Optimization of structures, biochemical properties of ketorolac and its degradation products based on computational studies

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Received: 28 August 2018 / Accepted: 10 January 2019 / Published online: 19 February 2019
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Abstract

Background Ketorolac (KTR) is used as an analgesic drug with an efficacy close to that of the opioid family. It is mainly used for the short term treatment of post-operative pain. It can inhibit the prostaglandin synthesis by blocking cyclooxygenase (COX).

Methods In this investigation, the inherent stability and biochemical interaction of Ketorolac (KTR) and its degradation products have been studied on the basis of quantum mechanical approaches. Density functional theory (DFT) with B3LYP/ 6-31G (d) has been employed to optimize the structures. Thermodynamic properties, frontier molecular orbital features, dipole moment, electrostatic potential, equilibrium geometry, vibrational frequencies and atomic partial charges of these optimized structures were investigated. Molecular docking has been performed against prostaglandin H2 (PGH2) synthase protein 5F19 to search the binding affinity and mode(s). ADMET prediction has performed to evaluate the absorption, metabolism and carcinogenic properties.

Results The equilibrium geometry calculations support the optimized structures. Thermodynamic results disclosed the thermal stability of all structures. From molecular orbital data, all the degradants are chemically more reactive than parent drug (except K3). However, the substitution of carboxymethyl radical in K4 improved the physicochemical properties and binding affinity. ADMET calculations predict the improved pharmacokinetic and non-carcinogenic properties of all degradants.

Conclusion Based on physicochemical, molecular docking, and ADMET calculation, this study can be helpful to understand the biochemical activities of Ketorolac and its degradants and to design a potent analgesic drug.

Keywords Ketorolac · Density functional theory · HOMO-LUMO · MEP · Molecular docking · Pharmacokinetics

Abbreviations

KTR	Ketorolac
DFT	Density functional theory
HOMO	Highest occupied molecular orbital
LUMO	Lowest unoccupied molecular orbital
MEP	Molecular electrostatic potential
ADMET	Absorption, distribution, metabolism, excretion, toxicity

Introduction

Ketorolac (KTR) is one of the most popularly used analgesic drugs all over the world. It is non-steroidal anti-inflammatory drug (NSAID) and member of heterocyclic acetic acid derivatives [1]. KTR shows analgesic, anti-inflammation, and antipyretic activities [2]. It is safe and effective analgesic agent for the short-term management of moderate to severe pain and can be used as an alternative to opioid therapy [3]. It is also used in migraine pain and postoperative cancer pain [4–6]. Long-term use of KTR may produce gastrointestinal ulceration and renal injury in human [7]. Primarily, KTR inhibits the prostaglandin synthesis by blocking cyclooxygenase (COX) [8, 9]. It is sensitive for patients with cardiac decompensation, hypertension or similar conditions and not recommended for pre-operative analgesia, long-term chronic pain, and renal dysfunction patients [10]. Some serious side effects including heart attack, heart failure, stroke, high blood pressure,

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Table 1 Stress conditions for optimum degradation of ketorolac tromethamine

Stress conditions	Concentration of stressors	Exposure condition (°C)	Duration	Percent degradation (%)	degradation products
Thermal	–	80	4 h	6	K1
Acid Hydrolysis	1.0 N HCl	Room temperature	4 h	20	K2
Base Hydrolysis	1.0 N NaOH	Room temperature	4 h	8	K3
Neutral	H ₂ O	Room temperature	4 h	5	K2/K3
Oxidation	3.0% H ₂ O ₂	Room temperature	4 h	30	K4/K5

bleeding and ulcers in the stomach and intestine, renal and hepatic injury are found cause of KTR [11, 12].

Drug modification is another alternative way to search better agent, which can increase the selective action of drug and reduce the side effect. Simple modifications of molecular structure significantly influence the structural properties including geometry, vibrational frequency, thermochemistry, molecular orbital, binding affinity and/ or mode(s). In pharmaceutical industry, force degradation study has its importance before starting a new formulation, which provides an insight into degradation pathways and impurity of the drug substance also helps in elucidation of the structure of the degraded products. The degradation products of KTR have been identified from the stress degradation study under hydrolysis (acid, base, and neutral), oxidation, photolysis, thermal conditions [13–15]. Few computational studies of KTR and its modified derivatives also were reported previously [16, 17]. In this study, attempts have been taken to optimize some selected stressed degradation products of KTR and to investigate their biochemical behaviour on the basis of quantum mechanical approaches. The free energy, electronic energy, enthalpy, dipole moment, electrostatic potential, bond distances, bond angles, vibrational frequencies, HOMO-LUMO gap, hardness, softness, chemical potential, and atomic partial charge have been studied. Molecular docking and nonbonding calculation have been performed to understand the binding affinity, mode(s) and interaction between drugs and the amino acid residues of human prostaglandin synthase protein (5F19). Some of the degradants show better thermal stability, chemical reactivity, binding affinity, nonbonding interactions, and improved pharmacokinetics properties than KTR, which can promote more potential analgesic drugs.

Forced degradation conditions

Forced degradation study of the standard ketorolac sample was performed under the stress conditions as tabulated in Table 1. Degradation products of ketorolac (KTR) obtained at different conditions have been presented in Fig. 1.

