FULL PAPER

Synthesis and Biological Evaluation of Some Newly Synthesized Barbiturates and Their Derivatives by Using Task Specific Ionic Liquid [Bmim]OH

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\textbf{Abstract:}

Eco-friendly synthesis of some selected barbiturates, thiobarbiturates and dimedone derivatives has been developed by using task specific ionic liquid Bmim[OH], which not only act as catalyst but also are best solvent media for the Knoevenagel condensation reaction between heteroaryl (pyrazole, 2-chloro-quinoline and Indole) aldehydes with barbituric/thiobarbituric acid and dimedone. High yield and less reaction time are the advantages of this methodology. All the synthesized compounds were tested for their antimicrobial activities. Most of the compounds showed very good antibacterial and antifungal activity.

\textbf{Keywords:} antimicrobial activity; barbituric acid; 1-butyl-3-methyl-imidazolium hydroxide; dimedone; quinoline

1. Introduction

Barbituric acid and its derivatives are associated with a number of biological activities such as antibacterial, hypotensive and sedative. They also used as hypnotic and anesthetic agent [1]. Thiobarbiturates are useful intermediates in synthesis of heterocyclic compounds [2]. As a result of their importance from a pharmacological, industrial and synthetic point of view, there has been increasing interest in the development of efficient methodologies for the synthesis of various barbiturates and thiobarbiturate derivatives.

The Knoevenagel condensation is a very useful reaction and has been widely employed for carbon-carbon bond formation in organic synthesis [3]. This reaction is usually catalyzed by a base, an acid or heterogeneous neutral support. Among many other procedures a few using titanium tetrachloride [4], 1,5,7-triazabicyclo[4.4.0]dec-5-ene immobilized in MCM-41 [5], modified Mg-Al hydrotalcite [6], rare earth exchanged NaY zeolite [7], USY zeolite [8], aluminium oxide [9], water [10], high pressure [11], ethylenediammonium diacetate in ionic liquid [12], Lewis acidic ionic liquid [bPy]ClAlCl\textsubscript{3} [13], glycine in ionic acid [14], hydrotalcites in ionic liquid [15], may be mentioned.

5-Arylidene barbiturates are generally prepared by condensation of barbituric/thiobarbituric acid with various aldehydes on refluxing in water by using acetic acid as catalyst [16]. Villemein \textit{et al} reported the synthesis of barbiturates in the presence of montmorillonite KSF clay under microwave irradiation [17]. Dewan has reported various catalysts like NH\textsubscript{4}OAc/AcOH, montmorillonite K-10, silica gel, basic alumina, NaCl, montmorillonite KSF, and KSF/NaCl for the synthesis of 5-arylidene barbiturates [18]. Grinding method has also been employed for the
synthesis of arylidene barbiturates [19]. The condensation reactions have also been reported in water only or without solvents using BiCl₃ [20-21], Ethyl ammonium nitrate (EAN) [22], [Bmim]BF₄ [23], amino sulfonic acid [24], L-proline [25], Indole-barbiturates [26], Nickel nanoparticles [27], NH₄Cl Mediated [28], sodium p-toluene sulfonate (NaPTSA) [29].

On the other side in organic synthesis alternatives to the volatile organic solvents is the use of green solvent medium. Furthermore, the task-specific ionic liquid (TSILs) where a functional group is covalently tethered to the cation or anion (or both) of the ionic liquid, are the latest generation of ionic liquids. The incorporation of this functionality should imbue the ionic liquid with capacity to catalyze in some reaction or processes [30]. TSILs nowadays are very fascinating to the researchers from a wide variety of fields; especially the area of organic synthesis and some of them have even been applied to the chemical industry. Our literature search revealed that although reactions of aromatic aldehydes are very facile and were addressed in all the procedures, condensation of heteroaryl aldehydes are difficult to achieve and were not adequately covered. Although there are isolated examples of condensation of heteroaryl aldehydes with barbituric acid [31-34] as one of the steps in course of synthesis of target compounds, the reactions are not very fast and high yielding. A couple of reports also addressed however; these reactions lack general applicability. In addition, the reactions are very slow, and the reaction conditions also vary with each substrate. This prompted us to develop a new synthetic protocol for synthesizing barbiturate derivatives using task-specific ionic liquid 1-butyl-3-methylimidazolium hydroxide.

