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Determination of ionization constant and drug-likeness prediction of synthetic isoniazid derivatives

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Abstract: Determination of ionization constant, commonly termed *p*Ka is of prime interest in a wide range of pharmaceutical Research fields. The *pK*a of a compound is critical as it influences on its physicochemical parameters in biological and environmental systems. The study of *pK*a is also essential not only for the formulation of drugs and optimization of a variety of novel analytical methods, establishing new pharmaceutical dosage forms yet the exploration of the mechanism of action of drugs. In this research work, we have determined *pK*a values of isoniazid (INH) derivatives; N′-[(4-methyl benzoyl)] pyridine-4-carbohydrazide (I) and [2-oxo-2-(4-phenyl phenyl) ethyl] (pyridine-4-yl formamido) azanium bromide (II) through UV- spectrophotometry, a method is known for the accuracy and precision of results. These two compounds (I and II) were synthetically prepared in our lab by derivatizing INH and reported by Naeem *et al* in the year 2014. The mean *pK*a values for compounds I and II were experimentally determined as 7.37 and 3.76 respectively. The study is helpful in understanding the physicochemical behavior of these compounds in a biological system. Different pharmacokinetic parameters were also predicted using online web tools which ensured significant drug-likeness for both compounds.

Keywords: Ionization constant, inflection, spectrophotometry, physicochemical, pharmacokinetics, drug-likeness.

INTRODUCTION

For a drug, ADME properties are crucial for its effective behavior in the body wherein it has to display its action. Married to this goal is the ionization constant (*pK*a) of a drug, an important physicochemical property that determines drug absorption and partition across biological compartments in the body. It has long been recognized that the acid-base dissociation constant, *pK*a, has an impact on the biopharmaceutical properties of drugs. Early in the drug development process, knowledge of experimental or predicted *pK*a is required to establish the rate of ADME (absorption, distribution, metabolism and excretion) of a drug or lead compound (Gaohua, Miao *et al*., 2021). Since most drugs are weakly acidic or basic, understanding the dissociation or *pK*a value aids in determining the ionized and unionized forms of a molecule over a *p*H range (Berkhout and Ram, 2019, Shalaby and Mohamed, 2020).

The physicochemical properties of a drug, particularly its *pK*a, must always be assessed when designing and selecting a suitable formulation vehicle for a drug, as well as considering its medicinal administration because a drug can only penetrate through cell membranes in the unionized (uncharged) state, which is less water soluble than its charged form (ionized) (Atalay, 2019). The *pK*a value can be used to calculate the drug's ionization at a certain *p*H. Therefore, the rate of drug penetration through the cell membrane can be regulated (Daldal and Demiralay, 2022, Silva, Resende *et al*., 2018).

The solubility, lipophilicity, permeability and protein binding of a drug, all are affected by its *pK*a and ADME properties (Martínez and Dardonville, 2013). The *pK*a values can be used to determine the nature of the contributing ions and the amount of drug ionized in the system. With the help of *pK*a, we can modify the chemical structure of a particular drug to improve pharmacokinetic behavior at different *p*H. To develop and establish effective pharmaceutical dosage forms, it is more appropriate to use *pK*a in conjunction with other molecular or physicochemical characteristics (Gaohua, Miao *et al*., 2021).

Spectrophotometry is considered as a reliable, efficient, and sensitive technique for *pK*a determinations at very low sample concentrations. The only requirement is that the compound must possess an ionizing functional group known as ‘chromophore’ (Dubey, Singhvi *et al*., 2017).

Isoniazid (INH) also known as 4-Pyridine carboxylic acid hydrazide is commonly used against tuberculosis. The present synthetic derivatives of isoniazid possessed good

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anti-inflammatory, cytotoxic and antibacterial activities (Oliveira, Guidetti *et al*., 2017). Here, in this study we have taken two synthesized bioactive derivatives, N′-[(4-methyl benzoyl)] pyridine-4-carbohydrazide (I) and [2-oxo-2-(4-phenyl phenyl) ethyl] (pyridin-4-yl formamido) azanium bromide (II) from our lab (Jahan, Akhtar *et al*., 2013, Naeem, Akhtar *et al*., 2014) to explore their ionization constant (*pK*a) values through UV-spectrophotometry, as their *p*H-dependent ionization behavior could help us to better understand the biopharmaceutical characteristics of these molecules. However, the predicted *pK*a values for isoniazid and its various derivatives were recently published (Naeem, Akhtar *et al*., 2020). The predicted values are not always reliable when considering the several parameters that influence a therapeutic molecule's ionization behavior and experimental validation is essential.

