Asian Journal of Chemistry; Vol. 36, No. 10 (2024), 2461-2466

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.32547

Rapid Iodination Kinetic Studies of *o*-Toluidine in Aqueous Medium: A Pharmacokinetic Insight from QSAR and Molecular Docking of its Iodo Product with Cytochrome P450

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Received: 18 August 2024; *Accepted*: 20 September 2024; *Published online*: 30 September 2024; AJC-21778

The kinetic data of uncatalyzed rapid iodination of *o*-toluidine using molecular iodine in aqueous medium was investigated by employing hydrodynamic voltammetry (HV) technique. The frequency factor, activation energy and entropy change accompanying the reaction were evaluated. The reaction followed second order kinetics that had a half-life of 90 s and specific reaction rate 888.87 M s^{-1} at 22.1 ºC. The iodo product was formed *via* the green route in the aqueous solvent. The physico-chemical characteristics, lipophilicity, water solubility and pharmacokinetic parameters were derived by QSAR analysis. The docking of the iodo product with cytochrome P450 exhibited steric and physiological complementarity of the ligand-protein interface through hydrogen bonding and pi-pi stacking interactions, indicating that the product exhibited metabolic activity was similar to the existing drugs.

Keywords: Iodoproduct, Rapid kinetics, Hydrodynamic voltammetry, Pharmacokinetics, QSAR, Molecular docking.

INTRODUCTION

Iodination of aromatic substrates are rapid electrophilic substitution reactions in aqueous medium devoid of iodide ions [1,2]. The organic iodoaromatic compounds produced have extensive uses in the pharmaceutical industry, advanced organic synthesis [3,4] and numerous coupling processes such as Wurtz, Ullmann, Kumada, Neigishi, Suzuki reactions [5-9]. Advanced techniques are necessary to analyze the rapid kinetics of these iodination reactions [10,11]. Aryl iodides are the important precursors for the C-C and C-N bond forming reactions [12]. Recently, iodination reactions have been reported using HgO–I_2 [12], I_2-HIO_3 [13], NH_4I-H_2O_2 [14], $NaClO_3$-NaI/HCl [15], KI/TBuOOH [16], benzyltrimethyl ammonium dichloroiodate [17], KI/$KBrO_3$/H^+ [18], KI-$NaIO_4$ [19], KI-DMSO [20], *etc*. *In vitro* model of iodination of tyrosine has been studied to explain hyper/hypo-thyroid behaviour by DarFarhad *et al.* [21].

Most of these experiments, meanwhile, take place in non-aqueous media, which is likely because reactions happen so quickly in the aqueous solutions. More recently, molecular iodine in aqueous medium has been used for iodination of the regioisomers of nitroaniline [22]. In view of the dearth of studies of iodination in aqueous medium using aqueous molecular iodine devoid of iodide ions, we have studied uncatalyzed rapid iodination of *o*-toluidine (OTD) at pH 7.0. Herein, we have used relatively inexpensive hands-on hydrodynamic voltammetry (HV) set-up [23]. The molecular docking of the iodo aromatic derivative formed with cytochrome P450 (CYP) has also been explored to provide an insight into the biological activity of the product. The drug like properties and pharmacokinetics of the iodo product of OTD were ascertained from QSAR study.

EXPERIMENTAL

All chemicals having 99% purity were purchased from Sigma-Aldrich, USA and used as supplied. Stock solutions of 1 mM *o*-toluidine (OTD), 1 mM aqueous iodine and 100 mM potassium nitrate were prepared in double distilled water. The exact concentration of iodine solution was determined by iodimetric titration. From these stock solutions, the required concentrations of different solutions were prepared as shown in Table-1. Stock solutions of 100 mM each of disodium phosphate (Na_2HPO_4) and monosodium phosphate (NaH_2PO_4), were prepared for the buffer solution of pH 7.0. Digital pH meter-Equiptronics Model EQ-610 was used to determine pH of the solutions. The hands-on hydrodynamic voltammetry (HV)

