REPORT

Determination of p*K*a values of new phenacyl-piperidine derivatives by potentiometric titration method in aqueous medium at room temperature (25±0.5°C)

Shaista Zafar*, Shamim Akhtar*, Talat Tariq, Noushin Mushtaq*, Arfa Akram***, Ahsaan Ahmed*, Muhammad Arif*, Sabahat Naeem* and Sana Anwar***

*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan
**Department of Chemistry, Federal Urdu University of Science and Technology Karachi, Pakistan
***Department of Pharmaceutical Chemistry, Federal Urdu University of Science and Technology, Karachi, Pakistan

Abstract: Dissociation constant (p*K*a) of ten novel phenacyl derivatives of piperidine were determined by potentiometric titration method in aqueous medium at room temperature (25 ±0.5°C). The sample solutions were prepared in deionized water with ionic strength 0.01M and titrated with 0.1M NaOH solution. In addition, ΔG values were also calculated. Different prediction software programs were used to calculate p*K*a values too and compared to the experimentally observed p*K*a values. The experimental and theoretical values were found in close agreement. The results obtained in this research would help to predict the good absorption of the studied compounds and can be selected as lead molecules for the synthesis of CNS active agents because of their lipophilic nature especially compound VII.

Keywords: Dissociation constant, phenacyl derivatives, CNS active agents, potentiometry, lipophilicity, pH partition theory.

INTRODUCTION

Hundreds of novel piperidine derivatives had been prepared in our lab since last two decades (Khan *et al* 2006, Saify *et al* 2005, 2006, Akhtar *et al* 2000, 2006, 2012, Taqvi *et al* 2006, Saied *et al* 1998, Jahan *et al* 2013). These compounds displayed promising pharmacological activities which initiated us to determine their physicochemical characteristics to make them useful medicinal agents.

The physicochemical characteristics played pivotal role to make new synthetic compounds acceptable to be used as medicines because many pharmacologically active compounds failed to become drugs. The reasons behind would be their poor bioavailability, unacceptable pharmacokinetics and/or unexpected safety problems (Caliaro and Herbots, 2001).

Acid dissociation constant is an important parameter to indicate the extent of ionization of molecules in solution at different pH values which was significantly found important in many analytical procedures (Zhao *et al* 1996 and Rochester, 1971, Kin *et al* 2001, Ornskov *et al* 2003).

The basic principal involved in the determination of p*K*a is the ratio of ionized and unionized forms of the functional group in the molecule and also the contribution of the aqueous medium. Different methods were reported by Barbosa 1991, Papanastasiou *et al.,* 1989, Saeeduddin *et al* 2004 and Song Li 1991. Potentiometry amongst them was commonly used technique because of its precision, accuracy and simplicity (Andrasi *et al.,* 2007).

Recently a group of scientists developed new methods to determine acid dissociation constant based upon calorimetry, voltammetry, solubility, fluorometry and polarimetry (Reijenga *et al.,* 2013).

General Theory

Drugs are either weak acid or weak base and contain the site that can dissociate or associate proton to form an anion or a cat ion along with unionized molecular form of the drug. The expression for the phenomenon can be written in the following manner:

$HA \leftrightarrow H^+ + A^-$ or $HB \leftrightarrow H^+ + B^-$

Sayle concluded that the p*K*a of a site could be thought of the pH at which the protonated and deprotonated fractions were equal. When the pH was higher than the p*K*a, the site was mostly protonated (Sayle, 2000).

The relationship between the p*K*a and the concentration of ionized and unionized drug as a function of pH was given by the buffer equations commonly known as Henderson-

**Corresponding author:* e-mail: shaistazafar02@hotmail.com

Hasselbalch equations. These equations for acidic and basic drugs are given as under:

For weak acids: p*K*a= pH + log Cu/Ci

For weak bases: p*K*a= pH + log Ci/Cu

Where Cu was the concentration of unionized form and Ci, the concentration of ionized from of the drug.

The two equations had been derived from a consideration of the ionization of weak acids and bases. For weak acids:

$HA \leftrightarrow H^+ + A^-$

By applying the equation of law of mass action and expressing molar concentration in square brackets the equation was written as:

$Ka = [H^+] + [A^-] / [HA]$

Where, *K*a is the dissociation constant of the acid.

