Determination of dissociation constant of some pyrimidine derivatives

Shipra Baluja*, Kajal Nandha and Paras Ramavat

Physical Chemistry Laboratory, Department of Chemistry, Saurashtra University, Rajkot-360005 (Gujarat), India.

Accepted 29 April, 2016

Some new pyrimidine derivatives have been synthesized and their structures have been confirmed by IR, $^1H$ NMR and mass spectral data. The thermodynamic dissociation constants of pyrimidine derivatives were studied in 1, 4-dioxane: water (50:50 v/v) solvent systems at different temperatures ranging from 25°C to 45°C at 10°C interval using Calvin–Bjerrum pH titration method. Further, some thermodynamic parameters such as enthalpy ($\Delta H$), Gibb’s free energy ($\Delta G$) and entropy ($\Delta S$) of solutions have also been evaluated at different temperatures for these systems.

Key words: Acidity constant, pyrimidine, 1, 4-dioxane, enthalpy, Gibb’s free energy, entropy.

INTRODUCTION

The constants, which are used to measure the extent of ionization of molecules in solutions, are known as dissociation constants or ionization constants. Dissociation in chemistry and biochemistry is a general process in which ionic compounds (complexes or salts) separate or split into smaller particles, ions, or radical, usually in a reversible manner (Kristine et al., 2010). The dissociation constants are used for choosing appropriate acidic or basic reagents in drug discovery and development, as knowledge of the dissociation state of a particular functional group is critical for understanding the pharmacokinetic and pharmacodynamic properties of new drug substances (Benet et al., 1967). This important physicochemical parameter also provides critical information about drug properties such as solubility, lipophilicity, acidity, transport behavior, bonding to receptors and permeability. Hence, the relationship between the acid dissociation constants and structure in drug design studies is important. Acid dissociation constants are also important parameters for the selection of the optimum conditions in the development of analytical methods (Hakli et al., 2008) and provides information about the stereo chemical and conformational structures of active centres of enzymes (Ogretir et al., 2003).

Literature survey shows that large array of pyrimidine derivatives possess a variety of pharmacological properties such as antifungal (Gholap et al., 2008), antihypertensive (Alam et al., 2010) antihistaminic (Prasad et al., 2008), anticancer (Dudhe et al., 2011), analgesic (Gupta et al., 2011), anti-tubercular (Chitre et al., 2011), antimicrobial (Reddy et al., 2011), central nervous activities (Fhid et al., 2002), antimalarial (Morgan et al., 2008), ant HIV (Liu et al., 2009) etc.

Thus, in the present work, some new pyrimidine derivatives are synthesized and their dissociation constants are determined in 1,4-dioxane-water medium at different temperatures (298.15 K to 318.15 K). Using these experimental data, some thermodynamic parameters such as enthalpy ($\Delta H$), Gibb’s free energy ($\Delta G$) and entropy ($\Delta S$) of solutions were evaluated to understand dissociation process.

Experimental

Materials

Different substituted benzaldehydes, isobutyl propionate, urea and phenacyl bromide used for the synthesis, was supplied from Spectrochem Pvt. Ltd. (Mumbai, India) and was used without any treatment. The methanol and acetone used were of AR grade supplied by...
Spectrochem Pvt. Ltd. (Mumbai, India) whereas the 1, 4-dioxane used to determine dissociation constant was of AR grade supplied by Spectrochem Pvt. Ltd. (Mumbai, India) and was purified according to the standard procedure (Riddick et al., 1986). The distilled 1, 4-dioxane was stored over molecular sieves. The purity of 1, 4-dioxane was confirmed by GC-MS (SHIMADZU-Model No.-QP-2010) equipped with column (DB-5MS, 25 m in length, 0.20 mm internal diameter and 0.33μm film) and was found to be 99.99%. The Milli-Q water (Millipore Pvt. Ltd. Bangalore, India) was used for the study.