TABLE-1
CONSTANT PARAMETERS IN THE KINETICS OF IODINATION OF *o*-TOLUIDINE IN AQUEOUS MEDIUM AT VARIOUS TEMPERATURES AT pH 7.0

Parameters	Values	Units
Potential applied at the RPE *vs.* SCE	0.10	V
Initial concentration of molecular iodine	1.25×10^{-5}	M
Initial concentration of *o*-toluidine	1.25×10^{-5}	M
Concentration of KNO_3	1.25×10^{-3}	M
Total volume of the reaction mixture	100	cm^3

setup was used wherein the indicator electrode was a rotating platinum electrode (RPE) and the reference electrode was the saturated calomel electrode (SCE).

Calibration and kinetics: Hydrodynamic voltammetry (HV) was used to study the rapid uncatalyzed iodination kinetics of *o*-toluidine (OTD) by aqueous molecular iodine at pH 7.0 and different temperatures *viz.* 12.0, 17.2, 22.1, 27.5 and 32.2 ºC. Iodine was the only electro-reducible species among the reactants and products in the reaction under study at the applied potential 0.10 V and its decaying concentration during the course of reaction at various time intervals was detected in terms of the diffusion current (I_D) at the rotating platinum electrode (RPE). Calibration of the diffusion current was carried out prior to the kinetic study. Calibration of $[I_2]$ at pH 7.0 and the temperatures 12.0, 17.2, 22.1, 27.5 and 32.2 ºC is reported in Table-2 and Fig. 1.

TABLE-2
CALIBRATION OF THE DIFFUSION CURRENT OF I_2 AT VARIOUS TEMPERATURES FOR IODINATION OF *o*-TOLUIDINE IN AQUEOUS MEDIUM AT 7 pH (± 0.2 nA error)

$[I_2]/10^{-5}$ (M)	Diffusion current (nA)				
	12.0 °C	17.2 °C	22.1 °C	27.5 °C	32.2 °C
0.25	6.4	6.6	6.8	7.0	7.3
0.50	12.8	13.2	13.6	14.0	14.7
0.75	19.2	19.9	20.4	21.1	22.0
1.00	25.6	26.5	27.2	28.1	29.4
1.25	32.1	33.0	34.0	35.2	36.8

Aqueous iodine and OTD solutions each 50 mL and 25 µM, containing the required supporting electrolyte and buffers were maintained in a thermostat. After attaining the desired temperature, both the reactants were added simultaneously to the reaction vessel containing the two electrodes. The initial concentration of each of two reactants became 12.5 µM after mixing due to double dilution. The fall in the concentration of iodine during the course of reaction was recorded in terms of

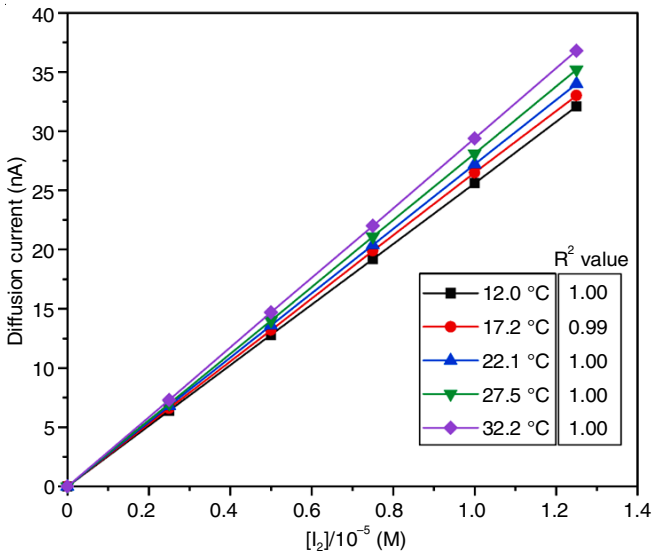


Fig. 1. Calibration of iodine solutions at different temperatures and pH 7.0

the diffusion current at the RPE and was recorded at every 10 s. The procedure was repeated at different temperatures and at 7.0 pH. Kinetics of iodination of OTD in aqueous medium at 22.1 ºC and pH 7.0 is reported as a typical set of observations in Table-3. The plots of $[I_2]^{-1}$ *versus* time were plotted for different temperatures to obtain the specific reaction rates at these temperatures as shown in Fig. 2.

