Investigation of the Detailed Internal Structure and Dynamics of Itraconazole by Solid-State NMR Measurements

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ABSTRACT: The structure and dynamics of itraconazole were investigated by $^{13}$C 2DPASS MAS SSNMR and spin-lattice relaxation time measurement to get an insight into its multiple biological activities, e.g., antifungal, antiviral, anticancer activities, etc. The molecular correlation time at chemically different sites of carbon nuclei was calculated by considering that the spin-lattice relaxation mechanism is mainly dominated by chemical shift anisotropy interaction and heteronuclear dipole–dipole interaction. The spin-lattice relaxation time is long for C35, C6, C5, and C34 carbon nuclei that participated in the 1, 2, 4-triazole ring. On the contrary, it is comparatively shorter for C1, C2, C3, and C4 carbon nuclei associated with the sec-butyl group in the triazolane side-chain region. Chemical shift anisotropy (CSA) parameters of C5, C6, C34, and C35 nuclei are much higher than those of C1, C2, C3, C4 nuclei, indicating that the relaxation mechanism at a high value of magnetic field is predominated by chemical shift anisotropy interaction. The molecular correlation time of carbon nuclei residing at the side-chain region is 2–3 orders of magnitude lesser than that of those participated in the 1,2,4-triazole ring. The spin-lattice relaxation time is very long for carbon nuclei C28 and C30 bonded with chlorine. Asymmetry and anisotropy parameters are also very high for the spinning CSA sideband pattern corresponding to the C28 and C30 nuclei. The molecular correlation time is on the order of $10^{-3}$ s for C28 and $10^{-4}$ s for C30, whereas for side-chain carbon nuclei, it is on the order of $10^{-2}$ s. This suggests that the effective magnetic field experienced by C28 and C30 nuclei is affected by the polarization of the chemical bond. A huge variation in molecular correlation time is observed for chemically different sites of carbon nuclei of the itraconazole molecule. These investigations vividly portrayed how the structure is correlated with the dynamics of a valuable drug, itraconazole, with multiple biological activities. This study will enlighten the way of inventing advance medicine for multiple biological activities in the pharmaceutical industry.

1. INTRODUCTION

Itraconazole is a trizole-containing drug prescribed for the prevention and treatment of fungal infection. The primary structural difference among itraconazole and other azole antifungals is the presence of a triazolone ring (the ring consists of three nitrogens) and a sec-butyl side chain, and these are responsible for its different biological activities like antifungal and anticancer activities, as well as its interesting pharmacokinetic behavior like strong protein binding, tissue penetration, prolonged half-life and bioavailability, etc. Nitrogen atoms of the azole ring interact with the heme iron of the fungal cytochrome P4503A (CYP3A). As a result, it inhibits the function of the lanosine 14-demethylase enzyme to stop the synthesis of ergosterol. It is the only drug in the azole class of antifungal agents that inhibits the hedgehog (Hh) signaling pathway and angiogenesis, responsible for the anticancer activity. It is also used for the treatment of dermatophyte infections, sporotrichosis, penicilliosis, allergic and invasive aspergillosis, histoplasmosis, superficial candidiasis, coccidioidomycosis, blastomycosis, etc. Itraconazole is a well-tolerated drug as the mammalian cytochrome P450 enzyme is less affected even at a high concentration of the drug. Hence, the sterol and steroid pathways of the human pituitary–adrenal–testicular axis are less affected by itraconazole. It is a weak base (pKa = 3.7). It can be ionized at a low pH. It is available as capsules, intravenous preparations, and oral suspensions. However, it is insoluble in water and dilute acid solutions. Therefore, it is difficult to extract the information about the structure and dynamics of itraconazole in the solution state.

The molecular structure of itraconazole shares a striking similarity with terconazole and ketoconazole. Although terconazole and ketoconazole possess antifungal activity like itraconazole, but they fail to restrain the human umbilical vein endothelial cell (HUVEC) proliferation and to persuade the vascular endothelial growth factor receptor 2 (VEGFR 2) glycosylation defect. The range of application of triazoles (itraconazole, fluconazole, voriconazole, and posaconazole) is broader than that of ketoconazole (with an azole ring associated with two nitrogens). Itraconazole can be used for the medication of both superficial and systemic fungal infections. A single drug with multiple biological activities is not so common, and itraconazole is one of them. Hence, it is fascinating to investigate the internal structure and spin dynamics of itraconazole to get an insight into the varying dynamics in different parts of the structure responsible for different biological and pharmacokinetic behaviors (Figure 5a). The structural details and molecular
dynamics of this unique azole were investigated by $^{13}$C CP-MAS NMR spectral analysis, $^{13}$C spin-lattice relaxation time measurements, two-dimensional phase-adjusted spinning sideband (2DPASS) magic-angle-spinning (MAS) nuclear magnetic resonance (NMR) experiment, and calculation of molecular correlation time at numerous carbon nuclei situated at various chemical environments.