2. Results and Discussion

In continuation with our previous research work to develop greener protocols for the synthesis of bioactive heterocyclic compounds [35-36], here we wish to report a convenient method for the synthesis of heteroaryl barbiturates and thiobarbiturates using task-specific ionic liquid 1-butyl-3-methylimidazolium hydroxide [Bmim]OH under neat conditions by using conventional and microwave irradiation technique as shown in (Scheme 1).

In order to develop the rational reaction conditions, initially a model reaction between Indole-3-carboxaldehyde (1 mmol) and barbituric...
Acid (1 mmol) in absence of catalyst but using different solvents was studied (Scheme 2).

The results obtained by screening of different solvents for the model reaction is as shown in Table 1, the reaction in organic solvents such as acetonitrile, methanol, and chloroform proceeded very slowly (entries 1–3). Then we investigated the efficiency of IL [Bmim]OH compared to the most commonly used ILs [Bmim]BF4 and [Bmim]PF6 by the model condensation (entries 4–6). The results summarized in (Table 1) show that the reaction go smoothly in IL [Bmim]OH rather than [Bmim]BF4 and [Bmim]PF6. This [Bmim]OH plays dual role as a catalyst and a solvent in the reaction. The volume of the IL was also tested for the same model reaction (Table 1, entries 7–9). The 2 mL volume of [Bmim]OH solvent was sufficient to carry out the reaction efficiently.

With the optimal reaction conditions in hand, we then synthesized the selected barbiturate and thiobarbiturate derivatives by treating aldehydes [3-(p-methoxy) pyrazole aldehyde, 2-chloro quinoline aldehyde, Indole-3-carboxaldehyde and N,N-dimethyl benzaldehyde] with active methylene group containing compounds as barbituric acid, thiobarbituric acid and dimedone using 1-butyl-3-methylimidazolium hydroxide (2 mL) as solvent medium. This knoevenagel condensation reaction was studied by two methods one is by classical heating (at 60 °C) method and under microwave irradiation (at 150W) method. The comparative data of these two methods is summarized in Table 2. It can be observed from table 2 that three active methylene ingredients could successfully react with various aldehydes / heteryl aldehydes forming the products in high percentage yield and in less reaction time. Out of these twelve synthesized derivatives, seven derivatives are newly synthesized 3am, 3an, 3bm, 3bn, 3bo, 3cm, 3do in Table 2.

**Antimicrobial activity**

Antimicrobial activity of synthesized compounds was checked by disc diffusion method using Ciprofloxacin (Antibacterial) and Fluconazole (antifungal) agent which served as positive controls. The antimicrobial data (Table 3, given in supplementary information) shows that thiobarbitones of p-N,N-dimethyl aldehydes (code. 3dn) Indole-3-carboxaldehyde (code.3co) show superior to equivalent antibacterial activities against the standard drug ciprofloxacin by gram negative bacteria. Thiobarbitones of 2-Cl-quinoline carboxaldehyde (code. 3bn) show good antibacterial activities while the remaining compound show moderate activities. The good antifungal activities are shown by derivatives of pyrazole aldehydes (code. 3bm) and 2-Cl-quinoline-3-carboxaldehyde against the standard drug fluconazole.

**Table 1. Effects of various solvents on the Knoevenagel condensation between Indole-3-carboxaldehyde and Barbituric acid at 60 °C.**

| Entry | Solvents (mL) | Time (min) | Yield, a (%) |
|-------|---------------|------------|--------------|
| 1     | Acetonitrile (2) | 30         | 32           |
| 2     | Methanol (2)    | 30         | 28           |
| 3     | Chloroform (2)  | 30         | 22           |
| 4     | [Bmim]BF4(2)    | 30         | 50           |
| 5     | [Bmim]PF6(2)    | 30         | 45           |
| 6     | TBAB (2)        | 30         | 55           |
| 7     | [Bmim]OH(1)     | 30         | 70           |
| 8     | [Bmim]OH(2)     | 30         | 92           |
| 9     | [Bmim]OH(3)     | 30         | 83           |
| 10    | [Bmim]OH(3.5)   | 30         | 80           |

Reaction condition: Indole-3-carboxaldehyde (1mmol), Barbituric Acid (1mmol) keeping time constant for half an hour at 60 °C. a Isolated yields of products.
Table 2. Synthesis of Knoevenagel products by conventional and microwave irradiation method using task specific ionic liquid [Bmim]OH.