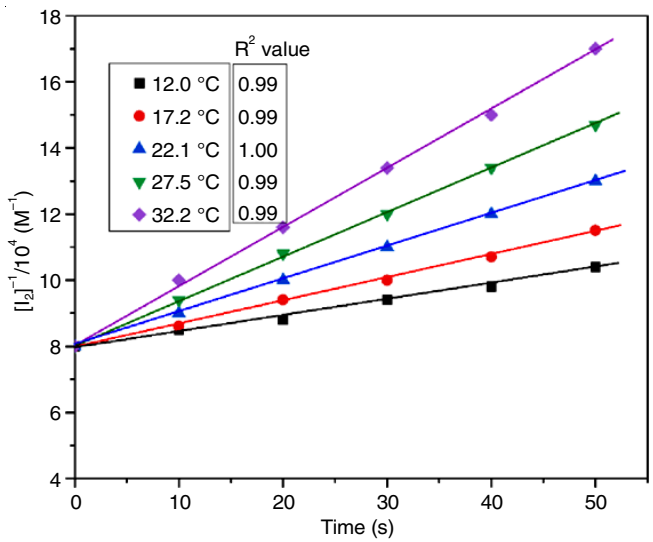


Fig. 2. Kinetics of uncatalyzed iodination of *o*-toluidine at different temperatures and pH 7.0

TABLE-3
KINETICS OF IODINATION OF *o*-TOLUIDINE AT VARIOUS TEMPERATURES IN AQUEOUS MEDIUM AT 7 pH (± 0.2 nA error)

Time (s)	Diffusion current (nA)					$[I_2]/10^{-5}$ (M)					$[I_2]^{-1}/10^4$ (M^{-1})				
	12.0 °C	17.2 °C	22.1 °C	27.5 °C	32.2 °C	12.0 °C	17.2 °C	22.1 °C	27.5 °C	32.2 °C	12.0 °C	17.2 °C	22.1 °C	27.5 °C	32.2 °C
0	34.0	34.0	34.0	34.0	34.0	1.25	1.25	1.25	1.25	1.25	8.0	8.00	8.0	8.0	8.0
10	30.0	31.0	30.2	29.0	28.0	1.17	1.16	1.11	1.00	0.95	8.5	8.60	9.0	10.0	10.5
20	29.0	26.8	27.1	25.0	23.0	1.13	1.06	1.00	0.86	0.78	8.8	9.40	10.0	11.6	12.8
30	26.0	26.4	24.8	21.4	20.4	1.06	1.00	0.90	0.74	0.68	9.4	10.0	11.0	13.4	14.7
40	25.5	24.6	21.6	21.0	20.0	1.02	0.93	0.80	0.66	0.62	9.8	10.7	12.0	15.0	16.1
50	24.6	22.8	19.0	18.6	17.6	0.96	0.86	0.70	0.58	0.56	10.4	11.5	13.0	17.0	17.8

Molecular docking: Molecular docking was performed using MGL tools 1.5.6 software with the Auto Grid 4.2.6 and Auto Dock 4.2.6 packages. Swiss TargetPrediction webserver (http://www.swisstargetprediction.ch) was used to predict the target molecule of product ligand *o*-iodo OTD. CYP was the best target suggested. 6cir, stable, high-expression variant of human CYP was downloaded from PDB (www.rcsb.org). The downloaded structure was processed in Discovery Studio to remove water, heteroatoms and ligands. The processed protein structure was saved as protein pdb file. In the MarvinSkech software 3D structures of *o*-iodo OTD were drawn and saved separately as ligand pdb file. The optimized pdbqt structure of enzyme CYP and ligands *o*-iodo OTD were used in the molecular docking study. Lamarckian genetic algorithm (GA) 4.2 was used in this docking study. Polar hydrogen and Kollman charges were added before starting molecular docking. Auto grid was used to set the grid point. All other parameters were set to the default setting 10 docking runs were carried out. The minimum negative binding energy of docking was -4.23 kcal.