**Synthesis**

**Synthesis of dihydropyrimidine derivatives**

A mixture of different substituted aldehyde (0.01 mol), isobutyl propionate (0.01 mol) and urea (0.012 mol) in methanol was refluxed for 16 hrs in the presence of copper chloride and concentrated sulphuric acid. The completion of reaction was confirmed by analytical thin layer chromatography (TLC) (Performed on aluminum coated plates Gel 60F$_{254}$ (E. Merck)) using (3:2-Hexane: Ethyl acetate) as mobile phase. After the completion of reaction, the reaction mixture was cooled and the resulting solid was filtered. This solid was washed with methanol to remove unreacted reagents and dried under vacuum to give crude product.

**Oxidation of dihydropyrimidine derivatives**

In 60% chilled nitric acid (10 ml), 0.1 mole of above synthesized compound was slowly added and the mixture was stirred at 273.15 K for 30 mins. The progress of reaction was monitored by thin layer chromatography. The reaction mixture was poured into cold water and was neutralized with saturated sodium bicarbonate solution. The precipitate was filtered, washed with water and dried. The crude product was directly used for the next step.

**Synthesis of methyl 4-isopropyl-2-(2-oxo-2-phenylethoxy)-6-phenylpyrimidine-5-carboxylate derivatives**

Equimolar solution of the above product and phenacyl bromide in dry acetone was refluxed in the presence of dry K$_2$CO$_3$ for 1 h. The progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, the solvent was evaporated and reaction mixture was poured into crushed ice to give solid product. The resulting solid was filtered, washed with cold water and dried under vacuum to give crude product. The reaction scheme is given in Figure 1.

**Dissociation constant measurement:**

All the synthesized compounds were purified and were recrystallized before measurement. For each compound, 0.1 M solution was prepared in 1, 4-dioxane.

The solutions of nitric acid (HNO$_3$) (1.0 M), sodium nitrate (NaNO$_3$) (1.0 M), potassium hydroxide (KOH) (0.5 M) were prepared in Milli-Q water.
An electrical balance (Mettler Toledo AB204-S) with an accuracy of ± 0.1 mg was used for solution preparation. The solutions of nitric acid and Potassium Hydroxide were standardized by titrating with 0.1 N NaOH and 0.05M Succinic acid solutions respectively.

The Calvin Bjerrum pH titration method (Calvin et al., 1945; Bjerrum et al., 1941) was used to determine dissociation constant. For this, glass and saturated calomel electrodes was used for dissociation constant. The Systronic pH meter (Model No. EQ-664) was calibrated before use with buffer solution of known pH. The buffers used were potassium hydrogen phthalate and sodium borate decahydate.

For the determination of dissociation constant, two sets of solution were prepared.

**Set I:** 4.0 ml HNO₃ (1.0 M) + 8.0 ml water + 20.0 ml 1, 4-Dioxane + 8.0 ml NaNO₃ (1.0 M)

**Set II:** 4.0 ml HNO₃ (1.0 M) + 8.0 ml water + 18.0 ml 1, 4-Dioxane + 2.0 ml compound solution (0.1 M) + 8.0 ml NaNO₃ (1.0 M)

Thus, the initial volume (V₀) in each set is 40.0 ml and 1, 4-Dioxane: water ratio is 50:50 (v/v).

The above mentioned solutions were allowed to attain a definite temperature by circulating water through the outer jacket of the vessel using a thermostat (NOVA NV-8550 E) maintained at desired constant temperature. The uncertainty in temperature is ± 0.05K. These solutions were titrated against 0.5 M KOH at different temperatures and the corresponding pH was recorded by Systronic pH meter for each compound. The accuracy of pH meter was ± 0.01 pH unit.

The pH correlation was done using the following relation (Van Uitert et al., 1953):

\[
\log[H^+] = pH + log f + log U^0_H
\]  

(1)

Where, \(f\) is the activity coefficient of the hydrogen ions in the solvent mixtures under consideration at the same temperature and ionic strength and \(U^0_H\) is a correction factor at zero ionic strength, which depends only on the solvent composition and temperature. \(U^0_H\) is taken as unity in aqueous media.

### RESULTS AND DISCUSSION

All the five compounds are synthesized and their physical properties (RP-1 to RP-5) are given in Table 1.