Chemical shift anisotropy provides valuable information about the molecular conformation and internal structure. There are various techniques to determine CSA parameters. They can be measured by the two-dimensional MAS/CSA NMR experiment and by SUPER (separation of undistorted powder patterns by effortless recoupling) MAS NMR at a magic-angle-spinning (MAS) of 2.5–5 kHz. ROCSA (recouping of chemical shift anisotropy) pulse sequence was applied to determine CSA parameters at MAS frequencies of 11–20 kHz. RNCSA (γ-encoded RN$^*$-symmetry-based chemical shift anisotropy) recoupling schemes were applied to extract CSA parameters of the system with weak homonuclear dipole–dipole interactions under a wide range of MAS frequencies. The two-dimensional magic-angle-flipping (2DMAF) experiment, two-dimensional magic-angle-turning (2DMAT) experiment, and two-dimensional phase-adjusted spinning sideband (2DPASS) magic-angle-spinning (MAS) SSNMR experiment can extract information about CSA for multiple site compounds at very low MAS speed. The total evolution period in indirect dimension for the 2DMAT experiment is not constant. Consequently, the spin–spin relaxation mechanism makes the spectrum so complicated that it would be difficult to extract the exact information about the relative abundance of chemically different sites of carbon nuclei. Thus, a probe is required for the 2DMAT experiment that can alter the orientation of the spinner during each scan. This type of probe is not commercially available. In these aspects, the two-dimensional phase-adjusted spinning sideband (2DPASS) magic-angle-spinning (MAS) SSNMR technique is more feasible as the total time during five π pulses remains constant, and this experiment can be performed using a standard commercial probe. This technique was employed to investigate the properties of glass compounds and biopolymers, but it is not yet exploited properly to investigate the internal structure and dynamic of such a valuable antifungal drug, itraconazole, with several ancillary biological activities. This study will enlighten the way of inventing advance medicine for fungal infections and design of potent drugs.

2. EXPERIMENTAL SECTION

2.1. NMR Measurements. An active ingredient of itraconazole, purchased from Sigma Aldrich, was used for solid-state NMR experiments. $^{13}$C CP-MAS solid-state NMR experiments were performed using a JEOL ECX 500 NMR spectrometer. The resonance frequency for $^{13}$C was 125.721 MHz. All of the experiments were carried out in a 3.2 mm JEOL double resonance MAS probe. The magic-angle-spinning (MAS) speed was 10 kHz for $^{13}$C CP-MAS spectrum and spin-lattice relaxation measurements. The condition of cross-polarization (CP) was maintained by keeping contact time 2 ms, and SPINAL-64 $^1$H decoupling was used during acquisition. The $^{13}$C spin-lattice relaxation experiment was conducted using the Torchia CP method.

2.2. CSA Measurements. During the slow MAS speed, the powder pattern breaks into several numbers of sidebands. The spacing among the sidebands is equal to the MAS speed. Using sideband intensities of the spinning CSA sideband pattern, CSA parameters can be measured by the Herzfeld and Berger integral method.

The pulse sequence of the 2DPASS MAS NMR experiment with five π pulses was established by Antzutkin et al. in 1995. The phase cycling for the desired coherence pathway was done by 13 steps cogwheel phase cycling. In the indirect dimension, data points were sixteen. The time evolution of five π pulses was calculated by PASS equations. The 2DPASS experiments were performed at two different values of spinning speed 600 and 2000 Hz. The CP condition for these two spinning speeds was optimized on glycin with 2 ms contact time. The 90° pulse length for $^{13}$C was 3 μs.

