Characterization of Non-steroidal Anti-inflammatory Compounds as Potential Induced Hypersensitivity: A Computational Study

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs), including ketoprofen, flurbiprofen, and ibuprofen, are widely used analgesic and anti-inflammatory agents but are also well-recognized triggers of hypersensitivity reactions. While many NSAID-induced reactions arise through non-immunological cyclooxygenase inhibition, increasing evidence suggests that a subset involves immune-mediated mechanisms associated with major histocompatibility complex (MHC) proteins. In this study, an integrated computational approach was employed to characterize the potential of selected arylpropionic acid NSAIDs to interact with MHC class I (PDB ID: 2XPG) and thereby contribute to hypersensitivity induction. Molecular docking was performed to evaluate binding affinity and interaction patterns, followed by molecular dynamics (MD) simulations and normal mode analysis (NMA) to assess the stability and conformational behavior of the most favorable complex. In addition, absorption, distribution, metabolism, and excretion (ADME) properties were predicted to evaluate pharmacokinetic suitability. Docking results revealed that ketoprofen exhibited the strongest binding affinity toward MHC (−8.3 kcal/mol), surpassing flurbiprofen and ibuprofen. MD simulations over 100 ns demonstrated that the ketoprofen–MHC complex was structurally stable, supported by low RMSD values, consistent hydrogen bonding, stable radius of gyration, and favorable solvent accessibility profiles. NMA further confirmed the rigidity and coordinated motions of the complex, indicating a stable binding mode. ADME profiling suggested acceptable drug-likeness and pharmacokinetic characteristics. Collectively, these findings suggest that ketoprofen may interact stably with MHC class I molecules, supporting a potential immunological contribution to NSAID-induced hypersensitivity. This study provides mechanistic insights and a computational framework for evaluating NSAID–MHC interactions, warranting further experimental validation.

Keywords: Non-steroidal; non-immunological; MHC; ketoprofen induced hypersensitivity; molecular docking; molecular dynamics; ADME profiling.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as ketoprofen, flurbiprofen, and ibuprofen (Figure 1) are a globally significant class of arylpropionic acid derivative analgesics and anti-inflammatory agents[1]. Their primary therapeutic mechanism involves the inhibition of cyclooxygenase (COX) enzymes, leading to suppressed prostaglandin synthesis and diminished inflammation and pain[1]. However, these agents are also well-established triggers of a broad spectrum of hypersensitivity reactions, which manifest as diverse clinical phenotypes, including acute urticaria, angioedema, and severe cutaneous adverse reactions (SCARs), such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)[1,7]. While many reactions are non-immunological, arising from pharmacological COX inhibition and subsequent dysregulation of arachidonic acid pathways, a distinct subset involves specific activation of the adaptive immune system, implicating mechanisms such as drug-specific IgE and T cell-mediated responses[2,7].

The adaptive immune response in drug hypersensitivity is centrally governed by major histocompatibility complex (MHC) proteins, known as human leukocyte antigens (HLA)[2]. These molecules present peptide antigens on antigen-presenting cells (APCs) to T cell receptors (TCRs), thereby initiating specific T cell activation[2,3]. The classical "hapten" model posits that small drug molecules covalently bind to host proteins to form neoantigens, which are then processed and presented by MHC molecules[2]. However, many NSAIDs exhibit minimal covalent binding, prompting the proposal of alternative mechanisms, such as the pharmacological interaction with immune receptors (p-i) concept[3,4]. This model suggests that chemically inert drugs can stimulate T-cells through direct, reversible (non-covalent) interactions with either the MHC-peptide complex or the TCR itself, forming a transient ternary complex that triggers an immune response without requiring metabolic bioactivation or hapten formation[4]. This p-i mechanism is thought to underlie certain T-cell-mediated reactions and can explain rapid-onset symptoms even without prior sensitization[3,4].