In addition to the experimental *pK*a values, some other physicochemical, pharmacokinetic features were also predicted to ensure the drug-likeness of these compounds.

MATERIALS AND METHODS

Materials

INH synthetic derivatives N′-[(4-methyl benzoyl)] pyridine-4-carbohydrazide (I) and [2-oxo-2-(4-phenyl phenyl) ethyl] (pyridin-4-yl formamido) azanium bromide (II) obtained from our laboratory (Jahan, Akhtar *et al*., 2013, Naeem, Akhtar *et al*., 2014). Reagents such as sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl), citric acid ($C_6H_8O_7$), di-sodium hydrogen phosphate (Na_2HPO_4), boric acid (H_3BO_3), potassium chloride (KCl), were obtained from Sigma Aldrich and of analytical grade purity. De-ionized water was used for all experiments.

UV Spectrophotometers Shimadzu (UV-1800 and UV-800) was used to record UV spectra of the compounds, *p*H was measured through *p*H-meter (Jenway, Germany) 3510, Standard buffer solutions of *p*H 4.0, 7.0 and 9.0 were used to calibrate the *p*H-meter.

Methods

*pK*a *determination*

The basic concept for determination of *pK*a was reported by Albert and Sergeant (Albert and Serjeant, 1962). The whole experiment was carried out at room temperature (25±1°C). Buffer solutions of *p*H from 2.0 to 11.0, were prepared by mixing appropriate amount of 0.2M, Na_2HPO_4 and citric acid (0.1M) according to the procedure reported earlier (Cole, 1955, NF, Perrin, 1974). HCl (0.2M) and NaOH (0.2M) solutions were used to adjust the *p*H of buffer solutions. The solutions of each compound in different *p*H ranging from 2.0 to 11.0 were analyzed on a spectrophotometer at the wavelength range of 200 to 400nm. The absorbance spectra were recorded at two different wavelengths for each compound. After determining the 'inflection point' by plotting absorbance vs *p*H, the series of five buffer solutions (with *p*H around inflection point) were prepared. Ionic strength was maintained at 0.02 for each solution by adding a suitable amount of NaCl. The sample solutions of 1µg/ml were prepared in each buffer solution and analyzed on a spectrophotometer. The spectra and absorbance values of samples were recorded carefully. The absorbance of the an-ionic and ionic species of both compounds was determined in the same way by employing HCl (0.01N) and NaOH (0.01N) solutions respectively. Each experiment was repeated thrice for each sample using the same buffer solutions to eliminate intrinsic errors.

The Henderson-Hasselbalch equation (Singh, Sharda *et al*., 1999) described below was used to calculate the experimental *pK*a values for each compound.

$$\mathrm{pKa} = \mathrm{pH} = \log \frac{\mathrm{di} - \mathrm{d}}{\mathrm{d} - \mathrm{dm}}$$

Where '*di*' is the absorbance of ionized, '*dm'* is the absorbance of unionized forms, and '*d*' represents the absorbance of molecule in buffer solution.

The obtained *pK*a results were validated by repeating the measurements by two different team members and using different spectrophotometers on two alternate days with the same strength of working and buffer solutions.

Drug-likeness prediction

The physicochemical, pharmacokinetic properties, and drug-likeness of both compounds were predicted by using an online web application "Swiss ADME" available by (SIB) Swiss Institute of bioinformatics (http://www.swissadme.ch).