For weak bases with only one ionizable group (Saify, 1984) the equation will be:

$B + H^+ \leftrightarrow BH^+$

$Ka = [H^+] [B] / [BH^+]$

p*K*a is the negative logarithm of dissociation constant, *K*a, i.e.

p*K*a = -Log *K*a

EXPERIMENTAL

Materials

Reagents and Chemicals

Distilled water, D.I. (Deionised) water, Carbon dioxide (CO_2) free water, (freshly prepared), NaOH (0.1M). All the reagents and solvents were of analytical grade and purchased from Sigma Aldrich and Merck respectively.

Compounds for analysis

Already synthesized piperidine derivatives (I-V) were obtained from our synthetic chemistry lab (Akhtar *et al* 2000, 2006 and 2012, Hina 2011, Azra, 2010 and Sajida, 2009). Their purity was confirmed by TLC and melting point.

Instruments and Glassware's

The instruments used in this study were pH Meter (Jenway, Germany) 3510, hotplate and stirrer (Jenway, Germany) 1000, weighing balance (Mettler Toledo AB204, Switzerland (precision ±0.1mg). Pyrex Glassware's (Burette and beaker) calibrated at room temperature (25±0.5°C)

Method

Preparation of solutions

The homogenous solutions of samples and standard were prepared by dissolving required quantity in the carbon dioxide free distilled water to make 0.01M. Nitrogen was passed through the titration solutions for 10 minutes to expel the dissolved gases.

Potentiometric titration

Before experimentation, the potentiometer was calibrated using standard aqueous buffers of pH 2, 5 and 7. Titration was carried out at 25±0.5°C using 0.1M NaOH solutions. Ionic strength of solutions was maintained by using 0.1M potassium chloride solution. The titration was carried out in small aliquots with the help of standardized NaOH solution and pH-titration curves were obtained, under constant stirring of solutions. NaOH was added in small volume starting with 0.1mL and up to 0.5mL near end point and the pH was measured after each addition. Then the p*K*a was calculated at pH of half neutralization point. Each sample was titrated three times and mean of three readings was used in calculation.

RESULTS

IUPAC Names of the compounds used in this study were presented in table 1. The p*K*a values of tested compounds were given in table 2 and Fig. 1. ΔG values were also calculated and reported in the same table. Table **3** was showing the theoretical p*K*a values calculated by various software programs. The correlation between calculated and experimental pKa and ΔG values were represented by Fig. 2 and 3 respectively.

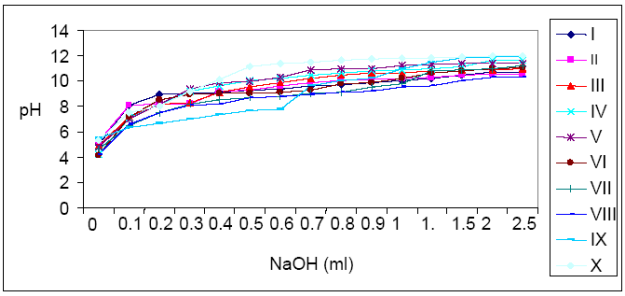


Fig. 1: Titration curves of phenacyl derivatives (I-X).

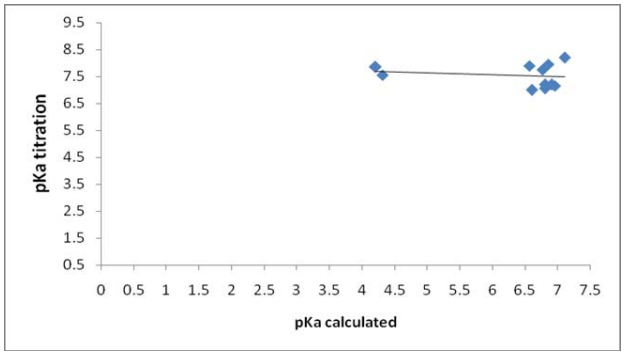


Fig. 2: Correlation p*K*a calculated/p*K*a titration.

DISCUSSION

It is evident from the table that the p*K*a values were obtained in the range of 5.8-8.2 and changing by the change in substitution of different functional groups on the phenyl ring as for example: Compound VII containing unsubstituted benzene ring at position 4 of the phenacyl

moiety expressed p*K*a 8.2. The high p*K*a value of this compound was indicative of less ionization and when one hydroxyl group was introduced on the phenyl ring at *para* position and one methyl group at *ortho* position (compound VI), the p*K*a was decreased (5.9). The low ionization value might be due to the presence of hydroxyl group, which made the compound more ionizable, whereas presence of nitro group caused the increase in the p*K*a values (7.70-7.75), in compounds I and II.