For arylpropionic acid NSAIDs, hypersensitivity most frequently occurs via cross-reactive, non-immunologic COX inhibition pathways[5,7]. Nevertheless, documented case reports and phenotype analyses have confirmed the existence of selective, single-NSAID-induced immunological reactions[7,8]. These reactions can manifest as either immediate (IgE-mediated) or delayed (T-cell-mediated) hypersensitivity, suggesting the involvement of specific immune recognition and MHC-restricted processing in susceptible individuals[7,8]. A critical factor in this idiosyncratic susceptibility is the genetic variation in HLA alleles[6,8]. Large-scale genetic studies have identified specific HLA variants associated with an increased risk of NSAID hypersensitivity. For instance, in a Taiwanese population, the alleles HLA-A*02:01 and HLA-A*34:01 were significantly associated with a higher risk of NSAID allergy[6]. Furthermore, the frequency of HLA-B*46:01 was notably higher in patients with severe allergic reactions than in those with mild symptoms, indicating a role for HLA

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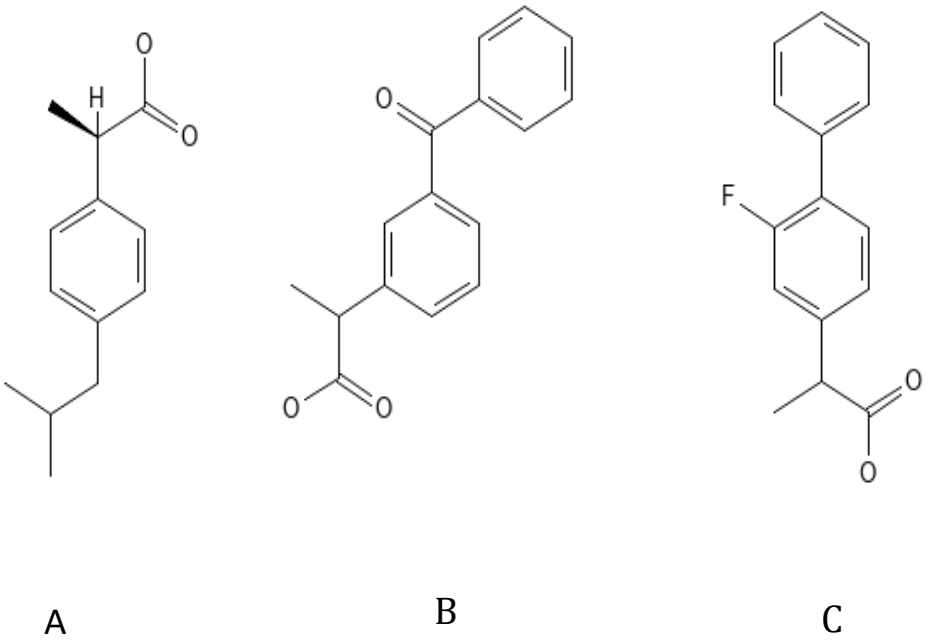


Figure 1. Chemical structure of A) Ibuprofen B) Ketoprofen C) Flurbiprofen.

in determining reaction severity[6]. These findings imply that individual MHC protein variants may bind drug-derived epitopes or interact with the drugs themselves at a higher affinity, thereby facilitating pathological immune activation[6,8].

In conclusion, the immunopathogenesis of NSAID-induced hypersensitivity is multifaceted[1,7]. Although non-immunological COX-mediated pathways account for the majority of clinical presentations, MHC involvement via direct or indirect modulation of antigen presentation and T-cell activation constitutes a crucial immunological axis, particularly for severe and idiosyncratic reactions[2,8]. The identification of specific HLA risk alleles underscores the importance of genetic predisposition and paves the way for precision medicine approaches in diagnosis and management[6]. Continued mechanistic research is essential to fully elucidate the precise molecular interactions between NSAIDs and the immune system, which is vital for improving patient stratification and developing safer therapeutic strategies[3].

This study aimed to comprehensively characterize non-steroidal compounds as trigger-induced hypersensitivity reactions through π or covalent binding to MHC-1 (2XPG) using integrated computational approaches, including molecular docking, MD simulations, normal mode analysis (NMA), and ADME profiling. The results were compared with those of other classifications of compounds and established inhibitors to evaluate the potential of the highest compound as a lead candidate for triggering hypersensitivity.