#### Spectral Data:

**RP-1:**

- **IR (cm⁻¹):** 1722.43 (carbonyl str. in COOCH₃), 1695.43 (Acyclic carbonyl str.), 1598.99 (Ar-C=C str.), 1390.68 (alkane C-H bending), 1334.78 (C-O str. of ether), 1066.64 (C-O str. of ether), 850.61 (p-di substituted aromatic ring).
- **¹H NMR (DMSO-d₆) δ(ppm):** 1.250 (6H, doublet, -CH₃ of isopropyl), 3.12 (1H, multiplet, C-H of isopropyl), 3.715 (3H, singlet, -COOCH₃), 5.983 (2H, singlet, -OCH₂), 7.017 (2H, doublet, Ar-CH), 7.520 (2H, doublet, Ar-CH), 7.600 (2H, triplet, Ar-CH), 7.744 (1H, triplet, Ar-CH) and 8.043 (2H, doublet, Ar-CH).
- **MS:** (m/z) = 408

**RP-2:**

- **IR (cm⁻¹):** 1722.43 (carbonyl str. in COOCH₃), 1695.43 (Acyclic carbonyl str.), 1546.91 (Ar-C=C str.), 1390.68 (alkane C-H bending), 1334.74 (C-O str. of ether), 1066.64 (C-O str. of ether), 850.61 (p-di substituted aromatic ring).
- **¹H NMR (DMSO-d₆) δ(ppm):** 1.258 (6H, doublet, -CH₃ of isopropyl), 3.129 (1H, multiplet, C-H of isopropyl), 3.716 (3H, singlet, -COOCH₃), 5.985 (2H, singlet, -OCH₂), 7.019 (2H, doublet, Ar-CH), 7.528 (2H, doublet, Ar-CH), 7.609 (2H, triplet, Ar-CH), 7.746 (1H, triplet, Ar-CH) and 8.048 (2H, doublet, Ar-CH).
- **MS:** (m/z) = 469

**RP-3:**

- **IR (cm⁻¹):** 1722.43 (carbonyl str. in COOCH₃), 1695.43 (Acyclic carbonyl str.), 1546.91 (Ar-C=C str.), 1390.68 (alkane C-H bending), 1334.74 (C-O str. of ether), 1008.77 (C-O str. of ether).
- **¹H NMR (DMSO-d₆) δ(ppm):** 1.261 (6H, doublet, -CH₃ of isopropyl), 3.026 (1H, multiplet, C-H of isopropyl), 3.546 (3H, singlet, -COOCH₃), 3.827 (6H, singlet, -OCH₃), 5.885 (2H, singlet, -OCH₂), 7.014 (1H, doublet, Ar-CH), 7.115 (1H, doublet, Ar-CH), 7.214 (1H, singlet, Ar-CH), 7.598 (2H, triplet, Ar-CH), 7.740 (1H, triplet, Ar-CH), 8.048 (2H, doublet, Ar-CH).
Figure 2. Variation of pH against volume of KOH for RP-1 at 298.15 K. ♦: Set I and ■: Set II.

8.040 (2H, doublet, Ar-CH).

**MS: (m/z) = 450**

**RP-4:**

IR (cm⁻¹): 1750.00 (carbonyl str. in -COOCH₃), 1541.12 (Acyclic carbonyl str.), 1446.61 (Ar-C=C str.), 1365.60 (alkane C-H bending), 1284.59 (C-O str. of ester), 1026.16 (C-O str. of ether), 840.99 (p-di substituted aromatic ring), 792.77 (m-di substituted aromatic ring).

**¹H NMR (DMSO-d₆) δ(ppm):** 1.260 (6H, doublet, -CH₃ of isopropyl), 3.024 (1H, multiplet, C-H of isopropyl), 3.536 (3H, singlet, -COOCH₃), 3.820 (1H, singlet, -OCH₃), 5.861 (2H, singlet, -OCH₂), 7.570 (7H, multiplet, Ar-CH) and 8.062 (2H, doublet, Ar-CH).

