Three and Four Membered Heterocyclic Rings Substituted Derivatives of Phenobarbital and Using CPE for Determination of PB (II)

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Abstract

A series of three and four member rings N-substituted Phenobarbital have been synthesized. The nitrogen atoms have been alkylated first to form N-carboxymethyl followed by cyclization reaction to form three and four heterocyclic rings. All the synthesized compounds have been identified using I.R, ¹H NMR, and then using its heterocyclic rings by determination of lead (II) by cloud point extraction (CPE) techniques.

Keywords: 5, 5-substituted Phenobarbital, 1, 2-Diazetidine, 1,2-diaziridin, Cloud point extraction, Triton X-114.

Introduction

The pharmaceutical population of barbituric acids has concerned the attention of for over 100 years due to their therapeutic value [1]. The biological activities shown by Phenobarbital derivatives are well known [2-4]. Along with aromatic heterocycles, the Oxazole and Imidazolunits constitute an useful biological active compounds [5]. Similarly, the Triazole and Oxadiazol rings are also associated with wide ranging physiological activity [6].

Along with aromatic heterocycles, the 1,2-Diazetidine and 1,2-diaziridin units constitutes an useful biological active compounds [7-8].

Structure activity relationship shows that heterocyclic/substituted aryl moieties at the 5th position of barbituric acid nucleus remarkably increase the anticonvulsant activity [9]. The anti-inflammatory drugs in use today [10] are characterized by presence of both hydrophilic and lipophilic groups. To increase the solubility in water-alkyl and 5,5-dialkyl barbituric acids were prepared having different substituent on nitrogen atom. Previously synthesized N-carboxyalkyl barbituric acids include 1-carboxymethylphenobarbiton, [11, 12]1-carboxymethylallobarbitone, [13]1-butyl, 1-cyclohexyl, 1-octyl and 1-phenyl-3-carboxymethylbarbituric acids [14] and 1, 3-dicarboxymethylallobarbiton. All the compounds tested are very weak inhibitors of prostaglandin synthetizes. On the other hand N-carboxyalkyl barbital derivatives have been synthesized by the reaction of haloacetel with barbital and methyl Phenobarbital in polar aprotic solvents in the presence of K₂CO₃. All the prepared compounds lack anticonvulsant slight sedative and analgesic action [15]. The antibacterial and antifungal activity of the N-isoxazolyl barbital has been studied recently [16]. Work have been done by our research group to synthesize some newer potent derivatives of barbituric acid in which both nitrogen atoms bonded to one or
two heterocyclic/substituted aryl groups [17,18]. Separation and pre concentration based on cloud point extraction were becomes an important and practical application of surfactants in analytical chemistry [19,20].

Cloud point extraction methods, the hydrophobic analyses are distributed between aqueous phases and surfactants (such as TritonX-114) that’s used in this procedure, solution is heated upper a critical temperature of nonionic surfactant, called cloud point temperature (CPT). Therefore, the hydrophobic species are in a position to interact with the micelles, thus being separated and concentrated in a small volume of the surfactant-rich phase [21-23].

Materials and Methods

All the chemical were purchased from Sigma Aldrich, BDH and Merck. Melting point determinations were performed by the open capillary method using a SMP 30 melting point apparatus and are reported uncorrected. The FT-IR spectra (KBr-discs) were recorded with a IRAFFINITY-1 CE Shimadzu spectrometer. ¹H NMR spectra were recorded on a jeol 400-Hz NMR spectrophotometer operating at 400 MHz for ¹H measurements. Spectrofluoro photometer RF-1501 (Shimadzu).UV-6100 PC Double beam Spectrophotometer, EMCLAB, and Germany. Thin layer chromatography was performed on pre-coated sheets with a 0.25 mm layer of Silica Gel GF254 of the Merck Company.

Synthesis of 2, 2′-(5-ethyl-2,4,6-trixo-5-Phenylidihydropyrimidine-1,3(2H,4H)- diyl)diacetic acid (A)[24]:

In a round bottom flask (5.27 gm, 0.2 ml) Phenobartal was melted with chloroacetic acid (3.5mol) for (1hr) with stirring then a solution of (Sodium hydroxide (8g) dissolved in distilled water (20ml)) added. The refluxing was continued for (6 hrs). This reaction was monitored by TLC. Then added to distilled water (150ml).