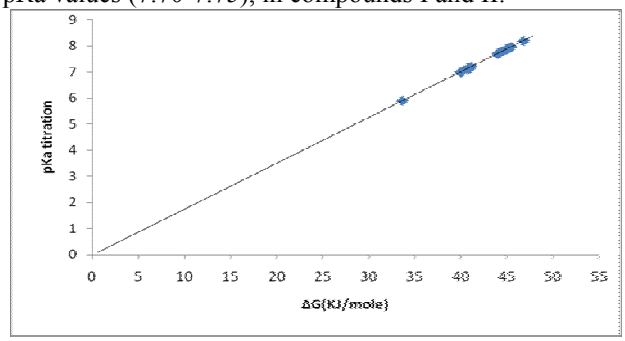


Fig. 3: Correlation p*K*a Experimental/ΔG.

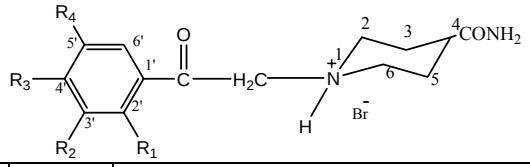
Among the halogenated derivatives, compound III and V exhibited the same p*K*a values (7.9), showing their more acidic character. These compounds contained halides such as flourine (F) and bromine (Br) substituted on phenyl ring. This effect would be explained with respect to the size and electronegativity of the substituents. Flourine had high electronegativity as compared to bromine but bromine was bigger in size than that of Florine. So both size and electronegativity contributed in p*K*a values.
Compound IV had p*K*a value slightly lower than that of compounds III and V, because, compound IV contained chlorine (Cl) substituted on phenyl ring, which was less electronegative than flourine. The slight difference was might be due to the electronegativity, size or position of these functional groups.

Compound I and II showed almost same p*K*a values. Both compounds contained one Nitro group on phenyl ring. Thus position of nitro group was contributing in slight change in p*K*a values. Compound II had nitro group at *para* position and it showed slightly higher p*K*a value 7.75, as compared to compound I with 7.70 p*K*a, which contained nitro group at *meta* position. It meant position of nitro group on phenyl ring had no remarkable effect on p*K*a.

Compound VIII and IX showed same p*K*a values i.e., 7.0. Both the compounds contained one methoxy group at different positions (*meta* and *para*) on phenyl ring. So the presence of methoxy group produced no change in the p*K*a value. It was also seemed that position of functional group (methoxy) did not affect the p*Ka* value. Compound X which contained two methoxy groups on *ortho* and *meta* position respectively on phenyl ring, showed higher p*K*a value 7.2 as compared to compound VIII which contained one methoxy group at meta position and compound IX which also contained one methoxy group at *para* position. Therefore, it was suggested that addition of one more methoxy group resulted in high p*K*a. This effect might be due to the increase in molecular size.

From the above discussion, it might be concluded that

Table 1: Structural Representation and IUPAC Names of phenacyl derivatives of Isonipecotamide



Compound #	R_1	R_2	R_3	IUPAC Names
I	H	$-NO_2$	H	4-Carbamoyl-1-[2-(3′-nitrophenyl)-2-oxoethyl)]-piperidinium bromide
II	H	H	$-NO_2$	4-Carbamoyl-1-[2-(4′-nitrophenyl)-2-oxoethyl])-piperidinium bromide
III	H	H	-Br	4-Carbamoyl-1-[2-(4′-bromophenyl)-2-oxoethyl)]-piperidinium bromide
IV	H	H	-Cl	4-Carbamoyl-1-[2-(4′-chlorophenyl)-2-oxoethyl)]-piperidinium bromide
V	H	H	-F	4-Carbamoyl-1-[2-(4′-fluorohenyl)-2-oxoethyl)]-piperidinium bromide
VI	$-CH_3$	H	-OH	4-Carbamoyl-1-[2-(4′-hydroxy-2-methylphenyl)]-piperidinium bromide
VII	H	H	$-C_6H_5$	4-Carbamoyl-1-[2-(4′-phenyl)-2-oxoethyl)]-piperidinium bromide
VIII	H	$-OCH_3$	H	4-Carbamoyl-1-[2-(3′-methoxyphenyl)-2-oxoethyl)]-piperidinium bromide
IX	H	H	$-OCH_3$	4-Carbamoyl-1-[2-(4′-methoxyphenyl)-2-oxoethyl)]-piperidinium bromide
X	$-OCH_3$	H	$-OCH_3$	4-Carbamoyl-1-[2-(4′-dimethoxyphenyl)-2-oxoethyl)]-piperidinium bromide

there was a relationship between atomic size and acidity. Increasing atomic size increased acidity and decreased p*K*a values. Similarly, there was also relationship between electronegativity and p*K*a. By increasing electro-negativity, increased acidity i.e. decreased p*K*a values (Ruiter 2005 and Naaliya, 2010) and the same was observed in this research work.