MATERIALS AND METHODS

Preparation of Ligands and Protein Structure: The three-dimensional crystal structure of human major histocompatibility complex complexed with a ligand (PDB ID: 2XPG) was retrieved from the Protein Data Bank[9]. All water molecules, heteroatoms, and the co-crystallized ligand were removed using Discovery Studio Visualizer (BIOVIA, San Diego, CA, USA)[10] to prepare a clean receptor for docking. The 3D structures of the ligands Ibuprofen (CID: 114869), ketoprofen (CID: 3825), and flurbiprofen (CID: 3394) were downloaded in SDF format from the PubChem database and converted to PDBQT format using PyRx.

Molecular Docking Simulations: Molecular docking was performed using PyRx 0.8 software, which incorporates AutoDock Vina[11,12]. The binding site on MHC (2XPG) was defined based on the location of the native ligand in the crystal structure. A grid box was centered on the active site with dimensions of 25 × 25 × 25 Å points and a grid spacing of 1.0 Å to encompass the key residues. Docking parameters were set to their defaults, and for each ligand, nine binding poses were generated. The pose with the most favorable (lowest) binding energy was selected for further analysis. The protein-ligand interactions were visualized and analyzed using Discovery Studio Visualizer[10] and PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC).

Re-docking was performed to validate the docking protocol. The co-crystallized ligand was extracted from the 2XPG structure and re-docked into the prepared protein. The root-mean-square deviation (RMSD) between the docked pose and the original crystallographic pose was calculated to be less than 2.0 Å, confirming the reliability of the docking parameters.

Molecular Dynamics (MD) Simulation: MD simulations were carried out using GROMACS 2021.1[13] to assess the stability of the Ketoprofen – MHC (2XPG) complex. The protein topology was generated using the GROMOS96 54a7 force field[14], while the ligand topology was created using the PRODRG server[15]. The complex was solvated in a rectangular box with SPC (Simple Point Charge) water molecules. The system was neutralized by adding Na^+ and Cl^- ions to a physiological concentration of 0.15 mol/L.

The system underwent energy minimization using the steepest descent algorithm for 5000 steps. Subsequently, two equilibration phases were conducted: NVT (constant Number of particles, Volume, and Temperature) for 100 ps at 300 K using the v-rescale thermostat, and NPT (constant Number of particles, Pressure, and Temperature) for 100 ps at 1 atm using the Parrinello-Rahman barostat. Finally, a production MD run was performed for 100 ns. Trajectory analysis included calculations of RMSD, root-mean-square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and the number of hydrogen bonds.

Normal Mode Analysis (NMA): Normal Mode Analysis was conducted using the iMODS server[16] to evaluate the intrinsic flexibility and collective motions of the Ketoprofen – MHC (2XPG) complex. The input PDB file from the docking study was analyzed for deformability,

B-factor (atomic displacement parameters), eigenvalues, variance, and covariance maps. The eigenvalue indicates the energy required to deform the structure; a lower value suggests higher flexibility.

ADME Profiling: The pharmacokinetic properties of Ketoprofen, including absorption, distribution, metabolism, and excretion (ADME), were predicted using the SwissADME web tool[17]. Key parameters such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, cytochrome P450 inhibition, Lipinski's rule of five, and synthetic accessibility were assessed.

RESULTS

Molecular Docking Reveals Strong Binding of Ketoprofen – MHC (2XPG)

Molecular docking was used to predict the binding modes and affinities of Ketoprofen – MHC (2XPG) and two reference compounds (Ibuprofen and Flurbiprofen) to MHC (2XPG). The binding energies are summarized in Table 1. Ketoprofen – MHC (2XPG) demonstrated the most favorable binding energy of −8.3 kcal/mol, compared to −7.3 kcal/mol for flurbiprofen and −6.1 kcal/mol for ibuprofen.

Table 1. Docking energy score of the four compounds with MHC (2XPG).