| Entry | Aldehyde | Active methylene | Product | Method A | Method B |
|-------|----------|------------------|---------|----------|----------|
|       |          |                  |         | Time (min) | Yield (%) | Time (min) | Yield (%) |
| 1     | 1a       | 2m               | 3am     | 25        | 85       | 03        | 90       |
| 2     | 1a       | 2n               | 3an     | 20        | 87       | 03        | 91       |
| 3     | 1a       | 2o               | 3ao     | 15        | 82       | 02        | 87       |
| 4     | 1b       | 2m               | 3bm     | 20        | 87       | 05        | 92       |
| 5     | 1b       | 2n               | 3bn     | 22        | 86       | 04        | 90       |
| 6     | 1b       | 2o               | 3bo     | 13        | 89       | 02        | 90       |
| 7     | 1c       | 2m               | 3cm     | 20        | 86       | 03        | 93       |
| 8     | 1c       | 2n               | 3cn     | 25        | 85       | 02        | 90       |
| 9     | 1c       | 2o               | 3co     | 15        | 88       | 02        | 90       |
| 10    | 1d       | 2m               | 3dm     | 18        | 88       | 04        | 94       |
| 11    | 1d       | 2n               | 3dn     | 16        | 87       | 03        | 90       |
Table 3. Antimicrobial activity of all synthesized compounds against the pathological strains based on disc diffusion assay

| Product Code | Zone of Inhibition in mm |
|--------------|--------------------------|
| Fungus culture | Gram positive bacteria | Gram negative bacteria |
|               | I | II | III | IV | V | VI | VII | VIII | IX |
| 3am          | 9 | -  | 2   | 1  | - | 7  | 10  | 14   | 12 |
| 3an          | 10 | 8 | 6   | 9  | 15| 7  | 8   | 9    | 6  |
| 3ao          | 10 | 5 | 5   | 10 | 14| 9  | 10  | 14   | 11 |
| 3bm          | 13 | 9 | 9   | -  | - | 5  | -   | -    | 5  |
| 3bn          | 8  | 6 | 4   | 7  | 13| 14 | 15  | 16   | 13 |
| 3bo          | 8  | 5 | 5   | 7  | 12| 10 | 12  | 15   | 15 |
| 3cn          | 6  | 10| 11  | 14 | 18| 7  | 10  | 12   | 10 |
| 3co          | 5  | 11| 9   | 9  | 14| 10 | 9   | 10   | 10 |
| 3cm          | -  | 8 | 8   | 10 | 19| 13 | 14  | 17   | 13 |
| 3dm          | -  | 8 | 9   | 8  | 13| 7  | 9   | 10   | 9  |
| 3dn          | -  | 5 | 5   | 19 | 12| 18 | 17  | 17   | 16 |
| 3do          | 3  | - | -   | -  | - | 16 | 14  | 15   | 17 |
| Control      | 7  | 3 | 2   | 6  | 11| 5  | 6   | 8    | 6  |
| Ciprofloxacin| -  | 20| 22  | 21 | 23| 16 | 13  | 17   | 16 |
| Fluconazole  | 14 | - | -   | -  | - | -  | -   | -    | -  |

3. Material and Methods

All the reagents were purchased from commercial supplier as Sigma Aldrich, Spectrochem Pvt. Ltd., Avra, and were used directly without purification. Melting points were determined in an open capillary tube and were uncorrected. Progress of the reaction was monitored by thin layer chromatography (TLC). $^1$HNMR spectra were recorded on Bruker Advance 400 MHz instruments using tetramethylsilane (TMS) as internal standard, $^{13}$CNMR spectra were recorded on Bruker Avl 100 MHz instruments and elemental analysis recorded on elemental analyzers Euro-E 3000.

3.1. Microwave oven Instrument

All the reactions were performed in 1.5 Cu. Ft. LG countertop domestic microwave oven of 1100 Watts and 10 power levels with LED display and touch Keypad.

3.2. Experimental

3.2.1 Preparation of 1-Butyl-3-Methylimidazoliumhydroxide [Bmim]OH [37-38]

This was prepared by modification of a reported procedure. Solid potassium hydroxide (2.3 g, 40mmol) was added to a solution of
[Bmim]Br (8.8 g, 40mmol) in dry methylene chloride (20mL) and the mixture was stirred vigorously at room temperature for 10h. The precipitated KBr was filtered off and the filtrate was evaporated to leave the crude [Bmim]OH as a viscous liquid that was washed with ether (2×20mL) and dried at 90°C for 10 h to prepare the pure ionic liquid for use.