Computational details

Optimization of KTR and its degradants using DFT

Quantum mechanical (QM) methods have greater attention on calculation of thermodynamic properties, molecular orbital

Fig. 1 Chemical and most stable optimized structures of Ketorolac (KTR) and its degradation products. Optimized at B3LYP/6–31 G (d) level theory

Table 2 The stoichiometry, molecular weight, change of electronic energy (ΔE), enthalpy (ΔH), Gibbs free energy (ΔG) in kcal/mol, and dipole moment ($\Delta \mu$, Debye) of Ketorolac and its degradation products

Drug	Stoichiometry	Molecular weight	ΔE	ΔH	ΔG	$\Delta \mu$
KTR	C ₁₅ H ₁₃ NO ₃	255.269	−7.84	−7.90	−8.59	1.14
K1	C ₁₄ H ₁₃ NO	211.259	−5.08	−5.08	−5.33	0.83
K2	C ₁₄ H ₁₁ NO	209.243	−5.02	−4.96	−5.21	0.70
K3	C ₁₄ H ₁₃ NO ₂	227.259	−6.71	−6.65	−6.96	1.13
K4	C ₁₇ H ₁₅ NO ₅	313.305	−11.72	−11.78	−12.10	1.95
K5	C ₁₆ H ₁₅ NO ₃	269.295	−7.03	−7.03	−7.47	1.19

features, dipole moment, geometries, vibrational frequencies, atomic partial charges, molecular electrostatic potential and as well as interpretation of different types of interactions [18]. Initial geometry of KTR was taken from the online structure database named ChemSpider [19] and proceed with S-isomer for further calculations. Molecular geometry optimization and further modification of all drugs carried out using Gaussian 09 program package [20]. All the structures were optimized using density functional theory (DFT) employing Becke's (B) [21] three-parameter hybrid model, Lee, Yang and Parr's (LYP) correlation functional [22] under Pople's 6-31G (d) basis set which has amply been proven to give very good ground state geometries [23]. Initial optimization of all drugs was performed in the gas phase. To include solvent effect, gas optimized geometries were re-optimized in water employing polarizable continuum model (PCM) at the same level of theory [24, 25]. In addition, vibrational frequencies (Table 4) were scaled using the scale factor [26].

Frontier molecular orbital features HOMO (highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbital) were calculated at the same level of theory. For each of the drugs, hardness (η), softness (S), and chemical potential were calculated from the energies of frontier HOMO and LUMO as reported [27] considering Parr and Pearson interpretation [28, 29] of DFT and Koopmans theorem [30] on the correlation of ionization potential (I) and electron affinities (E) with HOMO and LUMO energy (eV). The following equations are used to calculate hardness (η), softness (S), and chemical potential (μ);

$$\eta = \frac{[\varepsilon_{\text{LUMO}} - \varepsilon_{\text{HOMO}}]}{2}; S = \frac{1}{\eta}; \mu = \frac{[\varepsilon_{\text{LUMO}} + \varepsilon_{\text{HOMO}}]}{2}$$

Preparation of receptor protein

Three dimensional crystal structure of aspirin acetylated human cyclooxygenase-2 (PDB ID: 5F19) was collected in pdb format from online protein data bank (PDB) database [31]. All hetero atoms and water molecules were eliminated using PyMol (version 1.3) software packages [32]. Energy

minimization of the protein implemented by Swiss-Pdbviewer software (version 4.1.0) [33]. Then optimized drugs were subjected for molecular docking study against human prostaglandin synthase protein (5F19).

Molecular Docking simulation, analysis, and visualization

In computer aided drug design, binding affinity and mode(s) of ligand with target protein can predict by molecular docking simulation [34, 35]. Finally, molecular docking simulation was performed by PyRx software (version 0.8) [36] considering the protein as macromolecule and the drug as ligand. In this analysis, rigid docking was performed where, all rotatable bonds were converted into non-rotatable with the center grid box size 20.8612, 37.5501 and 59.3402 Å along x, y and z directions respectively. After docking, both the protein and ligand structures were saved in pdb format to perform non-bonding interactions. [37]. Non bonded interaction was performed to analyze, visualize, and interpret the docking result and different interactions among ligands and amino acid residues of target protein.

ADMET prediction

Absorption, distribution, metabolism, excretion, toxicity of KTR and its degradation products were predicted by utilizing AdmetSAR online database [38]. SDF (Structure Data File) and SMILES (simplified molecular-input line-entry system) strings were used throughout the generation process.

Table 3 Energy (eV) of HOMO, LUMO, Gap, hardness (η), softness (S), and chemical potential (μ) of Ketorolac (KTR) and its degradation products

Drug	HOMO	LUMO	Gap	η	S	μ
KTR	−5.93	−1.43	4.50	2.25	0.44	−3.68
K1	−5.74	−1.28	4.46	2.23	0.45	−3.51
K2	−5.72	−1.26	4.46	2.23	0.45	−3.49
K3	−5.98	−1.45	4.53	2.26	0.44	−3.72
K4	−5.91	−1.47	4.44	2.22	0.45	−3.69
K5	−5.87	−1.39	4.48	2.24	0.45	−3.63

Fig. 2 Frontier molecular orbital (HOMO and LUMO) and related energies of Ketorolac (KTR) and K4

Result and discussion

Thermodynamic properties analysis

Chemical and optimized structures of KTR and its degradation products are depicted in Fig. 1. Thermodynamic properties such as difference of electronic energy (ΔE), enthalpy (ΔH), Gibb's free energy (ΔG) and dipole moment ($\Delta \mu$) are calculated by subtracting gas phase values from water phase values are tabulated in Table 2. Spontaneity of a reaction and stability of a product can be predicted from Gibb's free energy, enthalpy, and electronic energy [39]. Free energy is a significant criterion to represent the interaction of binding partners, where negative value favourable for spontaneous binding and interaction. The free energy difference (ΔG) of KTR is -8.59 kcal/mol, where K4 shows the highest negative value -12.10 kcal/mol. With increasing the negative value from KTR to K4, hence suggesting energetically and configurationally more stable.