The solution acified with hydrochloric acid (10%). Then, the solvent evaporated in under reduced pressure (by rotary evaporator) and recrystallized from absolute ethanol. Color: Crystalline white; Yield:52%; m.p.79-81°C; IR(v, cm⁻¹): brod 3,400-2,990 (OHcarboxyl), 1,707(C=Ocarboxyl), 2,987-2,848 (C-H aliph), 3,248-3,151(NH),3,037 (C-Har), ¹HNMR(400MHz,DMSO-d₆) δ (ppm): 0.82 (t,3H,-CH₃), 2.13(q, 2H, CH₂-CH₃), 4.51(S,4H ,CH₂-CO), 7.00-7.33 (d, 5H, Ph), 7.97 (s , 2H, -OHcarboxyl). TLC Rf = 0.62 (DCM: n-hexane).

Synthesis of 1,3-bis((1H-diazirin-3-yl)methyl)-5-ethyl-5-phenylpyrimidine-4,6(1H,3H,5H)-trione (A1):

In a round bottom flask containing (0.078 g) of compound A, a mixture of hydrazine (0.024 mol), concentrated HCl (1.76 ml) and (5 ml) water have been added. The mixture has been refluxed with stirring for 6 hrs. A (25 ml) of ethanol added. The precipitate was filtered of and dried. Color: Crystalline white; Yield:62%; m.p.159-160°C; IR (v, cm⁻¹):1,654 (C=O), 2,987-2,848 (C-Haliph), 3,248-3,151 (NH), 3,037 (C-H ar); ¹HNMR(400MHz,DMSO-d₆)δ(ppm): 0.82 (t,3H,-CH₃), 2.13(q, 4H, CH₂-CH₃), 3.91 (S,4H, N-CH₂-C), 7.00-7.33 (d, 5H, Ph), 7.97 (s , 2H, -NH). TLC Rf = 0.62 (DCM: n-hexane).

Synthesis of 2′-(5-ethyl-2,4,6-trixo-5-phenyldihydropyrimidine-1,3(2H,4H)-diyl)(acetohydrazide) (A2):

In a round bottom flask containing (1.27 gm) of compound A, a mixture of hydrazine (2 ml) and ethanol (27 ml) have been added. The mixture refluxed for 8 hrs. The precipitate was filtered of and dried. Color: Crystalline white; Yield:58%; m.p.181-182°C; IR(v, cm⁻¹): 1,687 (C=O amid), 3,392 (NH), 3,053-3,167(NH₂), 2,987-2,848 (C-H aliph), 3,037 (C-Har); ¹HNMR (400MHz,DMSO-d₆) δ (ppm): 0.82 (t,3H,-CH₃), 2.13(q, 2H, CH₂-CH₃), 2.73 (d, 4H, NH₂), 3.91 (S,4H, N-CH₂-C), 7.00-7.33 (d, 5H, Ph), 7.97 (t , 2H, -NH). TLC Rf = 0.62 (DCM: n-hexane). TLC Rf = 0.62 (DCM: n-hexane).

Synthesis of 1,3-bis(2-(diaziridin-1-yl)-2-oxoethyl)-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (A3):

In around bottom flask containing (0.11 gm) of compound A2, a mixture of DCM (0.1 ml) and ethanol (0.7 ml) have been added. The mixture refluxed for 5 hrs. The precipitate was filtered of and dried. The precipitate was recrystallized from ethanol. Color: Crystalline white; Yield: 58%; m.p. 213-214
The reaction of melting of phenobarbital with chloroacetic acid in basic media gave 2, 2'- (5-ethyl-2, 4, 6-trioxo-5-phenylhydropyrimidine-1, 3 (2H, 4H) dipyl) diacetic acid (A) compound. The IR spectrum of the product indicated by the appearance of broad band (3400 2990) cm⁻¹ due to hydroxyl group of carboxylic derivatives. The ¹H-NMR spectrum of compound A shows the proton signals at 1.27 ppm due to methyl group, new signal at 3.76-3.77 ppm for 4H of CH2-CO and 8.20 ppm for hydroxyl group. The IR spectrum of compound A1 has shown the disappearance of absorption band due to Carbonyl group at 1,707 cm⁻¹ and presence of NH absorption at 3248–3151 cm⁻¹. The ¹H-NMR has shown singlet peak resonated at 7.97 ppm due to NH group. Compound A2 have been identified by IR spectrum through the appearance of absorption band at 1,687 cm⁻¹ due to the amide carboxyl group. Also the appearance of NH and NH2 absorption bands at 3,392 and 3,305–3,167 respectively was a good indication for the formation of compound A2. The ¹H-NMR spectra of synthesis of 1,3-bis(2-(3,4-dioxo-1,2-diazetidin-1-yl)-2-oxoethyl)-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (A4):