3. RESULTS AND DISCUSSION

3.1. Spin-Lattice Relaxation Measurements. Figure 1a shows that the itraconazole molecule is associated with three prominent regions: triazole-containing dioxolane region, phenyl–piperazine–phenyl linker region, and triazolone side-chain region. Although the phenyl–piperazine–phenyl linker region and triazolone side-chain region are not playing prominent roles in interaction with the heme group CYP51, they interact with amino acid residues in the substrate access channel. The side-chain region can easily be replaced by various functional groups like hydrazine carboxamides and meta-substituted amides. The triazole-containing dioxolane region is responsible for inhibition of CYP3A4 to thwart coordination of the molecular oxygen, essential for oxida-
(a−d) Show $^{13}$C spin-lattice decay curves of itraconazole at various resonance peak positions of carbon nuclei. (e) Shows the bar diagram of the spin-lattice relaxation time of carbon nuclei residing in various chemical environments.

| position of carbon atoms at which relaxation time is measured (ppm) | $^{13}$C spin-lattice relaxation time $T_1$ (s) of itraconazole | position of carbon atoms at which relaxation time is measured (ppm) | $^{13}$C spin-lattice relaxation time $T_1$ (s) |
|---------------------------------------------------------------|-------------------------------------------------|---------------------------------------------------------------|---------------------------------|
| C35 at 157.31 ppm                                             | 142 ± 10                                        | C7 at 155.88 ppm                                              | 162 ± 10                        |
| C6 at 154.1 ppm                                               | 197 ± 10                                        | C20 at 150.18 ppm                                             | 192 ± 10                        |
| C10 at 147.69 ppm                                             | 204 ± 10                                        | C27 at 139.5 ppm                                              | 178 ± 10                        |
| C34 at 141 ppm                                               | 90 ± 10                                         | C17 at 122.83 ppm                                             | 35 ± 5 and 2 ± 0.5              |
| C3 at 136.65 ppm                                              | 290 ± 10                                        | C30 at 132.73 ppm                                             | 196 ± 10                        |
| C28 at 131.46 ppm                                             | 330 ± 20 and 12 ± 2                            | C32 at 124.04 ppm                                             | 36 ± 5 and 2 ± 0.5              |
| overlap of C18 and C22 at 117.75 ppm                        | 45 ± 5 and 2 ± 0.5                             | Overlap of line C9 and C11 at 80.6                           | 36 ± 5                          |
| overlap of C8 and C12 at 112.04 ppm                         | 210 ± 20                                       | C16 at 55.46                                                  | 110 ± 10 and 7 ± 2             |
| overlap of line C14 and C33 at 56.52 ppm                     | 240 ± 10                                       | C3 at 56.52 ppm                                               | 32 ± 2                          |
| C23 at 71.47 ppm                                             | 180 ± 10                                       | C4 at 25.39 ppm                                               | 47 ± 2 and 2 ± 0.2              |
| C2 at 33.18 ppm                                               | 65 ± 2 and 2 ± 0.5                             | C1 at 15.46 ppm                                               | 50 ± 2 and 2 ± 0.5              |
| C25 at 74.322 ppm                                            | 73 ± 10                                        | C24 and C26 at 79.46 ppm                                      | 116 ± 10                        |
Stereocchemical orientation of the dioxolane ring plays a significant role in inhibition of the hedgehog signaling pathway. The antifungal action is due to the binding of the triazole nitrogen with cytochrome P45051 (CYP51). Figure 1b shows $^{13}$C CP-MAS NMR spectrum of itraconazole. Figure 2a–d shows $^{13}$C spin-lattice decay curves of various carbon nuclei situated in chemically and crystallographically different environments. The bar diagram of the spin-lattice relaxation time (as shown in Figure 2e) suggests that the spin-lattice relaxation time hugely varied due to the change of the chemical environment surrounding the nuclei. The spin-lattice relaxation time (as shown in Table 1) is very long for C5, C6, C34, and C35 carbon nuclei participated in the 1,2,4-triazole ring. Anisotropy parameters (Table 2) are also comparatively large for these specific sites of carbon nuclei. On the contrary, the relaxation time is shorter and CSA parameters are lower for C1, C2, C3, and C4 carbon nuclei, residing in the side-chain region.