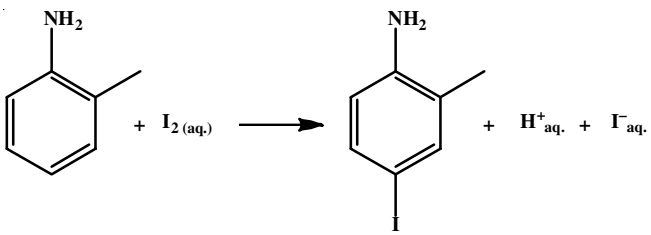
QSAR study: The physico-chemical properties, lipophilicity, water solubility, pharmacokinetics, drug likeness, medicinal chemistry of the iodo-product of OTD were obtained from online QSAR model SwissADME.

RESULTS AND DISCUSSION

Product formation: Halogenations of aromatic substrates in aqueous solution are pH-dependent reactions [24], hence, pH 7 was used in the iodination of OTD by molecular iodine in aqueous medium devoid of iodide ions. Molecular iodine undergoes hydrolysis in aqueous medium forming hydrogen ions, iodide ions and hypoiodous acid.

$$I_2 + H_2O \rightleftharpoons HOI + H^+_{aq.} + I^-_{aq.}$$

The equilibrium constant (5.4×10^{-13} M^2) for the hydrolysis of iodine indicates that the concentration of iodide ions in weak aqueous solutions employed in this investigation is insignificant, since the equilibrium is significantly shifted to backward [25]. Iodination of aromatic substrates are electrophilic substitution reactions. In the aromatic substrate OTD, $-NH_2$ group has a lone pair of electrons and is *ortho-para* directing. In OTD, of the two *ortho* positions, one *ortho* position is blocked by the methyl group and the other is not preferred by the introduction of bulky iodo group due to the steric compulsion of the proximity of $-NH_2$ and *ortho* $-CH_3$ group. Considering the donating nature and the steric compulsion of the exisiting groups of OTD, the product 4-iodo-2-methyl aniline *i.e. p*-iodo-*o*-toluidine (PIOTD) was formed. The reaction is reported in **Scheme-I**.



Scheme-I: Iodination of *o*-toluidine

Specific reaction rate (k): A plot of $[I_2]^{-1}$ *vs.* time was found to be linear confirmed that the reaction is of second order. The second order velocity constant for iodination of OTD was found to be 888.87 M^{-1} s^{-1} at 22.1 ºC and pH 7.0.

Activation energy (E_a): The E_a of iodination of OTD in aqueous solution was obtained from the Arrhenius plot of the reaction, which was studied at five different temperatures. The variation of specific reaction rates with temperatures at pH 7.0 is reported in Table-4 and Fig. 3 (plot of log k *vs.* T^{-1}). The E_a was calculated by using eqn. 1 and found to be 24.07 kJ mol^{-1}.

$$E_a = -2.303 \times R \times \text{Slope of the plot log k } vs.\ T^{-1} \quad (1)$$

TABLE-4
VARIATION OF SPECIFIC REACTION RATE OF IODINATION OF *o*-TOLUDINE BY I_2 IN AQUEOUS MEDIUM WITH TEMPERATURE AT pH 7.0 (±0.2 nA error)

Temp. (°C)	Temp. (K)	$[T]^{-1}/10^{-3}$ (K^{-1})	k (M^{-1} s^{-1})	log k
12.0	285.0	3.50	420.00	2.6232
17.2	290.2	3.44	640.00	2.8062
22.1	295.1	3.38	888.87	2.9488
27.1	300.5	3.32	1277.7	3.1064
32.2	305.2	3.27	1666.6	3.2218