**MS: (m/z) = 420.**

**Dissociation constant and thermodynamic study:**

Figure 2 shows the titration curves of blank and compound solutions for RP-1 at 298.15 K. It is clear from the figure that for the same volume of KOH, the titration curve for blank solution shows higher pH than that of compound solution. From these curves, the average number of protons associated with compound (\(\bar{n}_H\)) was evaluated using Irving and Rossotti equation (Irving et al., 1954).

\[
\bar{n}_H = Y - \frac{V' - V}{(N^0 + E^0)} \left[\frac{(N^0 + E^0)}{(N^0 + E^0 + 1)}\right] \tag{2}
\]

Where, \(Y\) is the number of replaceable proton per molecule. For all the studied compounds, the value of \(Y\) is one. \(V\) and \(V'\) are volume of KOH required for blank and compound titration curves at the same pH. \(N^0\) and \(E^0\) are the initial concentration of the alkali, acid and compound respectively. The values of \(\bar{n}_H\) is found to be between 0 to 1 for all the compounds indicating thereby that there is only one dissociation step.

For the evaluation of dissociation constants, two methods have been used:

**Half-integral method:** In this method, the dissociation constants were evaluated at \((\bar{n}_H) = 0.5\) from the plot of \((\bar{n}_H)\) verses pH.

**Average method:** Using the following equation,

\[
pK = pH + \log \left[\frac{\bar{n}_H}{\bar{n}_H - 1}\right] \tag{3}
\]

The dissociation constants were calculated at various pH values. The average of these pH values was calculated.

The evaluated values by both methods are given in Table 2 at different temperatures. It is clear from Table 2 that the dissociation constants evaluated by both methods are in good agreement. Further, dissociation of compounds increases with increase in the temperature.
Table 1. Some thermodynamic parameters of compounds.

<table>
<thead>
<tr>
<th>Compound code</th>
<th>T/K</th>
<th>Average method</th>
<th>Half-Integral method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔG kJ/mol</td>
<td>-ΔH kJ/mol</td>
<td>ΔS J/mol K</td>
</tr>
<tr>
<td>RP-1</td>
<td>298.15</td>
<td>15.86 ± 0.02</td>
<td>106.08 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>308.15</td>
<td>15.88 ± 0.01</td>
<td>102.70 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>15.87 ± 0.04</td>
<td>99.43 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>17.35 ± 0.01</td>
<td>124.73 ± 0.01</td>
</tr>
<tr>
<td>RP-2</td>
<td>308.15</td>
<td>16.90 ± 0.03</td>
<td>119.24 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>17.19 ± 0.01</td>
<td>116.39 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>18.09 ± 0.01</td>
<td>100.20 ± 0.01</td>
</tr>
<tr>
<td>RP-3</td>
<td>308.15</td>
<td>18.44 ± 0.02</td>
<td>11.78 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>18.51 ± 0.01</td>
<td>95.22 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>17.59 ± 0.01</td>
<td>111.90 ± 0.00</td>
</tr>
<tr>
<td>RP-4</td>
<td>308.15</td>
<td>17.67 ± 0.06</td>
<td>15.76 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>17.72 ± 0.02</td>
<td>105.25 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>38.24 ± 0.00</td>
<td>181.43 ± 0.01</td>
</tr>
<tr>
<td>RP-5</td>
<td>308.15</td>
<td>37.76 ± 0.02</td>
<td>15.84 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>38.37 ± 0.03</td>
<td>170.43 ± 0.03</td>
</tr>
</tbody>
</table>

Table 2. Dissociation constants of compounds at 298.15 K, 308.15 K and 318.15 K in 1, 4-dioxane-water system.