In drug design, improved dipole moment can enhance hydrogen bond and non-bonded interactions in drug receptor complexes which keep an important role to increase binding affinity. Elevated dipole moment improves the polar nature of a molecule [40]. The difference of dipole moment of KTR is found 1.14 Debye, where K4 (1.95 Debye) shows the highest value (Table 2). Therefore, increased binding affinity against 5F19 resulted for K4 with increased dipole moment.

Molecular orbital analysis

The HOMO and LUMO energies, gap, hardness, softness, and chemical potential, of all compounds are presented in Table 3. The electronic absorption relates to the transition from the ground to the first excited state and mainly described by one electron excitation from HOMO to LUMO (Fig. 2) [41]. From frontier molecular orbital theory, energies of HOMO and LUMO play an important role in chemical reactivity. The HOMO-LUMO gap also influence the chemical hardness,

Fig. 3 Molecular electrostatic potential map of KTR and K4

Table 4 Theoretical (gas phase) and experimental (X-ray diffraction) (a) bond distances and (b) bond angles ($^{\circ}$) of all compounds

(a)			
Name	Atom number	Bond distances ($\text{\AA}$)	
		Calculated	Experimental*
KTR	C12-O13	1.2348	1.2286
	N7-C11	1.3827	1.3860
	N7-C8	1.3581	1.3515
	N7- C6	1.4647	1.4633
	C2-O3	1.2101	1.2153
	O1-C2	1.3540	1.3143
	O1-H20	0.9761	–
K1	C9-O10	1.2360	1.2286
	N4-C8	1.3847	1.3860
	N4- C3	1.4639	1.4633
	N4-C5	1.3588	1.3515
K2	C9-O10	1.2374	1.2286
	N4-C8	1.3813	1.3860
	N4- C3	1.4590	1.4633
	N4-C5	1.3667	1.3515
K3	C9-O10	1.2344	1.2286
	N4-C8	1.3808	1.3860
	N4- C3	1.4662	1.4633
	N4-C5	1.3589	1.3515
K4	C1-O29	1.4347	–
	O29-H30	0.9701	–
	C12-O13	1.2342	1.2286
	N7-C11	1.3844	1.3860
	N7-C8	1.3588	1.3515
	N7- C6	1.4641	1.4633
	C2-O3	1.2103	1.2153
	C35-O36	1.2112	1.2153
	O1-C2	1.3537	1.3143
	O37-C35	1.3549	1.3143
K5	O1-H20	0.9763	–
	O37-H38	0.9760	–
	C12-O13	1.2348	1.2286
	N7- C11	1.3830	1.3860
	N7- C6	1.4649	1.4633
	N7-8C	1.3579	1.3515
	O1-C2	1.3485	–
	C2-O3	1.2113	1.2153
	O1-C31	1.4390	–
(b)			
Name	Atom number	Bond angles ($^{\circ}$)	
		Calculated	Experimental*
KTR	C12-C14-C15	117.54	117.64
	C12-C14-C19	123.27	123.00
	C10-C11-C12	133.02	132.46
	C6-N7-C8	114.20	114.65
	C6-N7-C11	135.61	135.46

Table 4 (continued)

	C8-N7-C11	110.11	109.72
	N7-C11-C12	120.78	121.23
	N7-C11-C10	106.06	105.99
	C11-C12-O13	120.47	–
	C14-C12-O13	119.79	–
	C11-C12-C14	119.72	–
	O3-C2-C4	125.82	–
	O3-C2-O1	123.04	–
	C4-C2-O1	111.13	–
	C2-O1-H20	106.33	–
K1	C9-C11-C12	117.57	117.64
	C9-C11-C16	123.27	123.00
	C9-C8-C7	133.08	132.46
	C3-N4-C5	114.07	114.65
	C3-N4-C8	135.43	135.46
	C5-N4-C8	110.45	109.72
	N4-C8-C9	120.86	121.23
	N4-C8-C7	105.87	105.99
	C11-C9-O10	119.62	–
	C8-C9-O10	120.64	–
	C8-C9-C11	119.73	–
K2	C9-C11-C12	117.58	117.64
	C9-C11-C16	123.25	123.00
	C9-C8-C7	134.03	132.46
	C3-N4-C5	112.00	114.65
	C3-N4-C8	137.49	135.46
	C5-N4-C8	110.50	109.72
	N4-C8-C9	120.00	121.23
	N4-C8-C7	105.84	105.99
	C11-C9-O10	119.87	–
	C8-C9-O10	119.89	–
	C8-C9-C11	120.22	–
K3	C9-C11-C12	117.55	117.64
	C9-C11-C16	123.25	123.00
	C9-C8-C7	132.92	132.46
	C3-N4-C5	113.69	114.65
	C3-N4-C8	135.96	135.46
	C5-N4-C8	110.32	109.72
	N4-C8-C9	120.83	121.23
	N4-C8-C7	106.09	105.99
	C8-C9-O10	120.38	–
	C11-C9-O10	119.84	–
	C1-O29-H30	107.60	–
	C8-C9-C11	119.77	–
K4	C12-C14-C15	117.46511	117.64
	C12-C14-C19	123.34477	123.00
	C10-C11-C12	132.64328	132.46
	C6-N7-C8	114.12567	114.65
	C6-N7-C11	135.73051	135.46
	C8-N7-C11	110.02211	109.72