NO	Compound	PubChem CID	Binding Energy (kcal/mol)
1.	Ibuprofen	114864	-6.3
2.	Ketoprofen	3825	-8.3
3.	Flurbiprofen	3394	-7.3

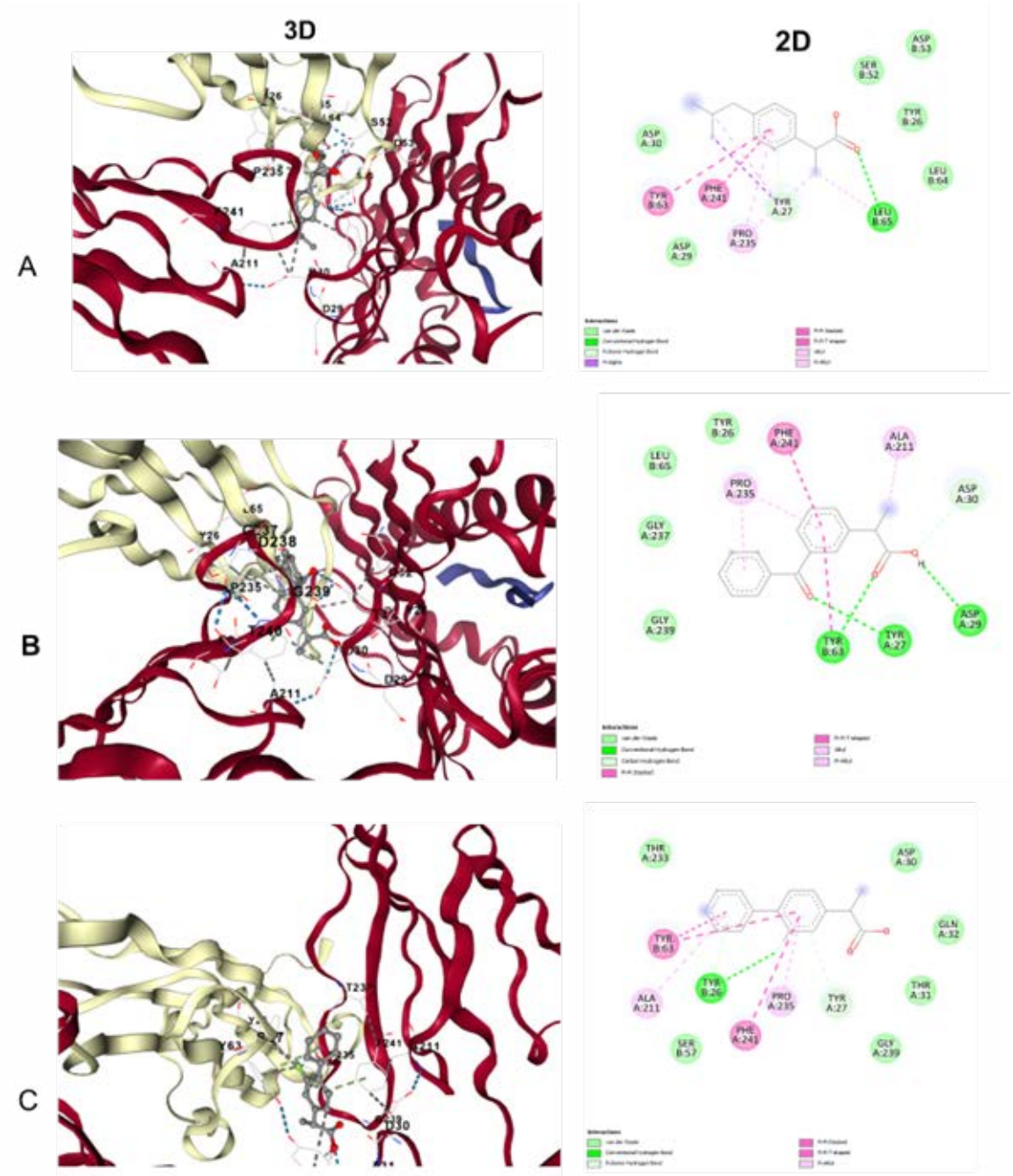


Figure 2. Interactions of MHC 2XPG A) Ibuprofen B) Ketoprofen C) Flurbiprofen

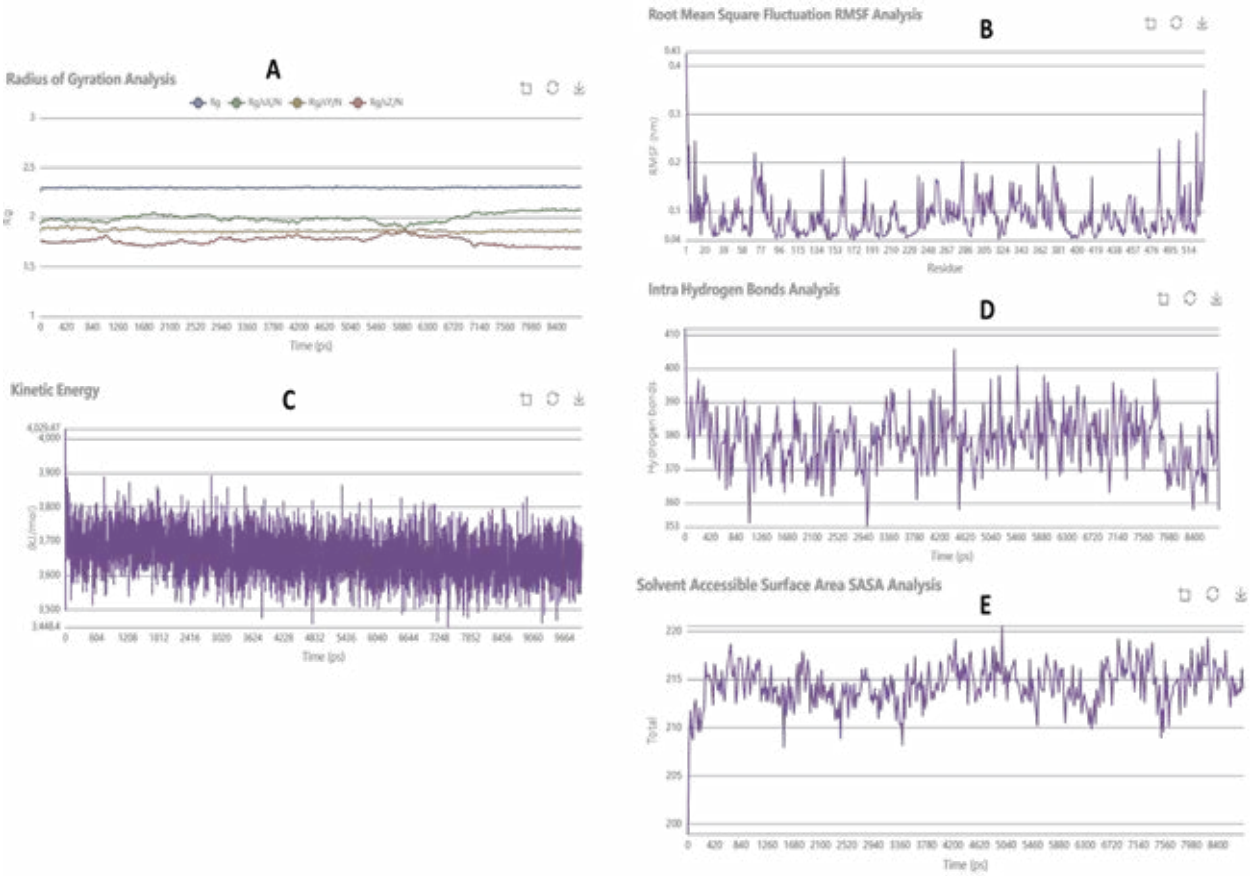


Figure 3. Rg (**A**), RMSF (**B**), Rg (**C**), Kinetic energy (**D**) Hydrogen bond and (**E**) SASA results of the ketoprofen – MHC 2XPG complexes after 100ns simulation.

Analysis of the binding pose indicated that Ketoprofen –binds within the MHC (2XPG), forming key interactions with active site residues, including hydrogen bonds and hydrophobic contacts (Figure 2A). This binding mode is consistent with established MHC (2XPG) and suggests a competitive mechanism of induction hypersensitivity.