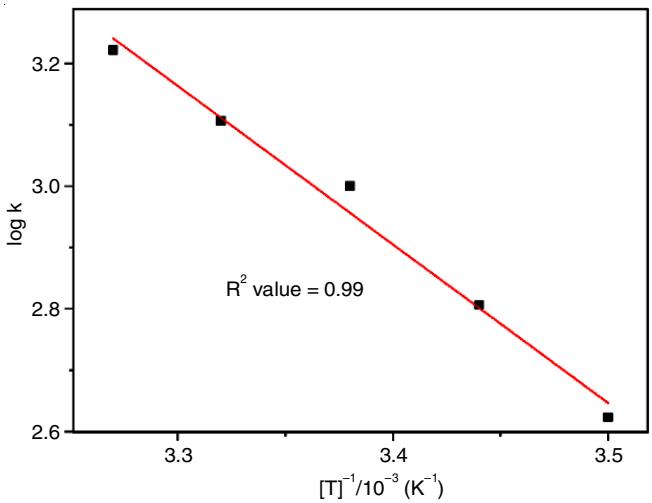


Fig. 3. Arrhenius plot for iodination of *o*-toluidine

Pre-exponential or frequency factor (A): The values of E_a and k were used to calculate the frequency factor (A) using Arrhenius equation. Using eqns. 2 and 3, the value of frequency factor (A) was calculated and found to be 1.62×10^{7} M^{-1} s^{-1}:

$$k = A.\exp\left(-\frac{E_a}{RT}\right) \quad (2)$$

$$\log A = \log k + \frac{E_a}{2.303RT} \quad (3)$$

Entropy of activation ($\Delta S^{\ddagger}$): All the reactions are in aqueous solution; hence, eqn. 4 was used to calculate $\Delta S^{\ddagger}$

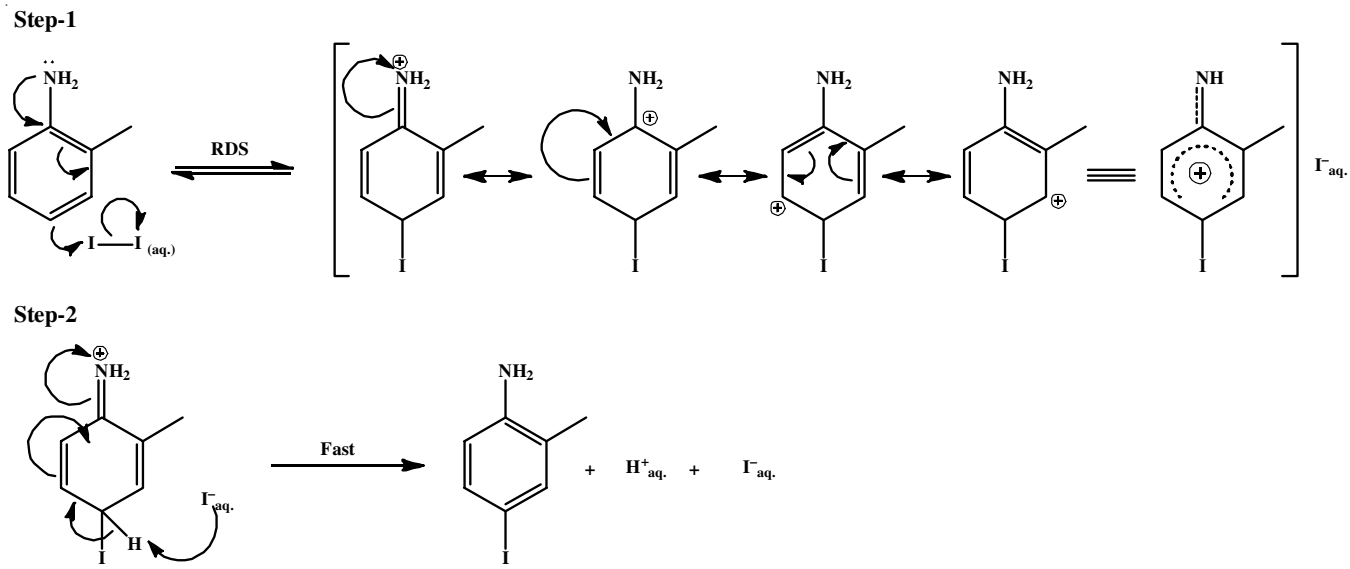
$$\Delta S^{\ddagger} = 2.303R \log k - 2.303R \log\left(\frac{ekBT}{h}\right) + \frac{E_a}{T} \quad (4)$$