Table 4 (continued)

K5	N7-C11-C12	121.21949	121.23
	N7-C11-C10	105.97105	105.99
	C11-C12-O13	120.79456	–
	C14-C12-O13	119.63527	–
	C11-C12-C14	119.56309	–
	O3-C2-C4	125.79716	–
	O3-C2-O1	122.84941	–
	C4-C2-O1	111.33714	–
	C2-O1-H20	106.34452	–
	C12-C14-C15	117.56	117.64
	C12-C14-C19	123.27	123.00
	C6-N7-C8	114.15	114.65
	C6-N7-C11	135.63	135.46
	C8-N7-C11	110.15	109.72
	C10-C11-C12	132.99	132.46
	N7-C11-C12	120.82	121.23
	N7-C11-C10	106.04	105.99
	C11-C12-O13	120.52	–
	C14-C12-O13	119.76	–
	C11-C12-C14	119.70	–
	O3-C2-C4	125.35	–
	O3-C2-O1	124.04	–
	C2-O1-C31	115.41	–
	O1-C2-4C	110.59	–

*Ref. [26]

softness, chemical potential and electrophilic index of a molecule [27, 42]. Large HOMO-LUMO gap is responsible for high kinetic stability and low chemical reactivity. A small HOMO-LUMO gap is important for low chemical stability, because addition of electrons to a high-lying LUMO and/or removal of electrons from a low-lying HOMO are energetically favourable in any potential reaction. In current analysis, most of the compounds (except K3) have lower HOMO-LUMO gap than KTR, where K4 has the lowest HOMO-LUMO gap (4.44 eV) with a chemical potential (−3.69 eV) which may contribute to show higher chemical activity and polarizability.

Molecular electrostatic potential analysis

Molecular electrostatic potential (MEP) was calculated to forecast the reactive sites for electrophilic and nucleophilic attack of all optimized structures. It also helps to interpret biological recognition process and hydrogen bonding interaction [43]. Red colour represent maximum negative area which favourable site for electrophilic attack, blue colour indicate the maximum positive area which favourable site for nucleophilic attack and green colour

represent zero potential area. MEP displays molecular size, shape as well as positive, negative and neutral electrostatic potential regions simultaneously in terms of colour grading. It is seen from MEP map, region having the negative potential are over electronegative atom (oxygen atoms) and having positive potential are over hydrogen atoms. Here, the maximum negative (−0.3046 a.u., deepest red) and positive (+0.2122 a.u., deepest blue) potentiality is found in K4 (Fig. 3).

Equilibrium geometry

Selected bond distances and angles of Ketorolac and its degradation products are summarized in Table 4a, b (for atoms number see the optimized structures). Equilibrium geometry is an important criterion for structural optimization of any chemical species. Structural modification and solvent effect may change in geometry as well as in vibrational frequencies [44]. In this study, though all the geometry optimizations

Table 5 Selected vibrational frequencies (cm^{−1}) calculated in gas phase (scaled)

Name	Assignment	Vibrational frequencies (cm ^{−1})
KTR	νO-H ^b stretch	3540
	νC-H ^a stretch	3082
	νC=O ^b stretch	1775
	νC=Ostretch	1634
	νC-Nstretch	1249
K1	νC-H ^a stretch	3081
	νC=Ostretch	1630
	νC-Nstretch	1245
K2	νC-H ^a stretch	3082
	νC=Ostretch	1623
	νC-Nstretch	1249
K3	νO-Hstretch	3598
	νC-H ^a stretch	3082
	νC=Ostretch	1636
	νC-Nstretch	1244
K4	νO-H ^b stretch	3542
	νC-H ^a stretch	3082
	νC=O ^b stretch	1773
	νC=Ostretch	1637
	νC-Nstretch	1252
	νC-Ostretch	1188
	νC-H ^a stretch	3082
K5	νC=O ^b stretch	1754
	νC=Ostretch	1634
	νC-Nstretch	1249
	νC-Ostretch	1193

A = aromatic, b = carboxylic

Fig. 4 IR spectra of KTR, K2, K3 and K4 in gas phase, calculated at 6–31 G (d) level theory

performed in gas phase and water phase, but only at gas phase results are presented due to the no significant geometry changes were observed in water phase. The calculated bond distances and angles were compared with X-ray diffraction data [45].

From Table 4a, it has been observed that the calculated bond distances are approximately same with the experimental data, which support the optimized structures. A noticeable change is observed for O1-C2, O37-C35 (carboxylic) bond length in KTR and K4. In KTR, calculated distance of O1-C2 (1.3540 Å) is larger than the experimental value (1.3143 Å). Similarly in K4, calculated distances of O1-C2 (1.3537 Å), O37-C35 (1.3549 Å) are higher than experimental (1.3143 Å).

Similarly from Table 4b, the calculated bond angles are approximately same with the experimental data, which support the optimized structures. A deviation is observed in K2 compound. In C9-C8-C7, C3-N4-C5 and C3-N4-C8 the noticeable changes arise due to the presence of double bond at C1-C2.