The spin-lattice relaxation time is very long for carbon nuclei C28 and C30 bonded with the chlorine atom. Asymmetry and anisotropy parameters are also very high for the spinning CSA sideband pattern corresponding to the C28 and C30 nuclei. This suggests that the relaxation mechanism is greatly affected by chemical shift anisotropy interaction. The role of chemical shift anisotropy in the spin-lattice relaxation mechanism can be expressed as:

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

where correlation time $\tau_s = 3 \tau_{2J}$, $B$ is the applied magnetic field, $S^2 = (\Delta \delta)^2 \left(1 + \eta^2/3\right)$, and $\Delta \delta = \delta_{33} - \left(\frac{\delta_{32} + \delta_1}{2}\right)$.

### 3.2. Chemical Shift Anisotropy

Both isotropic and anisotropic components of the chemical shift are correlated with chemical bonding. The anisotropic component of chemical shift depends on the orientation and conformation of the molecule. Chemical shift anisotropy can be represented by a second-rank tensor with nine components. In the principal axis system (PAS), off-diagonal components are cancel out and three diagonal terms survive. The expressions of these diagonal components of the chemical shift anisotropy tensor ($\delta_{11}, \delta_{22},$ and $\delta_{33}$) are given by

| carbon from different chemical environments with isotropic chemical shift ($\delta_{iso}$) (ppm) | $\delta_{11}$ | $\delta_{22}$ | $\delta_{33}$ | span (ppm) | skew | $\Delta S = \delta_{33} - \left(\frac{\delta_{32} + \delta_1}{2}\right)$ | anisotropy $\eta$ | asymmetry $\eta_0$ |
|---|---|---|---|---|---|---|---|---|
| 15.51 (C11) | 26.1 | 10.2 | 10.2 | 15.9 | -1 | 15.9 | 0 |
| 25.77 (C4) | 43.9 | 21.1 | 12.3 | 31.7 | -0.5 | 27.3 | 0.5 |
| 32.12 (C2) | 46.8 | 25.1 | 24.5 | 22.4 | -0.9 | 22.1 | 0.6 |
| 33.09 | 47.7 | 31.3 | 20.4 | 27.3 | -0.2 | 21.8 | 0.8 |
| 30.65 | 42.9 | 32.3 | 16.8 | 26.1 | 0.2 | -20.9 | 0.8 |
| 49.88 (C13) | 83.8 | 50.2 | 15.7 | 68.1 | 0.0 | -51.3 | 1 |
| 55.07 (C16) | 71.7 | 58.8 | 34.7 | 36.9 | 0.3 | -30.5 | 0.6 |
| 56.78 (C14) | 77.8 | 55.9 | 36.7 | 41.1 | -0.1 | 31.5 | 0.9 |
| 57.03 (overlap of C33, C3 and C14) | 78.2 | 55.5 | 37.4 | 40.9 | -0.1 | 31.8 | 0.9 |
| 71.19 (C23) | 119.3 | 59.9 | 34.4 | 84.8 | -0.4 | 72.1 | 0.5 |
| 73.14 (C25) | 100.4 | 59.5 | 59.5 | 40.8 | -1 | 40.8 | 0 |
| 79.25 (C24 and C26) | 118.1 | 66.9 | 52.8 | 65.3 | -0.6 | 58.3 | 0.4 |
| 80.23 (C11) | 118.4 | 65.5 | 56.8 | 61.5 | -0.7 | 57.2 | 0.2 |
| 82.91 (C9) | 123.1 | 63.2 | 62.5 | 60.6 | -1 | 60.3 | 0.0 |
| 111.97 (overlap of C19 and C21) | 138.4 | 113.8 | 83.7 | 54.7 | 0.1 | -42.4 | 0.9 |
| 112.46 (overlap of C8 and C12) | 138.4 | 114.9 | 84.1 | 54.3 | 0.1 | -42.5 | 0.8 |
| 117.68 (overlap of C18 and C22) | 198.9 | 143.3 | 10.8 | 118.1 | 0.4 | -160.3 | 0.5 |
| 122.99 (C17) | 233.8 | 85.4 | 47.8 | 188 | -0.6 | 169.2 | 0.3 |
| 124.25 (C32) | 233.7 | 87.9 | 49.1 | 186.7 | -0.6 | 167.2 | 0.4 |
| 126.75 (C31) | 209 | 158.6 | 12.7 | 196.3 | 0.5 | -171.1 | 0.4 |
| 129.56 (C29) | 213.3 | 144.9 | 30.5 | 182.7 | 0.3 | -148.6 | 0.7 |
| 131.12 (C28) | 223.4 | 132.2 | 37.7 | 185.7 | 0.0 | -140.1 | 1 |
| 132.69 (C30) | 221.4 | 129.7 | 46.87 | 174.6 | -0.05 | 133.2 | 0.9 |
| 134.25 | 214.4 | 125.85 | 62.5 | 151.9 | -0.2 | 120.2 | 0.8 |
| 136.75 (C5) | 234.4 | 135.8 | 40.1 | 194.3 | -0.02 | 146.5 | 1 |
| 139.57 (C27) | 224.7 | 148.9 | 45 | 179.7 | 0.2 | -141.8 | 0.8 |
| 141.13 (C34) | 237 | 128.2 | 58.2 | 178.8 | -0.2 | 143.8 | 0.7 |
| 147.69 (C10) | 197.9 | 155.5 | 89.6 | 108.3 | 0.2 | -87.1 | 0.7 |
| 150.19 (C20) | 237.7 | 131 | 81.9 | 155.7 | -0.4 | 131.2 | 0.6 |
| 154.26 (C6) | 251.6 | 136.1 | 75.1 | 176.5 | -0.3 | 146 | 0.6 |
| 155.82 (C7) | 255.1 | 135.6 | 76.7 | 178.4 | -0.3 | 149 | 0.6 |
| 157.07 (C35) | 215.4 | 171.2 | 84.6 | 130.8 | 0.3 | -108.7 | 0.6 |
| 158.01 | 211.1 | 175.9 | 87.1 | 124 | 0.4 | -106.4 | 0.5 |
| 158.64 | 227.4 | 164.7 | 83.8 | 143.5 | 0.1 | -112.2 | 0.8 |
| 159.26 | 228.5 | 164.7 | 84.5 | 144 | 0.1 | -112.1 | 0.9 |
Figure 3. $^{13}$C 2DPASS MAS NMR spectrum of itraconazole. The direct dimension of the 2D spectrum represents pure isotropic spectrum, and the indirect dimension represents anisotropic spectrum. (a–f) Spinning CSA sideband pattern for various carbon nuclei situated in chemically different environments.