Table 2: p*K*a values of phenacyl derivatives by potentiometric method and ΔG values at room temperature (25±0.5°C) in aqueous medium.

Compound #	p*K*a values	ΔG (kJ/mole)
VI	5.9	33.65
VIII	7.0	39.96
IX	7.0	39.96
X	7.2	41.07
I	7.7	43.93
II	7.75	44.21
IV	7.8	44.49
III	7.9	45.06
V	7.9	45.06
VII	8.2	46.78

Table 2 was also showing ΔG (Gibbs free energy) values in kJ/mol in aqueous medium. ΔG was found an indicator of spontaneity of a reaction or physical change at controlled temperature and pressure. The positive values of ΔG showed that the process of neutralization was spontaneous under the given condition. This thermodynamic constant (ΔG) also affected ionization and p*K*a (Whitten *et al*, 2000). The p*K*a value was found directly proportional to the standard Gibbs energy change for the reaction (Saeeduddin and Khanzada, 2004). The same was observed in this research. The compounds possessing larger ΔG values expressed higher p*K*a values. Larger ΔG values meant less ionization and hence the high p*K*a.

The relationship between ΔG and p*K*a was also studied and displayed in Fig. 3. The aqueous-phase ΔG and experimental p*K*a of the compounds under study at 25°C expressed a good correlation between these two parameters. In the field of medicinal chemistry, a lucid well defined theoretical approach would be very useful for research scientists in the prediction of acidity without experimentation (Charif *et al* 2007).

Most weak acidic drugs are predominantly in the un-ionized form at lower pH of the gastric fluid and therefore may be absorbed from the stomach as well as from the intestine. The fraction of the drug existing in its un-ionized form in a solution is a function of both the dissociation constant of a drug and the pH of the solution at the absorption site. The relationship between pH and p*K*a and the extent of ionization in terms of percentage was given by equations described elsewhere in the introductory part.

Table 3: Computed p*K*a values by different software programs.

Compound #	ACD Labs	Marvin	Pallas
I	6.46±0.46	5.86	5.88±1.4
II	6.76±0.46	5.886	5.886±1.4
III	6.53±0.43	6.09	6.13±1.4
IV	6.53±0.43	6.078	6.11±1.4
V	6.56±.43	6.06	6.06±1.4
VI	7.35±0.42	4.63	4.4±1.4
VII	7.1±0.46	6.148	6.63±1.40
VIII	6.56±0.46	4.145	6.31±1.4
IX	6.6±0.43	4.23	6.34±1.4
X	6.9±0.43	6.29	5.99±1.4

The significance of this research is based upon the fact that drug absorption is affected by many physiological factors. It also depends upon many physicochemical properties of the drug itself. Most drugs are absorbed from gastrointestinal tract by a process of passive diffusion in the un-ionised form across a lipid membrane. Furthermore, the dissociation constant, lipid solubility and pH of the fluid at the absorption site determine the extent of absorption from a solution.

The inter-relationship among these parameters was described in the literature as pH-partition theory (Lemke *et al* 2008). This theory provided a basic framework for the understanding of drug absorption from the GIT (Gastro-intestinal Tract) and drug transport across the biological membrane. Important points of this theory included, the GIT and other biological membranes acted like lipid barriers, the un-ionized form of the acidic or basic drug was preferentially absorbed and weak acidic and neutral drugs might be absorbed from the stomach.

Table 3 represented the computed p*K*a values of compounds by different software programs. All the p*K*a values found from ACD, Marvin and Pallas were in the range of 4.0-7.0, except one compound VIII which showed high p*K*a 8.1 by Marvin. Values by ACD were found closer to the experimental values obtained by this method.

Correlation between p*K*a calculated and p*K*a titration was demonstrated in Fig. 2.

CONCLUSION

Potentiometric titration measurement techniques can be used effectively to determine a p*K*a value which is one of the important physicochemical factors in development of a new drug substance.

From the aforementioned results, it can be suggested that in drug designing, compounds showing high p*K*a values might be used as molecules for the synthesis of CNS

active agents owing to the less % ionization at physiologic pH. As a result of this study, compound VII was emerged for further exploitation as lead molecule for the synthesis of CNS active medicinal agents/compounds due to its very high lipophilic behavior.

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