Vibrational frequencies

Selected IR vibrational frequencies and IR spectra are depicted in Table 5 and Fig. 4 respectively. All the vibrational frequencies are calculated in gas phase at the same level of theory then multiplied by the scale factor (0.9613) [26]. The infrared (IR) spectra for all compounds were measured in the 0–4000 cm^{-1} range and helped to indicate regions of absorption due to the respective vibrations. The band found in the region 3081–3082 cm^{-1} assigned to the aromatic $\nu\text{C-H}$ stretching. The band observed between 1623 and 1636 cm^{-1} due to symmetric stretching of $\nu\text{C=O}$ (ketone) and another band in the region 1754–1775 cm^{-1} assigned to symmetric stretching of $\nu\text{C=O}$ (carboxylic) group. The band observed at 3598 cm^{-1} due to the symmetry stretching of $\nu\text{O-H}$ and band between 3540 and 3545 cm^{-1} confirms the symmetry stretching of $\nu\text{O-H}$ (carboxylic) group. The peak between 1244 and 1249 cm^{-1} has been assigned to the $\nu\text{C-N}$ stretching, and band found between 1192 and 1193 cm^{-1} due to the presence of $\nu\text{C-O}$ stretch (Figs. 5, 6 and 7).

Fig. 5 Atomic partial charges of KTR

Fig. 5 Atomic partial charges of KTR

Fig. 6 Docked conformation of KTR at inhibition bounding site of receptor protein 5F19

Atomic partial charge

Two different methods (Mulliken and NBO) have been utilized to compute the partial charges of all drug atoms including both quantum chemical and empirical schemes [46]. They are the most recognised population analysis methods and have a significant role in the application of quantum chemical calculations to molecular system because dipole moment and molecular polarizability also related to atomic charges [47]. Here, all the hydrogen atoms show positive charge in both methods and other electronegative atoms (N, O) negative charge in both methods as expected. In KTR C-2 and C-12 show greater positive charge found due to the presence of highly electronegative oxygen (O-3, O-13) atoms and H-20 shows the highest positive value than other hydrogen because of oxygen atom of hydroxyl group. Similarly, in K1, K2, K3 (C-9), K4 (C-2, C-12, C-35), K5 (C-2, C-12) show positive charge in both methods due to the presence of oxygen atom of carbonyl group. All the nitrogen atoms show negative charge in both methods as expected.

Binding affinity and interactions with 5F19

Investigation of binding properties of degradation products of KTR as prostaglandin inhibitor is performed by molecular docking calculation by PyRx software. Binding affinities and drug-protein interactions of all drugs are summarized in Table 6. The binding affinity of KTR is -8.9 kcal/mol where, K1, K2 and K4 have considerably increased to -9.1 , -9.1 and -9.4 kcal/mol respectively. Decreased binding affinity found in case of K3 (-8.0 kcal/mol) and K5 (-7.6 kcal/mol). Hydrogen bond is essential for DNA structure in biological system. Improved hydrogen bonding observed in K4, which not only contributes in increasing binding affinity but can also increase binding speciality. In K4-5F19 docked structure, multiple nonbonded interactions were observed. From theoretical data, it is mentioned that, hydrogen bond of <2.3 Å are able to increase the binding affinity by several magnitude [48]. An effective oxygen hydrogen bond with Asn34 (1.99 Å) and other strong hydrogen bond with Cys47 (2.72 Å) are observed in K4-5F19 complex. Thus, strong hydrogen bonding is most significant contributing factor in increasing binding affinity of

Fig. 7 Nonbonding interactions of KTR, and K4 with 5F19 generated by Discovery Studio

Table 6 Binding affinity and nonbonding interaction of KTR and its degradants with 5F19 after rigid docking

Name	Binding affinity (kcal/mol)	Residues in contact	Interaction type	Distance (Å)
KTR	−8.9	Ser353	C	2.5478
		Ser353	C	2.8902
		Leu531	Pc	4.7246
		Leu534	PA	5.1872
		Leu352	PA	5.0357
		Ala527	PA	5.0360
		Phe529	Pa	4.4996
		Val349	PA	4.9348
K1	−9.1	Met522	C	3.0880
		Leu531	Pc	4.8974
		Tyr385	Pps	5.7573
		Val523	A	4.4674
		Met522	A	5.4728
		Phe518	PA	4.7216
		Leu534	PA	5.3114
		Val349	PA	4.7745
		Leu352	PA	4.8948
		Leu531	Pc	4.8237
K2	−9.1	Tyr385	Pps	5.7095
		Trp387	Pps	5.3610
		Leu352	A	5.0832
		Val523	A	4.6480
		Phe518	PA	4.8490
		Leu534	PA	5.3256
		Tyr130	H	2.4983
		Ala151	H	3.0027
K3	−8.0	Cys41	PA	5.0602
		Cys47	PA	4.6500
		Pro153	PA	5.2026
		Arg44	PA	4.5221
		Leu152	PA	5.3544
		Asn34	H	1.9933
		Cys47	C	2.7231
		Asp157	Pa	3.5684
K4	−9.4	Tyr136	Pps	5.4665
		Pro154	PA	4.6983
		Gln203	H	2.8028
		His207	C	2.8947
K5	−7.6	His388	C	2.4520
		Phe210	C	2.4509
		His207	Pps	4.5060

H Conventional hydrogen bond, *C* Carbon hydrogen bond, *Pa* Pi-anion, *Pc* Pi-cation, *A* Alkyl, *PA* Pi-alkyl, *Pps* Pi-pi T shaped

K4 with 5F19. Non-covalent interactions such as hydrogen bond, hydrophobic interaction and electrostatic interaction are involved in the binding of K1 with 5F19. Some others important hydrogen bonds Tyr130 (2.49 Å) in K3 and His388 (2.45 Å), Phe210 (2.45 Å) in K5 found. The K4-

5F19 structure is stabilized by two hydrogen bonds (Table 6) and three hydrophobic interactions including a Pi- Pi T-Shaped at Tyr136 (5.46 Å). Where, K3 shows two hydrogen bonds and five hydrophobic interaction and K5 shows Pi- Pi T-Shaped at His270 (4.50 Å) beside three of hydrogen bonds.