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

\[
\delta_{11} = \frac{e^2}{2m} \left\langle 0 \left| \frac{y^2 + z^2}{r^3} \right| 0 \right\rangle 
- \left( \frac{e\hbar}{2m} \right)^2 \sum_n \left\langle 0 \left| \frac{nL_z}{r^2} \right| n \right\rangle \frac{\langle nL_z|n\rangle}{(E_n - E_0)} 
+ \left\langle 0 \left| \frac{nL_z}{r^2} \right| n\rangle \frac{\langle nL_z|n\rangle}{(E_n - E_0)} \right\rangle
\]
where \( L_x, L_y, \) and \( L_z \) represent the components of angular momentum along \( x, y, \) and \( z \) directions, respectively. The first part of these three equations generates from those electrons that constitute spherically symmetric charge distribution. There arise distortions in this spherically symmetric charge distribution, when electrons are lifted to the excited state from the ground state. The second term mainly arises for those electrons that reside in the \( p \) or \( d \) orbital.

The center of gravity of the spinning CSA sideband pattern is represented as an isotropic chemical shift \( \delta_{\text{iso}} = \frac{\delta_{11} + \delta_{22} + \delta_{33}}{3} \). Changes in the isotropic chemical shift have a great influence on the breadth of the CSA tensor. Generally, small changes in the isotropic chemical shift correspond to a larger change in the chemical shift anisotropy. Span \( (\Omega = \delta_{11} - \delta_{33}) \) represents the maximum width of the spinning CSA sideband pattern. According to Haeberlen convention, the anisotropy \((\Delta \delta)\) and asymmetry \((\eta)\) parameters are defined as \( \Delta \delta = \delta_{33} - \frac{(\delta_{11} + \delta_{22})}{2} \) and \( \eta = \frac{\delta_{22} - \delta_{11}}{\delta_{33} - \delta_{11}} \), respectively. Anisotropy represents the largest separation from the center of gravity of the spinning CSA sideband pattern. The sign of the anisotropy tells on which side of the center of gravity one can find the largest separation. When the spinning CSA pattern is axially symmetric (i.e., \( \delta_{22} \) is equal to \( \delta_{11} \) or \( \delta_{33} \)), then the value of the asymmetry parameter is zero. Hence, the asymmetry parameter basically shows whether the CSA pattern deviates from its axially symmetric shape or not. As shown in Table 2, the asymmetry parameter is small (<0.5) for C1, C2, C4, C9, C11, C17, C18, C22, C23, C24, C25, C31, and C32 resonance lines, which indicates that the sideband patterns for these resonance lines are axially symmetric. On the contrary, the sideband patterns are highly asymmetric for these resonance lines for which the asymmetry parameter is greater than 0.5, especially for C5, C19, C22, C28, and C30 carbon resonance lines. The orientation of the asymmetry is represented by a parameter referred to as “skew” \( k = \frac{3(\delta_{22} - \delta_{11})}{\Omega} \). The position of \( \delta_{22} \) with respect to the center of gravity \( (\delta_{\text{iso}}) \) of the spinning CSA sideband pattern determines the sign of “skew”. Skew is zero when \( \delta_{22} \) coincides with \( \delta_{\text{iso}} \).