In around bottom flask containing (0.11 gm) of compound A2, a mixture of oxalic acid (0.1 gm) and ethanol (0.7 ml) have been added. The mixture refluxed for 5 hrs. The precipitate was filtered and dried. The precipitate was recrystallized from ethanol. Color: Crystalline white; Yield: 58%; m.p. 163-164 °C; IR(v, cm⁻¹): 1,687 (C=O amid), 3,392 (NH), 2,987-2,848 (C-H aliph.), 3,037 (C-Har); ¹H-NMR (400MHz, DMSO-d6) δ (ppm): 0.82 (t, 3H,-CH3), 2.13(q, 2H, CH2-CH3), 3.91 (s, 4H, -N-CH2-C), 7.00-7.33 (d, 5H, Ph), 7.97 (s, 2H, -NH). TLC Rf = 0.62 (DCM: n-hexane).

Results and Discussion
compound A2 has shown doublet peak resonated at 2.73 ppm due to NH$_2$ group and triplet peak resonate at 7.97 due to NH group. Compound A3 have been identified by IR spectrum through the appearance of absorption band at 1,687 cm$^{-1}$ due to the amide carboxyl group. Also the appearance of NH absorption bands at 3,392 and the disappearance of the NH$_2$ absorption band at 3,305–3,167 was a good indication for the formation of compound A3.

The $^1$H-NMR spectra of compound A3 has shown the disappearance of the doublet peak resonated at 2.73 ppm due to NH$_2$ group and remaining the triplet peak resonates at 7.97 due to NH group. Also, Compound A4 have been identified by IR spectrum through the appearance of absorption band at 1,687 cm$^{-1}$ due to the amide carboxyl group. Beside the appearance of NH absorption bands at 3,392 and the disappearance of the NH$_2$ absorption band at 3,305–3,167 was a good indication for the formation of compound A4. The $^1$H-NMR spectra of compound A4 has shown the disappearance of the doublet peak resonated at 2.73 ppm due to NH$_2$ group and remaining the triplet peak resonates at 7.97 due to NH group.

Cloud Point Extraction Procedure (CPE)

For the CPE procedure, 5.0 mL of a solution containing of the Pb (II), Triton X-114, A2 and a buffered solution at a suitable pH was kept in the water bath at 50-60 °C for a 20 min. For the decrease the density of its surfactant, then the centrifuging for 20 min at 5000 rpm accelerated the phase separation, and then make a cooling in ice bath to increasing the separation methods and to isolation two phases easily, the surfactant rich phase became viscous and was still at the lower of the centrifuge tube and the other phase will in upper. Decantation procedure for the tube can readily discard the aqueous phases. In order to decrease the viscosity and facilitate sample handing prior to the spectrophotometer instrument, 3 mL of UN organic solvent such as ethanol was added to the small surfactant rich phase. At last, its solution was used into a quartz cell to complete its measurement by cloud point extraction (CPE).

Optimum Conditions

Under the optimum experimental conditions which are represented in the coming results. The lead (II) will reacts with A2 to form hydrophobic complex, which is subsequently trapped in surfactant micelles after making critical micelle concentration.

Effect of PH

Cloud point extraction of lead-A2 was studies at different pH solutions for reach to a best extraction for its ion into an organic phase. The pH of the solution was optimized for the non-ionic CPE in order to reach to the optimum signal for the selected intensity; the pH was adjusted to the desired value by addition of buffer solution. Following the experimental process described in the procedure section it was found that the extraction efficiency is almost dependent on pH conditions for the pH range of (4-6.5) for its complex, represented in (Fig. 1).

![Fig. 1: Effect of pH on the extraction percent of Pb (II) - A2 complex](image-url)
Effect of A2 concentration on CPE

The effect of A2 reagent concentration on the determination of lead (II) was optimized by changing the volume of \((10^{-3}) \text{ mol/L}\) of its reagent in the range of \((0.1–1) \text{ mL}\). The signal was found increased up to the reagent (A2) volume of \((0.8) \text{ mL}\) for the lead (II), and reach to the complete extraction of its complex in a small volume of surfactant rich phase. A volume of \(0.2 \text{ mL}\) was chosen as the optimum volume for the studies and its value was represented in Fig. (2).