In eqn. 4, R is the molar gas constant (8.314 J K^{-1} mol^{-1}); k = specific reaction rate at temperature T (Kelvin); e = Euler's

number = 2.718; k_B = the Boltzmann's constant (1.38×10^{23} J K^{-1}); and h = the Planck's constant (6.67×10^{-34} Js). $\Delta S^{\ddagger}$ for the reaction studied was calculated to be -114.61 J K^{-1} mol^{-1}. Entropy of activation is negative for the reaction studied indicating an associative mechanism and gives evidence of the formation of stable arenium ion intermediate in the iodination reaction studied [26]. The plausible mechanism for iodination of OTD in aqueous medium is suggested in **Scheme-II**.

QSAR studies: The QSAR data of the product PIOTD in terms of physico-chemical properties, lipophilicity, water solubility, pharmacokinetics, medicinal chemistry are suggestive of its drug like nature [27]. Physico-chemical properties of PIOTD revealed the presence of 9 heavy atoms of which 6 are aromatic carbons and that there are no rotatable bonds. Fraction of Csp^3 is 0.14 and there is one donor atom. This is suggestive of binding ability of the PIOTD with biological entities. The logarithm of the partition coefficient *n*-octanol to water system (log $P_{o/w}$) and its consensus value was found to be 2.25 for PIOTD, which indicates its solubility in lipids. The obtained water solubility data reveal the favourable aqueous solubility of PIOTD. These data are reported in Table-5.

Pharmacokinetics data of PIOTD indicate its high gastrointestinal (GI) absorption, permeant blood brain barrier (BBB) and good skin permeability having log K_p value -6.32 cm/s.



Scheme-II: Plausible mechanism of iodination of *o*-toluidine

TABLE-5
QSAR DATA OF THE IODO PRODUCT

Physico-chemical properties	Lipophilicity	Water solubility	Pharmacokinetics	Drug likeness	Medicinal chemistry
Formula: C_7H_8IN	Log $P_{o/w}$ (iLOGP): 1.83	Log *S* (ESOL): -3.02	GI absorption: High	Lipinski: Yes; 0 violation	PAINS: 0 alert
Molecular weight: 233.05 g/mol	Log $P_{o/w}$ (XLOGP3): 1.97	Solubility: 2.23e-01 mg/mL; 9.56e-04 mol/L	BBB permeant: Yes	Ghose: No; 1 violation: #atoms<20	Brenk: 2 alerts: aniline, iodine
Num. heavy atoms: 9	Log $P_{o/w}$ (WLOGP): 2.19	Class: Soluble	P-gp substrate: No	Veber: Yes	Leadlikeness: No; 1 violation: MW<250
Num. arom. heavy atoms: 6	Log $P_{o/w}$ (MLOGP): 2.72	Log *S* (Ali): -2.14	CYP1A2 inhibitor: Yes	Egan: Yes	Synthetic accessibility: 1.43
Fraction Csp^3: 0.14	Log $P_{o/w}$ (SILICOS-IT): 2.55	Solubility: 1.68e+00 mg/mL; 7.21e-03 mol/L	CYP2C19 inhibitor: No	Muegge: No; 1 violation: Heteroatoms<2	–
Num. rotatable bonds: 0	Consensus Log $P_{o/w}$: 2.25	Class: Soluble	CYP2C9 inhibitor: No	Bioavailability score: 0.55	–
Num. H-bond acceptors:0	–	Log *S* (SILICOS-IT): -3.38	CYP2D6 inhibitor: No	–	–
Num. H-bond donors:1	–	Solubility: 9.65e-02 mg/mL; 4.14e-04 mol/L	CYP3A4 inhibitor: No	–	–
Molar refractivity: 48.53	–	Class: Soluble	Log K_p (skin permeation): -6.32 cm/s	–	–
TPSA: 26.02 Å^2	–	–	–	–	–