Fig. 8 Hydrogen bond surface of 5F19 with KTR and K4

Hydrogen bond interaction with residue Met522 observed in K1 and K2 has no hydrogen bond. Nonbonding interactions of KTR and its degradation products (K3, K4 and K5) in 5F19 are involved the following amino acids residues Val523, Arg469, Sar353, Met522, Tyr130, Tyr385, Ala151, Gln461, Cys36, Cys47, His39, Gln203, His388, Phe210 and many more. Hydrogen bond surface shows residues such as Phe529, Phe210, Tyr385, Tyr130, His207, His388, Met522, Cys36, Cys47 help in creating strong acceptor regions and residues such as Leu531, Ala151, Tyr130, Gln203, Gln461 help in creating strong donor regions on the drug-protein interaction surface (Fig. 8) and most of them can bound the active site of COX-2 [49]. This observation indicates that, KTR and its degradents (especially K4) are able to bind at the desired binding site of the protein 5F19. Alkyl- π interactions are observed in all drugs except K5. π - π stacked interactions also found for Tyr385 (at K1 and K2), Trp387 (at K2) and His207 (at K5) plays an important role for the drugs. Two more significant hydrogen bonds are observed at K4 than KTR. Strong π -cation interaction of Leu531 is detected at KTR, K1 and K2 under electrostatic interactions. Where a π -anion interaction of Phe529 (4.50 Å) is observed at KTR. There are several π -alkyl interactions observed in most of the drugs except K5.

ADMET analysis

From AdmetSAR calculation (Table 7), all the structures exhibit positive result for blood brain barrier (BBB) criteria, predicting that degradents can pass through the BBB. All degradents are non-carcinogenic and show III category acute oral toxicity. Where, KTR shows II category acute oral toxicity. So, all the degradents are relatively harmless than KTR for oral administration. All drugs are P-glycoprotein non-inhibitor where, P-glycoprotein inhibition can interrupt the absorption, permeability and retention of the drugs [50]. Moreover, all the analogues (except K4) show improved human intestinal absorption values. Rat acute toxicity of all drugs is higher than KTR. So, all degradents show higher median lethal dose (LD_{50}) values compared to parent drug. However, all the drugs show weak inhibitory feature for human ether-a-go-go-related gene (hERG) which can lead to long QT syndrome [51], so further more study of this aspect is necessary.

Conclusion

In this study, five degradation products of Ketorolac have been studied for the exploring of molecular interaction and binding

Table 7 Selected pharmacokinetic parameters of KTR and its degradation products. (Probability values related to each of the parameters are given in the parenthesis)

Parameters	KTR	K1	K2	K3	K4	K5
Blood brain barrier	+ (0.7918)	+ (0.9858)	+ (0.9919)	+ (0.9513)	+ (0.6234)	+ (0.9351)
Human intestinal absorption	+ (0.9919)	+ (1.0000)	+ (1.0000)	+ (1.0000)	+ (0.9218)	+ (0.9958)
P-glycoprotein inhibitor	NI (0.9624)	NI (0.9183)	NI (0.9281)	NI (0.9190)	NI (0.9555)	NI (0.8532)
Human ether α -go-go-related gene inhibition	WI (0.9478)	WI (0.8370)	WI (0.9323)	WI (0.9032)	WI (0.9582)	WI (0.9189)
Carcinogen	NC (0.9592)	NC (0.9464)	NC (0.9103)	NC (0.9687)	NC (0.9083)	NC (0.9471)
Acute oral toxicity	II	III	III	III	III	III
Rat acute toxicity LD_{50} mol/kg	2.2902	2.4010	2.3887	2.3068	2.3927	2.4805

NI Non-inhibitor, WI Weak inhibitor, NC Non-carcinogenic

affinity with prostaglandin H2 (PGH2) synthase protein 5F19. Equilibrium geometry and vibrational frequency calculation support the structures. From quantum calculation, all the degradation products are thermally stable and having lower HOMO-LUMO gap and higher softness than parent drug (except K3). From molecular docking result, strong binding affinity is found for K4-5F19 complex due to the substitution of carboxymethyl radical. Pharmacokinetic result predicts all drugs are non-carcinogenic and relatively harmless than KTR for oral administration. Considering present investigation, K1, K2 and K4 can be potent new possible candidate for better performance.

Acknowledgements Authors are thankful to Dr. Mohammad A. Halim (COE, The Red –Green Research Centre, Dhaka, Bangladesh) for his valuable suggestion.

Authors contribution Mohammad Nasir Uddin (MNU) and Moniruzzaman (M) conceived the idea and prepared the manuscript. M performed all quantum chemical calculations and data collection. All authors read and approved the manuscript.

Compliance with ethical standards

Conflicts of interest Authors declare no conflict of interest.