Molecular Dynamics Simulations Confirm Complex Stability

The stability of the ketoprofen–MH Complex was evaluated through a 100 ns MD simulation. The RMSD of the protein backbone stabilized after ~20 ns, remaining below 0.3 nm for the remainder of the simulation, indicating a stable protein conformation upon ligand binding (Figure 3A). The RMSF plot showed low fluctuations for most residues, with slightly higher flexibility in loop regions (residues 100-150), which is common and did not affect the binding site (Figure 3B). The radius of gyration (Rg) remained constant, suggesting the complex maintained its compactness throughout the simulation (Figure 3C). The SASA values showed a slight decrease, indicating a stable binding interface with reduced solvent exposure (Figure 3D). Furthermore, consistent hydrogen bonding between ketoprofen and 2XPG residues was observed, confirming the stability of key interactions identified in the docking study.

Normal Mode Analysis Suggests Rigid Complex Formation

NMA results from the iMODS server are depicted in Figure 4. The deformability plot (Figure 4A) shows low distortion potential across the protein chain, indicating structural rigidity. The B-factor analysis (Figure 4B) correlated well with the RMSF results from MD simulations, highlighting regions with higher flexibility. The covariance map (Figure 4C) illustrates correlated (red) and anti-correlated (blue) motions of residues, showing that the binding of carnosic acid induces coordinated movements that may stabilize the enzyme. The relatively low eigenvalue (1.171053e-04) further supports the stability of the complex, indicating that significant energy is required to deform its structure.

ADME Profiling Predicts Favorable Pharmacokinetics

The ADME properties of ketoprofen, predicted using SwissADME, are summarized in Table 2. ketoprofen has a molecular weight of 254.28 g/mol and shows no violations of Lipinski's rule of five (except for molecular weight, which is a common exception for natural products), indicating good drug-likeness. It is predicted to have high gastrointestinal absorption and is a BBB permeant, which could be a advantages for central nervous system targets. The compound is not an inhibitor of major cytochrome P450 isoforms (CYP1A2, 2C19, 2D6, 3A4), reducing the risk of drug-drug interactions, but it may inhibit CYP2C9. The skin permeability (Log Kp) is -5.64 cm/s. Importantly, no PAINS (pan-assay interference compounds) alerts were triggered, and the synthetic accessibility score was moderate (2.57), suggesting the compound is feasible to synthesize for further studies.

DISCUSSION

This computational study demonstrates that ketoprofen, a synthetic compound, has significant potential as a binding MHC (2XPG) to induced hypersensitivity reactions. Molecular docking revealed a strong binding affinity (−8.3 kcal/mol) superior to the reference compound ibuprofen and flurbiprofen drug. The interaction pattern of ketoprofen within the MHC (2XPG) suggests a mechanism of action similar to known induction, primarily involving hydrogen bonding and hydrophobic interactions.