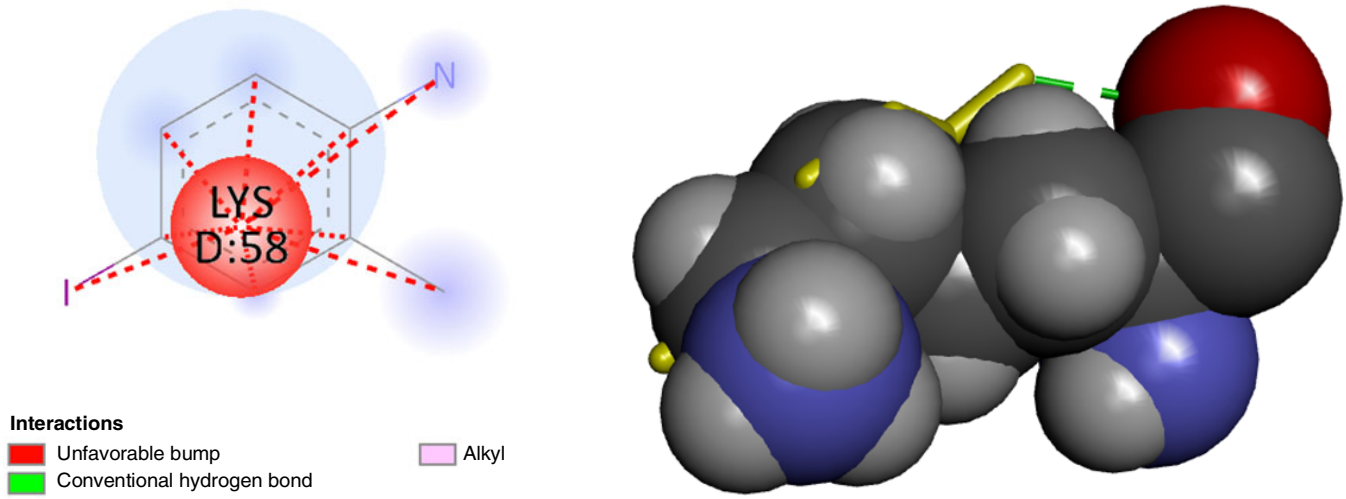


Fig. 4. 2D and 3D interaction images of 4-iodo-*o*-toluidine with CYP

The Lipinski, Veber and Egan values collectively demonstrate the drug-like properties of PIOTD.

Molecular docking: Molecular docking is the method of molecular modelling and used to predict the drug metabolism by studying cytochrome P450 (CYP) interactions with drug ligand [28]. Considering that PIOTD is a drug like molecule, its metabolism is of significance. This was ascertained by molecular docking of PIOTD with CYP. The 2D and 3D interactions of a molecular docking structure of PIOTD with CYP enzyme are shown in Fig. 4. The steric and physiological complementarity of ligand PIOTD-protein CYP interface was evident through hydrogen bonding and pi-pi stacking interactions suggesting the drug metabolism (Table-5).

Conclusion

The uncatalyzed iodination of *o*-toluidine (OTD) using molecular iodine in aqueous medium devoid of iodide ions was found to be rapid and its kinetics were investigated employing hydrodynamic voltammetry (HV). The reaction was found to be of the second order having specific reaction rate 888.87 M s^{-1} at 22.1 ºC and pH 7. The frequency factor, activation energy and entropy change accompanying the reaction were evaluated to explore the reaction dynamics. The product PIOTD was formed in the benign solvent water adhering to the green route synthesis. The QSAR study of the product revealed its drug likeness. When the drug like iodo product was docked with cytochrome P450, the steric and physiological complementarity of the ligand-protein interface was evident through hydrogen bonding and pi-pi stacking interactions suggesting its metabolic activity.

ACKNOWLEDGEMENTS

The authors acknowledge the Management and Principal of the Modern Education Society's Nowrosjee Wadia College, Pune for facilitating this work in the laboratories of the Department of Chemistry. The authors are thankful to Dr. V.T. Dangat, Retired Head, Department of Chemistry, Nowrosjee Wadia College, Pune, India for helpful discussions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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