RESULTS

The chemical structures of compounds I and II were illustrated in Scheme 1. The absorbance values of compounds at two different wavelengths in buffer solutions of *p*H ranging from 2 to 11 are listed in table 1 and the respective absorbance plots are shown in fig. 1. Graphs between Absorbance and different *p*H are displayed in fig. 2. The absorbance values of both compounds in their respective five different buffer solutions along with calculated *pK*a values and their ionized and un-ionized forms are depicted in table 2. The overlay of UV-spectra at different *p*H is displayed in fig. 3. The validation results are listed in table 3. The comparison of experimental and predicted *pK*a values were given in table 4. Predicted physicochemical, pharmacokinetic and drug-likeness parameters are presented in table 5.

Table 1: Absorbance values of compound I and II in buffer solution of *p*H 2.0 to 11.0.

Compound	λ (nm)	*p*H						
		2.0	3.5	5.0	6.0	7.4	9.0	11.0
I	238	0.400	0.445	0.460	0.354	0.489	0.358	0.481
	240	0.416	0.463	0.464	0.362	0.493	0.371	0.490
II	293	0.405	0.512	0.369	0.378	0.397	0.411	0.376
	295	0.420	0.534	0.389	0.388	0.402	0.430	0.390

Table 2: Experimental values of *pK*a of compounds I and II at respective *p*H

Compound I			Compound II		
*p*H	Absorbance (236 nm)	*pK*a	*p*H	Absorbance (296 nm)	*pK*a
7.1	0.468	7.15	3.1	0.56	4.31
7.2	0.490	7.40	3.2	0.48	3.59
7.3	0.440	7.17	3.3	0.47	3.63
7.4	0.507	7.71	3.4	0.43	3.52
7.5	0.453	7.44	3.5	0.46	3.78
Mean *pK*a		7.37 ± 0.168	Mean p*K*a		3.76 ± 0.222

For Compound-I, *di* = 0.328, *dm* = 0.593, for Compound-II, *di* = 0.230, *dm* = 0.580

Table 3: Validation of ruggedness of *pK*a values

Compound	Instrument 1		Instrument 2	
	Person1, day 1	Person1, day2	Person 1, day 3	Person 2, day 4
	Drug strength =1µg/ml	Drug strength =1µg/ml	Drug strength =1µg/ml	Drug strength =1µg/ml
I	7.360 ± 0.125	7.367 ± 0.173	7.373 ± 0.172	7.362 ± 0.179
II	3.760 ± 0.205	3.776 ± 0.216	3.759 ± 0.164	3.746 ± 0.181

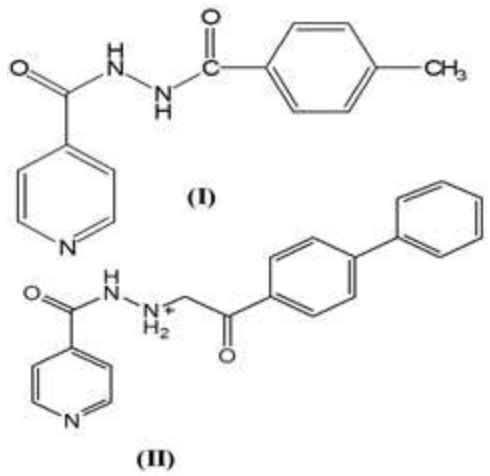
Table 4: Experimental and predicted values of *pK*a of compounds I and II

Compound	*pK*a values	
	Experimental (UV spectrophotometry)	Predicted (Marvin)
I	7.37	6.78
II	3.76	3.10

Table 5: Predicted physicochemical, pharmacokinetics and drug-likeness parameters of compounds I and II

Physicochemical parameters	Compound I	Compound II
MW	255	332
TPSA	71.09A2	75.67A2
RO5	Pass	Pass
Log *P*	2.02	2.43
H-bond donor	2	2
H- bond acceptor	3	3
Pharmacokinetics		
GI absorption	High	High
BBB permeability	Yes	No
Drug likeness		
Lipinski	Yes	Yes
Bioavailability score	0.55	0.55

Log *P*= partition coefficient, MW= molecular weight, TPSA= topological polar surface area, R05= Lipinski rule of Five, % ABS= percentage of absorption