Figure 3 shows the \(^{13}\text{C}\) 2DPASS MAS NMR spectrum of itraconazole. The direct dimension and indirect dimension of the 2D spectrum represent, respectively, the infinite spinning speed spectrum and the anisotropic spectrum. The spinning CSA sideband patterns for chemically different carbon sites are also shown in (a) C31, (b) C17, (c) C5, (d) C23, (e) C15, and (f) C27. Table 2 shows that the values of CSA parameters are varied for numerous carbon nuclei situated in different chemical environments. The values of both chemical shift anisotropy parameter \( (\Delta \delta) \) and the spin-lattice relaxation time is shorter for carbon nuclei (C1, C2, C3, and C4) associated with the sec-butyl group in the triazolane side-chain region compared to other carbon nuclei. The spinning CSA sideband patterns of C1 and C4 are axially symmetric \((\eta \approx 0)\) and span of C1 and C4 is also very low. These data suggest that the CSA parameters of the sec-butyl (C1, C2, C3, and C4) group are greatly influenced by the side-chain conformation and dynamics. On the other hand, anisotropy parameters are very high for carbon nuclei (C35, C6, C5, and C34) situated between two heteroatoms in a five-membered 1,2,4-triazole ring due to the strong deshielding effect. Magnetic shielding and deshielding effects arise due to the existence of the nonbonded electron, which manifest as a large value of anisotropy. The electrons revolving along the clockwise direction can generate a magnetic field, which is along the direction of the external magnetic field (paramagnetic current). Consequently, the magnitude of the resultant magnetic field is increased—the deshielding effect. On the contrary, electrons revolving along the counterclockwise direction can generate a magnetic field along the opposite direction of the external magnetic field (diamagnetic current). Hence, the resultant magnetic field is decreased—the shielding effect. As a consequence, the values of magnetic susceptibilities \((X_{\nu}, X_{\nu}', X_{\nu}'')\) are not the same along the three directions in the principal axis system. Moreover, there exist two components of magnetic susceptibilities—one parallel to the magnetic field \((\Delta X_{\|} = X_{\nu}' - X_{\nu})\) and another perpendicular to the magnetic field \((\Delta X_{\perp} = X_{\nu}' - X_{\nu}')\). The magnetic anisotropy in terms of these parallel and perpendicular components of magnetic susceptibilities can be represented by the McConnell equation

\[
\delta_{\text{anis}} = \frac{(\Delta X_{\|} (3 \cos^2 \theta_1 - 1) + \Delta X_{\perp} (3 \cos^2 \theta_2 - 1))}{3R^3}
\]

where \( \theta_1 \) is the angle between the radius vector and \( x \)-axis and \( \theta_2 \) is the angle between the radius vector and \( z \)-axis. This anisotropic magnetic susceptibility gives rise to the direction-dependent magnetic field. Magnetic shielding/deshielding effect and electrostatic effect are the reasons behind the large value of anisotropy for carbon nuclei surrounded by nonspherical distribution of charges.

The stereochemical orientation of the dioxolane ring (C24, C25, and C26 reside on that ring) plays a significant role in H-pathway inhibition and compound stability. For carbon nuclei in the dioxolane ring, the CSA parameters are not as high as those of the carbon nuclei associated with the 1, 2, 4-triazole ring.