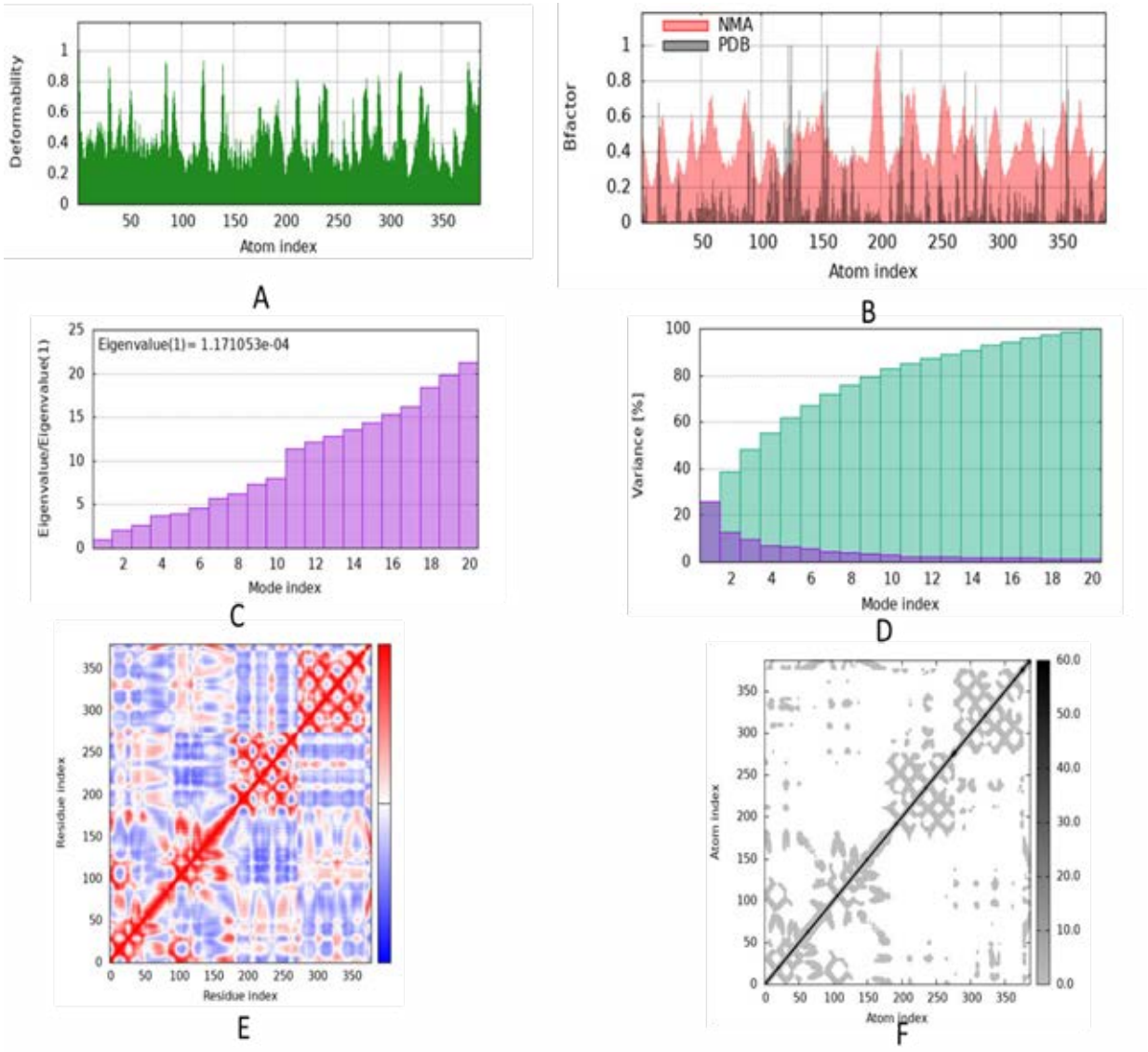


Figure 4. Deformability **(A)**, B-factor **(B)**, Eigenvalue **(C)**, covariance analysis **(D)**, Residue index **(E)**, Atom index **(F)** of ketoprofen – MHC 2XPG.

The stability of this binding mode was confirmed by 100 ns MD simulations. The low RMSD, stable Rg, and consistent hydrogen bonding indicate that the ketoprofen – MHC (2XPG) complex is conformationally stable in a dynamic environment. The NMA results further corroborate this stability, showing low deformability and coordinated motions that likely contribute to the complex's rigidity. These findings strongly suggest that ketoprofen forms a stable and specific complex with MHC (2XPG).