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References

- Mallinson TE. A review of ketorolac as a prehospital analgesic. *J Paramed Pract.* 2017;9:522–6.
- Litvak KM, McEvoy GK. Ketorolac, an injectable nonnarcotic analgesic. *Clin Pharm.* 1990;9:921–35.
- H.P. Rang, Rang and Dale's pharmacology, Churchill Livingstone, (2007).
- Myers KG, Trotman IF. Use of ketorolac by continuous subcutaneous infusion for the control of cancer-related pain. *Postgrad Med J.* 1994;70:359–62.
- Forget P, Bentin C, Machiels J-P, Berlière M, Coulie PG, De Kock M. Intraoperative use of ketorolac or diclofenac is associated with improved disease-free survival and overall survival in conservative breast cancer surgery. *Br J Anaesth.* 2014;113:i82–7.
- R. Reddy, S.J. Kim, Critical appraisal of ophthalmic ketorolac in treatment of pain and inflammation following cataract surgery, clinical ophthalmology (Auckland, NZ) 5 (2011) 751.
- Reinhart DJ. Minimising the adverse effects of ketorolac. *Drug Saf.* 2000;22:487–97.
- Galán-Herrera JF, Poo JL, Maya-Barrios JA, de Lago A, Oliva I, González-de la Parra M, et al. Bioavailability of two sublingual formulations of ketorolac tromethamine 30 mg: a randomized, open-label, single-dose, two-period crossover comparison in healthy Mexican adult volunteers. *Clin Ther.* 2008;30:1667–74.
- Wamer TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *FASEB J.* 2004;18:790–804.
- Strom BL, Berlin JA, Kinman JL, Spitz PW, Hennessy S, Feldman H, et al. Parenteral ketorolac and risk of gastrointestinal and operative site bleeding: a postmarketing surveillance study. *JAMA.* 1996;275:376–82.
- C. on S. of Medicines. Ketorolac: new restrictions on dose and duration of treatment. *Current Problems in Pharmacovigilance.* 1993;19:5–6.
- Uddin MN. A novel validated uplc method for the estimation of ketorolac tromethamine in pharmaceutical formulation. *Research.* 2014.
- Salaris M, Niedo M, Rubattu N, Testa C, Luongo E, Rimoli MG, et al. Acid and base degraded products of ketorolac. *J Pharm Biomed Anal.* 2010;52:320–2.
- Leo G, Hi-Shi C, Johnson D. Light degradation of ketorolac tromethamine. *Int J Pharm.* 1988;41:105–13.
- Kalariya PD, Raju B, Borkar RM, Namdev D, Ganadhamu S, Nandekar PP, et al. Characterization of forced degradation products of ketorolac tromethamine using LC/ESI/Q/TOF/MS/MS and in silico toxicity prediction. *J Mass Spectrom.* 2014;49:380–91.
- Samal SK, Routray S, Veeramachaneni GK, Dash R, Botlagunta M. Ketorolac salt is a newly discovered DDX3 inhibitor to treat oral cancer. *Sci Rep.* 2015;5:9982. <https://doi.org/10.1038/srep09982>.
- Uzzaman TM, Mohammed JH, Mohammad NU. Amrin a, quantum chemical, molecular Docking, and ADMET predictions of ketorolac and its modified analogues. *Biomedical Journal of Scientific & Technical Resesarch.* 2018;11:1–7.
- Gleeson MP, Gleeson D. QM/MM calculations in drug discovery: a useful method for studying binding phenomena? *J Chem Inf Model.* 2009;49:670–7. <https://doi.org/10.1021/ci800419j>.
- Pence HE, Williams A. ChemSpider: an online chemical information resource. *J Chem Educ.* 2010;87:1123–4. <https://doi.org/10.1021/ed100697w>.
- R.A. Gaussian09, MJ Frisch, GW Trucks, HB Schlegel, GE Scuseria, MA Robb, JR Cheeseman, G. Scalmani, V. Barone, B. Mennucci, GA Petersson et al., Gaussian, Inc, Wallingford CT. (2009).
- Becke AD. Density-functional exchange-energy approximation with correct asymptotic behavior. *Phys Rev A.* 1988;38:3098–100. <https://doi.org/10.1103/PhysRevA.38.3098>.
- Lee C, Yang W, Parr RG. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys Rev B.* 1988;37:785–9. <https://doi.org/10.1103/PhysRevB.37.785>.
- Kruse H, Goerigk L, Grimme S. Why the standard B3LYP/6-31G* model chemistry should not be used in DFT calculations of molecular thermochemistry: understanding and correcting the problem. *J Organic Chem.* 2012;77:10824–34. <https://doi.org/10.1021/jo302156p>.
- Tomasi J, Mennucci B, Cammi R. Quantum mechanical continuum solvation models. *Chem Rev.* 2005;105:2999–3094.
- Onawole AT, Popoola SA, Saleh TA, Al-Saadi AA. Silver-loaded graphene as an effective SERS substrate for clotrimazole detection: DFT and spectroscopic studies. *Spectrochim Acta A Mol Biomol Spectrosc.* 2018;201:354–61.
- Halim MA, Shaw DM, Poirier RA. Medium effect on the equilibrium geometries, vibrational frequencies and solvation energies of sulfanilamide. *J Mol Struct THEOCHEM.* 2010;960:63–72.
- Azam F, Alabdullah NH, Ehmedat HM, Abulifa AR, Taban I, Upadhyayula S. NSAIDs as potential treatment option for preventing amyloid β toxicity in Alzheimer's disease: an investigation by docking, molecular dynamics, and DFT studies. *J Biomol Struct Dyn.* 2017:1–19.
- Calais J-L. Density-functional theory of atoms and molecules. R.G. Parr and W. Yang, Oxford University press, New York, Oxford, 1989. IX + 333 pp. Price £45.00. *Int J Quantum Chem.* 1993;47: 101. <https://doi.org/10.1002/qua.560470107>.
- Pearson RG. The HSAB principle — more quantitative aspects. *Inorg Chim Acta.* 1995;240:93–8. [https://doi.org/10.1016/0020-1693\(95\)04648-8](https://doi.org/10.1016/0020-1693(95)04648-8).