Scheme 1: Molecular structures of compounds I and II

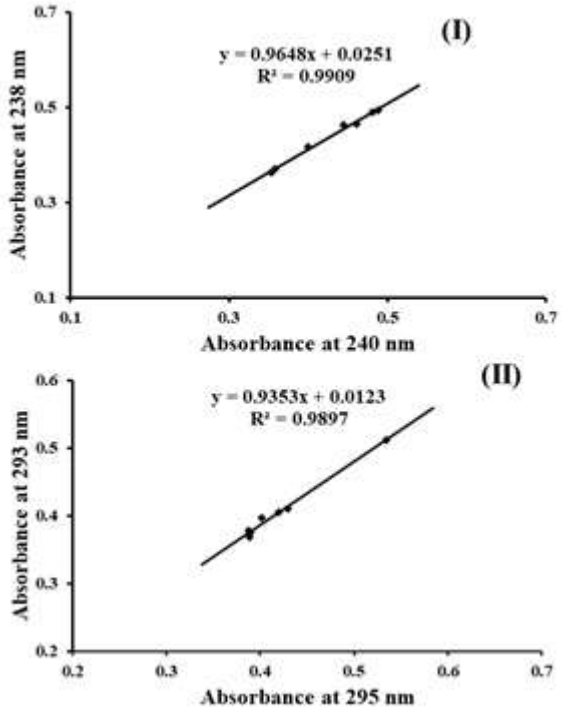


Fig. 1: Graphs showing Absorbance of compounds I and II.

DISCUSSION

The plots of absorbance values at one wavelength vs another, display a strong linear correlation without any variation in its course, which confirms the presence of a single equilibrium or single *pK*a value in the ionization state (Dubey, Singhvi *et al*., 2017) as shown in fig. 1. Inflection method was used to determine the approximate *pK*a value, where the absorbance values were plotted against different *p*H for each compound. The resulting 'inflection point' served as the approximate value of *pK*a.

For this purpose, the absorbance values of both compounds I and II when plotted against the corresponding *p*H values, the major deflection was noticed at *p*H 7.4 for the compound I and at *p*H 3.5 for compound II as displayed in fig. 2. These inflection points indicated the approximate value of *pK*a for the respective compound. Hence, to determine the exact value of *pK*a, the five buffer solutions were prepared around the 'approximate *pK*a value' of each compound. The experimental *pK*a values were calculated using the Henderson-Hasselbalch equation at each *p*H ranging from 7.1 to 7.4 for the compound I and 3.1 to 3.5 for compound II as depicted in table 2. The mean of the calculated five *pK*a values was taken as the final value of *pK*a for each sample compound. The obtained final *pK*a values were 7.37 and 3.76 for compounds I and II respectively. To ensure the precision of results and to avoid chances of error in calculation, first, we converted the mean *pK*a value to antilogarithm and then the logarithm of this average was taken as the final value of *pK*a. UV spectra in fig. 3 indicated that the compounds exhibited *pH*-dependent UV absorption behavior.

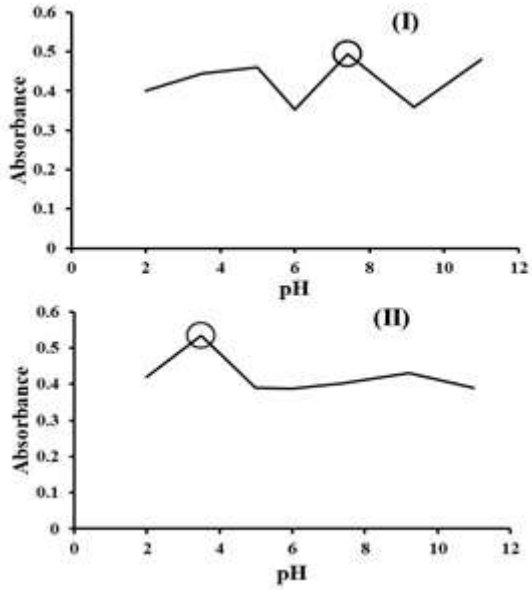


Fig. 2: Graph between absorbance and different *p*H of compounds I and II.

The validation of the ruggedness of *pK*a values was performed to ensure the preciseness of the results. The experimentally determined *pK*a values were found closer to the predicted *pK*a values previously reported (Naeem, Akhtar *et al*., 2020). Experimental values are always considered more significant and reliable compared to values predicted by software as they rely on chemical structures and public datasets that are probably included in the available tools' training sets.