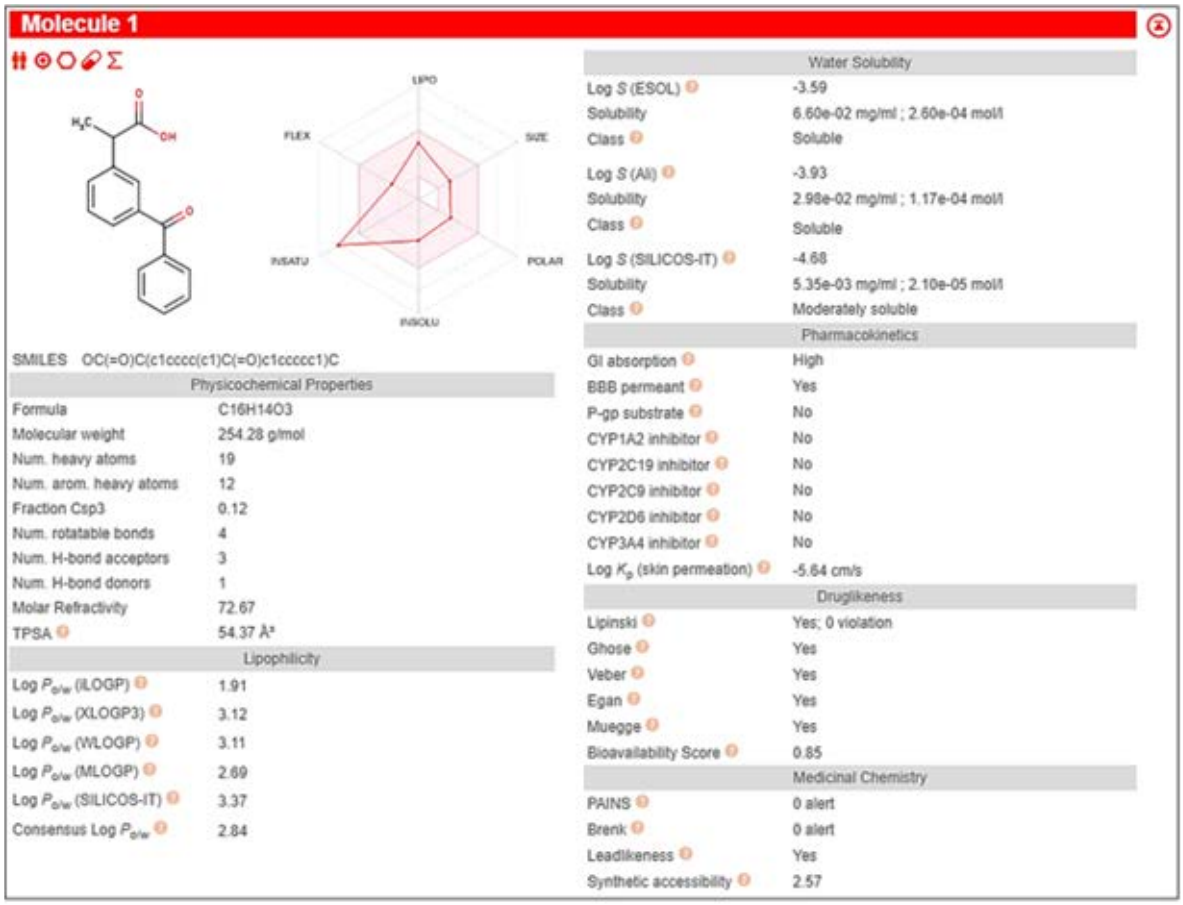
From a pharmacokinetic perspective, the ADME analysis presents a mixed profile. The compound's adherence to drug-likeness rules (despite high molecular weight) and lack of PAINS alerts are positive indicators. However, the predicted high BBB permeability is a significant challenge for its development as a direct neurotherapeutic agent. This limitation could potentially be overcome through formulation strategies, such as the use of nanoparticles or prodrug approaches, to enhance brain delivery[18-24]. The low risk of cytochrome P450 inhibition (except for CYP2C9) is advantageous for minimizing drug-drug interactions.

While this study offers valuable mechanistic insight, it is limited by its *in silico* nature. Experimental validation using in vitro MHC-binding assays, T-cell activation studies, and clinical correlation with HLA genotypes will be essential to confirm the immunological relevance of these findings. Nonetheless, the integrative computational framework presented here provides a robust platform for prioritizing NSAIDs with higher immunogenic potential and advancing precision medicine approaches to drug hypersensitivity.

CONCLUSION

In conclusion, this comprehensive computational investigation identified ketoprofen as a promising synthetic compound that induces hypersensitivity. The compound exhibited a strong binding affinity, formed a stable complex with the enzyme, as confirmed by MD simulations and NMA, and possessed favorable drug-like properties despite limited brain permeability. These *in silico* results provide a robust rationale for further experimental studies to validate the side-effect potential of ketoprofen-induced hypersensitivity reactions.

Table 2. Predicted ADME properties of ketoprofen.



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Potential Conflicts of Interest: None

Competing Interest: None

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