA parameters at the crystallographically distinct site of ketoconazole were determined by two-dimensional phase adjusted spinning sideband (2D PASS) cross-polarization magic angle spinning (CP-MAS) solid-state NMR experiment. The site-specific spin-lattice relaxation time was determined by the Torchia CP method. The values of CSA parameters are large for the carbon nuclei involved in sp^2 hybridization and bonded with electronegative oxygen, nitrogen, and chlorine atoms. The presence of π -electrons induced nonspherical distribution of electronic charge density surroundings the carbon nuclei, which leads to the higher values

of the CSA parameters. On the contrary, CSA parameters are lower for the carbon nuclei reside on the piperazine ring, 1,3-dioxolane ring, and the methyl group i.e. for those carbon which are in sp^3 hybridization. The spin-lattice relaxation rate is faster for the carbon nuclei C2, C3, C4, C5, and C26, whose CSA parameters are lower compared to other carbon nuclei of ketoconazole molecule. On the other hand, the spin-lattice relaxation rate is slower for the carbon nuclei reside on the benzene ring, 1,3-dioxolane ring, imidazole ring, and the 2,4-dichlorobenzene ring and the keto group of ketoconazole. The CSA parameters of those carbon nuclei are also very large. This suggests the close-pack arrangement of the molecule due to the $\pi - \pi$ stacking interaction. The molecular correlation time of all the carbon atoms reside on the benzene ring, 1,3-dioxolane ring, imidazole ring, 2,4-dichlorobenzene ring, and the keto group is of the order of 10^{-4} s, while it is of the order of 10^{-7} s for carbon nuclei reside on the piperazine ring. Hence, motional degrees of freedom of the carbon nuclei reside on the piperazine ring are much higher than those who reside on the other four rings of ketoconazole. The piperazine rings have chair conformations, and it has no contribution to interact with the heme group CYP51. It interacts with amino acid residues in the substrate access channel. The antifungal action of the azole group ketoconazole is due to the binding of the imidazole nitrogen with cytochrome P45051. The CSA parameters are very large for the carbon nuclei reside on the imidazole ring. The spin-lattice relaxation rate is slow, and the molecular correlation time is of the order of 10^{-4} s. The long side chain of ketoconazole played a vital role in adjusting the physicochemical properties of the molecule to improve the pharmacokinetic and pharmacodynamic behaviour. The CSA parameters are very high for carbon nuclei reside on the side-chain of the molecule like benzene ring, imidazole ring, and the 2,4-dichlorobenzene ring due to the presence of π -electrons. A remarkable variation of the motional degrees of freedom is observed for side-chain carbon nuclei. The spin-lattice relaxation time varies from 500 s to 8 s; and the molecular correlation time varies in the range

of 10^{-4} s to 10^{-7} s. These types of in-depth analysis of the structure and dynamics of theazole class antifungal agent will help to develop advanced antifungal drugs. Additionally, the CSA information of the drug molecules will be immensely useful for NMR crystallography.

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Figures

Figure 1

(a) Chemical structure of ketoconazole molecule. It is associated with five rings – piperazine ring, benzene ring, 1,3-dioxolane ring, imidazole ring, and 2,4-dichlorobenzene ring. It forms a close-pack arrangement by $\pi\cdots\pi$ stacking interaction. (b) ^{13}C CP-MAS NMR spectrum of ketoconazole.

Figure 2

^{13}C 2DPASS CP-MAS NMR spectrum of ketoconazole. Direct dimension represents infinite spinning speed spectrum, and the indirect dimension represents chemical shift anisotropy spectrum.

Figure 3

Spinning CSA sideband pattern of crystallographically different carbon sites of ketoconazole.

Figure 4

Spinning CSA sideband pattern of crystallographically different carbon sites of ketoconazole.

Figure 5

The bar-diagram of (a) anisotropy and (b) asymmetry parameter of ketoconazole.

Figure 6

The longitudinal magnetization decay curves at (a) C1, (b) C9, (c) C6, and (d) C23, C25 nuclei sites of ketoconazole. (e) Bar-diagram of spin-lattice relaxation time at chemically different carbon nuclei sites of ketoconazole.

Figure 7

Bar-diagram of molecular correlation time at various carbon nuclei sites of ketoconazole.