The electrostatic interaction of a specific molecule with the surrounding molecule generates polarization on the electron density. This polarization particularly influences the strength of the induced magnetic field. As a result, the induced magnetic field is different along different directions. As shown in Figure 4, the CSA parameters of C28 and C30 nuclei bonded with a chlorine atom are also high because the effective magnetic field experienced by these nuclei is influenced by the polarization of the chemical bond with which those atoms are attached. The spin-lattice relaxation time is also very long for these nuclei. Even the CSA parameters are also very high for those nuclei (C27, C29, C31, and C32) that reside near the polar bonds because the neighboring polar bonds also polarize the electron cloud surrounding C27, C29, C31, and C32 nuclei. As a result, the local shielding or deshielding become direction-dependent—that means the local field experienced by nuclei may increase in a certain direction or decrease in other directions. The effect of
The spin-lattice relaxation rate for $^{13}$C can be written as
\[
\frac{1}{T_1} = \frac{1}{T_1^{\text{CSA}}} + \frac{1}{T_1^{\text{diff}}}
\]
\[
= \frac{2}{15} \gamma^2 B^2 S \left( \frac{\tau_2}{1 + \omega^2 \tau_2^2} \right) + \frac{1}{10} \frac{\gamma_C h}{r_{CX}^2} \left( \frac{3}{1 + \omega_C^2 \tau_2^2} \right)
\]
(8)

The relaxation mechanism of nonprotonated carbon nuclei is predominated by chemical shift anisotropy (CSA) in the presence of a high value of magnetic field. The molecular correlation time of the itraconazole molecule is calculated using eq 8.

Table 3 shows the molecular correlation time of numerous carbon nuclei situated in different chemical environments of itraconazole. It varies in the range of $10^{-3}$–$10^{-6}$ s. It is clear from Tables 1–3 that the spin-lattice relaxation rate, CSA parameters, and molecular correlation time hugely varied for the same carbon nuclei placed in different electronic surroundings and numerous molecular conformations. From Figure 5 and Table 3, it is clear that the molecular correlation time of C1, C2, C3, and C4 carbon nuclei is on the order of $10^{-3}$ s for C28 and $10^{-6}$ s for C30. For side-chain carbon nuclei, the molecular correlation time is on the order of $10^{-4}$ s. The spin-lattice relaxation times of C6, C20, C23, C24, and C26 carbon nuclei linked with an oxygen atom are also significantly long. CSA parameters are high for C6 and C20 nuclei. The molecular correlation times for C6, C20, and C23 nuclei are on the order of $10^{-4}$ s and for C24, C25, and C26 nuclei on the order of $10^{-3}$ s.

Perhaps, these substantial variations of CSA parameters and degrees of motion at different regions of this molecule are responsible for its different biological activities like antifungal and anticancer activities, as well as interesting pharmacokinetic behavior like strong protein binding, tissue penetration, and

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**Table 3. Molecular Correlation Time of Itraconazole for Various Carbon Nuclei**

| carbon nuclei | molecular correlation time (s) | carbon nuclei | molecular correlation time (s) |
|--------------|--------------------------------|--------------|--------------------------------|
| C1           | $2 \times 10^{-6}$           | C2           | $5.1 \times 10^{-6}$         |
| C3           | $6.6 \times 10^{-6}$         | C4           | $6 \times 10^{-6}$           |
| C5           | $1.3 \times 10^{-3}$         | C6           | $7.5 \times 10^{-4}$         |
| C7           | $6.4 \times 10^{-4}$         | C8           | $7.4 \times 10^{-5}$         |
| C9           | $2.1 \times 10^{-5}$         | C10          | $2.9 \times 10^{-4}$         |
| C11          | $2.1 \times 10^{-5}$         | C12          | $7.4 \times 10^{-5}$         |
| C14          | $6.6 \times 10^{-6}$         | C16          | $1.8 \times 10^{-5}$         |
| C17          | $1.7 \times 10^{-4}$         | C18          | $2 \times 10^{-4}$           |
| C19          | $7.7 \times 10^{-5}$         | C20          | $5.9 \times 10^{-4}$         |
| C21          | $7.7 \times 10^{-5}$         | C22          | $2 \times 10^{-4}$           |
| C23          | $1.6 \times 10^{-4}$         | C24 and C26  | $6.6 \times 10^{-5}$         |
| C25          | $1.9 \times 10^{-3}$         | C27          | $6.9 \times 10^{-4}$         |
| C28          | $1.4 \times 10^{-3}$         | C29          | $1.7 \times 10^{-4}$         |
| C30          | $7.1 \times 10^{-4}$         | C31          | $1.7 \times 10^{-4}$         |
| C32          | $1.7 \times 10^{-4}$         | C33          | $6.6 \times 10^{-6}$         |
| C34          | $3.5 \times 10^{-4}$         | C35          | $3 \times 10^{-4}$           |
proteins of diverse biological activities by interacting with the enzyme/motions in its structure, like itraconazole, is capable of producing prolonged half-life and bioavailability. In essence, the influences of the local environment on the structure and dynamics of the itraconazole molecule are vividly portrayed by this type of investigation.