<table>
<thead>
<tr>
<th>Compound code</th>
<th>T/K</th>
<th>Average method</th>
<th>Half-Integral method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pK</td>
<td>ΔG kJ/mol</td>
<td>ΔH kJ/mol</td>
</tr>
<tr>
<td>RP-1</td>
<td>298.15</td>
<td>6.40 ± 0.02</td>
<td>6.49 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>308.15</td>
<td>6.20 ± 0.01</td>
<td>6.28 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>6.00 ± 0.04</td>
<td>6.07 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>7.00 ± 0.02</td>
<td>7.07 ± 0.02</td>
</tr>
<tr>
<td>RP-2</td>
<td>308.15</td>
<td>6.60 ± 0.01</td>
<td>6.65 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>6.50 ± 0.04</td>
<td>6.52 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>7.30 ± 0.02</td>
<td>7.33 ± 0.02</td>
</tr>
<tr>
<td>RP-3</td>
<td>308.15</td>
<td>7.20 ± 0.01</td>
<td>7.28 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>7.00 ± 0.04</td>
<td>7.08 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>7.10 ± 0.03</td>
<td>7.10 ± 0.04</td>
</tr>
<tr>
<td>RP-4</td>
<td>308.15</td>
<td>6.90 ± 0.01</td>
<td>6.93 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>6.70 ± 0.04</td>
<td>6.72 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>6.70 ± 0.02</td>
<td>6.73 ± 0.02</td>
</tr>
<tr>
<td>RP-5</td>
<td>308.15</td>
<td>6.40 ± 0.01</td>
<td>6.42 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>318.15</td>
<td>6.30 ± 0.04</td>
<td>6.34 ± 0.04</td>
</tr>
</tbody>
</table>

By using $\Delta H$ and $\Delta G$ values, entropy change ($\Delta S$) is calculated by the equation (5) (Meloun et al., 2011):

$$\Delta S = \frac{(\Delta H - \Delta G)}{T}$$  \hspace{1cm} (5)

All the calculated thermodynamic parameters evaluated by dissociation constants (for both average and half integral methods) are given in Table 3. It is evident from Table 3 that enthalpy and entropy values are negative whereas Gibb’s free energy values are positive for all the compounds. The negative enthalpy suggests exothermic dissociation process whereas the positive Gibb’s free energy indicates non-spontaneous dissociation. The negative entropy change proves greater solvation (Sarmini et al., 1999) which causes more ordered structure (Hassanein et al., 2000).

(Mubarak et al., 2010). It is observed that order of pH values for the studied compounds are: RP-3 > RP-4 > RP-2 > RP-5 > RP-1. All the compounds have the same central moiety but different substitutions (Table 1). Thus, RP-3 having 3,4-di-methoxy group is the most basic whereas RP-1 containing fluoro group is the most acidic in nature.

Using these dissociation constant data, some thermodynamic parameters such as enthalpy change ($\Delta H$), Gibb’s energy change ($\Delta G$) and entropy change ($\Delta S$) have been evaluated.

Van’t Hoff relation (Van’t Hoff 1884) is used for the evaluation of enthalpy change and Gibb’s free energy is calculated by the equation:

$$\Delta G = RTpK$$  \hspace{1cm} (4)
Conclusion

The dissociation of studied compounds increases with increase in temperature. The dissociation is affected by the nature of substitution. The thermodynamic parameters suggest that for all the studied compounds, dissociation process is exothermic and non-spontaneous.

Nomenclature

\( \bar{n}_{H} \)  
Average number of proton associated with the compound

\( V' \)  
Volume of KOH required for blank titration curve

\( V'' \)  
Volume of KOH required for ligand titration curve

\( Y \)  
Number of displaceable protons per molecule

\( V^0 \)  
Initial volume of the test solution

\( N^0 \)  
Initial concentration of the alkali

\( E^0 \)  
Initial concentration of the acid

\( \chi' \)  
Initial concentration of the compound solution

\( pK \)  
Dissociation constant

\( \Delta H \)  
Enthalpy change (kJ mol\(^{-1}\))

\( \Delta G \)  
Gibb’s energy change (kJ mol\(^{-1}\))

\( \Delta S \)  
Entropy change (J K\(^{-1}\) mol\(^{-1}\))

\( R \)  
Universal gas constant (J mol\(^{-1}\) K\(^{-1}\))

\( T \)  
Temperature

\( f \)  
Activity coefficient of the hydrogen ions

\( \alpha_{H} \)  
Correction factor at zero ionic strength

REFERENCES


Van’t Hoff JH (1884). Etudes de Dynamique Chimique, Muller: Amsterdam, 114.