Drug-likeness and pharmacokinetic properties were predicted for compounds I and II by employing an on-line

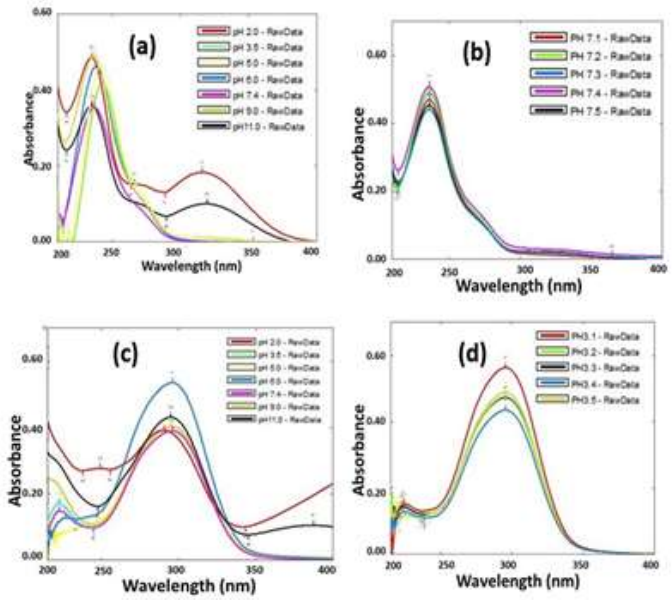


Fig. 3: UV-spectra of compound I (a and b), compound II (c and d) at different *p*H

"SwissADME" web application (Yadav and Mohite, 2020). As per the Lipinski rule of five, compounds must have molecular weight of less than 500 and Log $P_{O/W}$ of less than 5, donor H-bond less than 5, acceptor H-bonds less than and equal to 10 for oral administration. Drug-likeness properties were shown in table 5. Both compounds followed Lipinski rule of five. TPSA (Topological polar surface area) associated with polar atoms in the compound. The TPSA values of compounds I and II were found to be 71.09A2 and 75.67A2 respectively. It was discovered that smaller the TPSA value greater will be the absorption (Fernandes and Gattass, 2009). Molecular weight of compounds was found to be less than 500. H- bond donor and H-bond acceptor was less than 5. The log *P* values of both compounds I and II were less than five, which indicated the lipophilic nature of these compounds and can readily absorbed and distributed in the body. Absorption percentage (%ABS) was computed from TPSA using formula %ABS= 109 – (0.345 × TPSA) (Panyatip, Nunthaboot *et al*., 2020, Prasanthi, 2014). The absorption percentage obtained was more than 80% for both compounds indicating good absorption behavior and can be suitable for oral administration. Compound I was permeable to BBB (blood-brain-barrier) while compound II was non permeable. Both compounds exhibited significant bioavailability score. Physicochemical, pharmacokinetic predictions of drug-likeness assured that both compounds were promising candidate in pharmacokinetic aspect.

CONCLUSION

The *pK*a (ionization constant) values of N′-[(4-methyl benzoyl)] pyridine-4-carbohydrazide (I) and [2-oxo-2-(4-phenylphenyl) ethyl] (pyridin-4-yl formamido) azanium bromide (II) were determined through spectrophotometry. The mean p*K*a values for molecule I and II were found to be 7.37 and 3.76 respectively. The obtained p*K*a values including validation results were found to be within a ±0.250 spread, confirming the precision of our findings.

This study adds to existing knowledge about the physicochemical properties of synthetic derivatives of isoniazid and helpful in understanding the physicochemical behavior of these compounds. Since, *pK*a has an impact on drug transport into cells and across biological membranes, it must be considered when making structural alterations to bioactive compounds. Study of *pK*a is also required in the development and optimization of a variety of analytical methods aimed at bringing innovative ideas to commonly used procedures. pK_a influenced on the overall performance and biopharmaceutical properties of a drug. Predicted physicochemical, pharmacokinetic parameters revealed that being promising candidate both compounds fulfil good orally active drug criteria as they passed Lipinski rule of five and confirmed drug likeness.

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