4. CONCLUSIONS

Extraction of CSA parameters by the $^{13}$C 2DPASS MAS NMR experiment, determination of spin-lattice relaxation time by the Torchia CP method, and calculation of molecular correlation time at 35 crystallographically and chemically different sites of carbon nuclei of itraconazole provide the information about the correlation between the structure and dynamics of this valuable antifungal drug. Substantial difference in the spin-lattice relaxation time (shown in Figure 2e), CSA parameters (as shown in Table 2 and Figure 4), and molecular correlation time (as shown in Table 3 and Figure 5b) is observed for different structural parts of this molecule. The spin-lattice relaxation time is long for carbon nuclei (C35, C6, C5, and C34) that participated in the 1, 2, 4-triazole ring. On the contrary, the spin lattice relaxation time is comparatively short for C1, C2, C3, and C4 carbon nuclei that reside at the side-chain region. CSA parameters of C5, C6, C34, and C35 nuclei are much higher than those of C1, C2, C3, and C4 nuclei, indicating that the relaxation mechanism at a high value of magnetic field is predominated by chemical shift anisotropy interaction. The molecular correlation time of C1, C2, C3, and C4 regions is 2−3 orders of magnitude lesser than that of C5, C6, C34, and C35. The spin-lattice relaxation time is very long for carbon nuclei C28 and C30 bonded with chlorine. Asymmetry and anisotropy parameters are also very high of the spinning CSA sideband pattern corresponding to the C28 and C30 nuclei. The molecular correlation time is on the order of $10^{-3}$ s for C28 and $10^{-4}$ s for C30, whereas for the sec-buty1 group carbon nuclei, the molecular correlation time is on the order of $10^{-6}$ s. It may be possible that a molecule with different degrees of motions in its structure, like itraconazole, is capable of producing many biological activities by interacting with the enzyme/proteins of different structures and dynamics. This type of investigation will elucidate the way of inventing advanced medicine with multiple biological activities in the pharmaceutical industry.

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**MANUSCRIPT**

Figure 5. (a) Activities of different regions of the itraconazole molecule. (b) Molecular correlation time at chemically different sites.

Table 4. Bond Distances of the Itraconazole Molecule

| bond      | distance (Å) | bond      | distance (Å) |
|-----------|--------------|-----------|--------------|
| C1−H1     | 1.095        | C16−N4    | 1.463        |
| C1−H2     | 1.095        | C13−N4    | 1.463        |
| C1−H3     | 1.094        | C13−H15   | 1.097        |
| C2−H4     | 1.097        | C13−H16   | 1.097        |
| C2−H5     | 1.097        | C14−H17   | 1.097        |
| C3−H6     | 1.097        | C14−H18   | 1.097        |
| C4−H7     | 1.096        | C15−H19   | 1.097        |
| C4−H8     | 1.096        | C15−H20   | 1.097        |
| C4−H9     | 1.095        | C16−H21   | 1.097        |
| C3−N1     | 1.493        | C16−H22   | 1.097        |
| N1−N2     | 1.363        | C14−N5    | 1.463        |
| C5−H10    | 1.094        | C15−N5    | 1.463        |
| C5−N3     | 1.349        | C17−N5    | 1.392        |
| C6−N3     | 1.386        | C18−H23   | 1.087        |
| C6−O1     | 1.223        | C19−H24   | 1.087        |
| C8−H11    | 1.084        | C21−H25   | 1.086        |
| C9−H12    | 1.087        | C22−H26   | 1.087        |
| C11−H13   | 1.087        | C20−O2    | 1.371        |
| C12−H14   | 1.081        | C23−O2    | 1.42         |
| C10−N4    | 1.392        | C23−H27   | 1.098        |
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