30. Pearson RG. Absolute electronegativity and hardness correlated with molecular orbital theory. *Proc Natl Acad Sci*. 1986;83:8440–1 <http://www.pnas.org/content/83/22/8440.abstract>.
31. Lucido MJ, Orlando BJ, Vecchio AJ, Malkowski MG. Crystal structure of aspirin-acetylated human Cyclooxygenase-2: insight into the formation of products with reversed stereochemistry. *Biochemistry*. 2016;55:1226–38. <https://doi.org/10.1021/acs.biochem.5b01378>.
32. W.L. DELANO, The PyMOL molecular graphics system. De-Lano scientific, San Carlos, CA, USA, <http://www.pymol.org>. (2002). <http://ci.nii.ac.jp/naid/10025409089/en/>.
33. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb viewer: an environment for comparative protein modeling. *Electrophoresis*. 1997;18:2714–23. <https://doi.org/10.1002/elps.1150181505>.
34. Seeliger D, De Groot BL. Conformational transitions upon ligand binding: holo-structure prediction from apo conformations. *PLoS Comput Biol*. 2010;6:e1000634.
35. G.M. Morris, M. Lim-Wilby, Molecular Docking, in: A. Kukul (Ed.), Molecular modeling of proteins, Humana press, Totowa, NJ, 2008: pp. 365–382. https://doi.org/10.1007/978-1-59745-177-2_19, Molecular Docking
36. Dallakyan S, Olson AJ. Small-molecule library screening by Docking with PyRx. In: Hempel JE, Williams CH, Hong CC, editors. *Chemical biology: methods and protocols*, springer New York, New York, NY; 2015. p. 243–50. https://doi.org/10.1007/978-1-4939-2269-7_19.
37. A.D.S. Version, 4.0, Accelrys, San Diego, USA, (2017).
38. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, et al. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J Chem Inf Model*. 2012;52:3099–105. <https://doi.org/10.1021/ci300367a>.
39. Cohen N, Benson SW. Estimation of heats of formation of organic compounds by additivity methods. *Chem Rev*. 1993;93:2419–38.
40. Lien EJ, Guo Z-R, Li R-L, Su C-T. Use of dipole moment as a parameter in drug-receptor interaction and quantitative structure-activity relationship studies. *J Pharm Sci*. 1982;71:641–55. <https://doi.org/10.1002/jps.2600710611>.
41. Saravanan S, Balachandran V. Quantum chemical studies, natural bond orbital analysis and thermodynamic function of 2, 5-dichlorophenylisocyanate. *Spectrochim Acta A Mol Biomol Spectrosc*. 2014;120:351–64.
42. Parr RG, Zhou Z. Absolute hardness: unifying concept for identifying shells and subshells in nuclei, atoms, molecules, and metallic clusters. *Acc Chem Res*. 1993;26:256–8. <https://doi.org/10.1021/ar00029a005>.
43. Politzer P, Murray JS. Molecular electrostatic potentials and chemical reactivity. *Rev Comput Chem*. 1991:273–312.
44. Pappalardo RR, Sanchez Marcos E, Ruiz-Lopez MF, Rinaldi D, Rivail JL. Solvent effects on molecular geometries and isomerization processes: a study of push-pull ethylenes in solution. *J Am Chem Soc*. 1993;115:3722–30.
45. JASINSKI JP, Butcher RJ, Narayana B, SWAMY MT, Yathirajan HS. Crystal structure of (±)-5-Benzoyl-2, 3-dihydro-1H-pyrrolidine-1-carboxylic acid, ketorolac. *Analytical Sciences: X-Ray Structure Analysis Online*. 2008;24:x205–6.
46. Gross KC, Seybold PG, Hadad CM. Comparison of different atomic charge schemes for predicting pKa variations in substituted anilines and phenols. *Int J Quantum Chem*. 2002;90:445–58. <https://doi.org/10.1002/qua.10108>.
47. Mulliken RS. Electronic population analysis on LCAO–MO molecular wave functions. I. *J Chem Phys*. 1955;23:1833–40.
48. Wade RC, Goodford PJ. The role of hydrogen-bonds in drug binding. *Prog Clin Biol Res*. 1989;289:433–44 <http://europepmc.org/abstract/MED/2726808>.
49. Viegas A, Manso J, Corvo MC, Marques MMB, Cabrita EJ. Binding of ibuprofen, ketorolac, and diclofenac to COX-1 and COX-2 studied by saturation transfer difference NMR. *J Med Chem*. 2011;54:8555–62. <https://doi.org/10.1021/jm201090k>.
50. Amin ML. P-glycoprotein inhibition for optimal drug delivery. *Drug Target Insights*. 2013;2013:27–34. <https://doi.org/10.4137/DTI.S12519>.
51. Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. *Nature*. 2006;440:463–9.