![Fig. 2: Effect the volume of A2 reagent on the extraction percent of Pb (II)- A2 complex](image1)

Effect the Volume of Triton X-114

In cloud point extraction method, the concentration of the surfactant such as a nonionic types its play an important rule for reach to the complete extraction. Triton X-114 was chosen for the formation of the surfactant-rich phase due to its high density and low cloud point temperature of the surfactant-rich phase by which phase separation will be possible for separation two phases aquaese and organic phases using centrifugation. Extraction of lead (II) in the presence of TritonX-114 by the range of \((0.05–0.4) \text{ Ml}\) which is shown in Fig. 3. The optimum surfactant volume used for the Pb (II) was \((0.25) \text{ mL}\) for its ion its used for all experimental methods for reach to the highest possible extraction efficiency.

![Fig. 3: Effect the volume of TritonX-114 on the extraction percent of Pb (II)- A2 complex](image2)

Effect of the Incubation Time and Cloud Point Temperature

In the method (cloud point extraction system), the temperature of extraction is a one parameter studies to formation of micelle
by reach to the critical micelle concentration. The effect of temperature and time in the CPE system was investigated at 30 to 80 °C. At temperatures lower than 30 °C the separation of the two phases at the cloud point temperature was not complete. After 60 °C, the signal intensity was approximately same value because this complexes will be dissociated at high temperature (Fig. 4 a). The time effect was studies for sty in the water bath was regulator between 20 and 25 min (Fig. 4 b).

Thermodynamic Parameters

The stability constant value was dependence on temperature and its dependence on the extraction efficiency. The thermodynamic parameters: enthalpy changes (ΔH), entropy changes (ΔS), and free energy changes (ΔG) are the main evidences to determine the binding mode. The free energy change (ΔG) can be estimated from the following equation, based on the binding constant at different temperatures.

\[ ΔG = −2.303RT \log K \]  (1)

Where \( R \) is the gas constant, \( T \) is the experimental temperature; \( K \) is the stability constant at the corresponding temperature. From the value of stability constant at different temperatures, the enthalpy changes can be calculated using equation (2):

\[ \log \frac{K_2}{K_1} = \frac{2.303R}{T_1−T_2} (\Delta H) \]  (2)

The entropy changes can be calculated by using equation (3):

\[ ΔG = ΔH − TΔS. \]  (3)

The stability constant of Pb–A2 complex and other thermodynamic parameter are shown in Table (1) [25].

From the results in Table (1) the ΔH indicating that the extraction reactions are endothermic and the negative values of ΔG dictates that the solubilization are spontaneous phenomena.

Method validation of the spectrophotometric determination of pb (II) by cloud point extraction procedure

<table>
<thead>
<tr>
<th>Metal - complex</th>
<th>Log K</th>
<th>ΔG,KJ/mol</th>
<th>ΔH,KJ/mol</th>
<th>ΔS,KJ/mol K</th>
</tr>
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<tbody>
<tr>
<td>Pb²⁺</td>
<td>6.31</td>
<td>6.431</td>
<td>34.21</td>
<td>35.82</td>
</tr>
<tr>
<td></td>
<td>50 °C</td>
<td>60 °C</td>
<td>50 °C</td>
<td>60 °C</td>
</tr>
</tbody>
</table>

Table 2: Statistical data of calibration graph using proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value of pb(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( λ_{max} ) (nm)</td>
<td>304.382</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y=6.118x+0.321</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.993</td>
</tr>
<tr>
<td>Concentration rang (ng mL⁻¹)</td>
<td>5.00-15.00</td>
</tr>
<tr>
<td>LOD (ng mL⁻¹)</td>
<td>0.082</td>
</tr>
<tr>
<td>LOQ (ng mL⁻¹)</td>
<td>0.305</td>
</tr>
<tr>
<td>Enrichment factor</td>
<td>82.44</td>
</tr>
</tbody>
</table>

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Conclusion
In this method, used of the cloud point extraction for the determination and pre concentration of the lead (II) its procedure was sensitive, inexpensive, and simple and agreement with the green chemistry by using a non-ionic micelle instead of an organic solvent and its surfactant was used as detergent, and also provides low detection limits comparable or better than those of another methods, therefore we can use its method for determination lead in deferent samples.

References