3.2.2 A typical general procedure for the synthesis of 5-(1H-indol-3-yl) methylene) pyrimidine-2,4,6 (1H,3H,5H)-trione by conventional heating (Method A)

Indole-3-carboxaldehyde (0.145gm, 1mmol) and barbituric acid (0.128gm, 1mmol) were mixed together in the presence of 2mL IL 1-butyl-3-methylimidazolium hydroxide the reaction mixture was heated at 60°C temperature with stirring in an oil bath for appropriate time as shown in (Table 2). Upon the completion of the reaction (monitored by TLC), 2-3mL water was added to it and stirred for 5 minutes. The mixture was filtrated and the solid obtained was the condensational product with high purity, which did not need further purification. From the filtrate (including IL and water) water was removed by evaporation and the recovered IL was reused for subsequent reactions. The product was analyzed by melting point, 1HNMR, 13CNMR and Mass spectra.

3.3. Spectral data of some representative compounds

2-(((3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-5,5-pyrimidine-2,4,6,5H,5H)-trione(3am): Dark orange solid; M.F: C21H18N4O4; Mp (C) 295-298°C; 1HNMR(DMSO-d6, 400MHz) δ (ppm): 2.35(s, 3H, CH3), 7.27-8.51 (m, 4H, Ar-H), 9.10(s, 1H, vinyl CH ), 11.31(broad S, 2H, NH); 13CNMR (DMSO-d6, 100 MHz) δ (ppm): 21.34 (CH3), 125.50, 134.34, 136.23, 135.78, 130.55, 145.77, 150.44 (Arc), 150.45 (C=CH ), 124.54, 160.09, 162.73(C of barbituric acid), 124.33, 167.56,
178.59; m/z = (315) 338 [M+Na]+; Found, %: C 57.04, H 3.16, N 13.29; Calculated %: C 57.06, H 3.19, Cl 11.23, N 13.31.

5-(2-chloro-6-methylquinolin-3-yl)methylene)-dihydro-2-thioxopyrimidine-4,6(1H,5H)-dione (3bn): Cream solid; M.F.: C_{18}H_{17}ClNO; Mp (C) 280-282; ¹HNR(MS-DO-d₆, 400MHz) δ (ppm): 2.35 (s,3H,CH₃), 7.47-8.03(s, 4H, ArH), 8.01 (s,1H, vinyl CH), 8.15 (Broad S, 2H, NH), 13CNMR (MS-DO-d₆, 100 MHz) δ (ppm): 24.36(CH₃), 126.45, 132.38, 136.77, 130.54, 145.14, 148.33(Arc), 148.80(C=CH), 124.56, 150.67, 165.34, (C of barbituric acid), 124.55, 167.43, 178.45; Mass spectrum, m/z = 331 [M]+. Found, %: C 54.27, H: 3.03, N: 12.65, S: 9.63; Calculated, %: C 54.30, H 3.04, Cl 10.69, N 12.67, S 9.66.

2-(2-chloro-6-methylquinolin-3-yl)methylene)-5,5-dimethylcyclohexane-1,3-dione (3bo): Cream solid; M.F.: C_{19}H_{18}ClNO; Mp (C) >300; ¹HNR(MS-DO-d₆, 400MHz) δ (ppm): 1.40 (s, 6H, 2CH₃), 2.38 (s,3H, CH₃), 2.98 (s,4H,CH₂), 7.47-8.40 (m, 4H, ArH), 8.38 (s,1H, vinyl CH), 13CNMR (MS-DO-d₆, 100 MHz) δ (ppm): 24.12, 26.32, 30.46, 53.89, 126.65, 132.78, 135.78, 135.90, 145.40, 148.66, 146.55, 194.57; Mass spectrum, m/z = 327 [M]+. Found, %: C 59.59, H 5.50, N 4.24; Calculated, %: C 59.62, H 5.53, C 10.82, N 4.27.

5-(1H-indol-3-yl)pyrimidinyl-2,4,6(1H,3H,5H)-trione (3cm): Dark yellow solid; M.F.: C_{18}H_{17}NO; Mp (C) >300; ¹HNR(MS-DO-d₆, 400MHz) δ (ppm): 7.17-7.41(m, 4H, ArH), 8.88 (s, 1H, CH Indole), 9.57 (s, 1H, =CH), 11.15 (br s 2H, NH-barbituric acid), 10.01 (s, 1H, NH Indole), 13CNMR (MS-DO-d₆, 100 MHz) δ (ppm): 111.45, 113.52, 117.09, 122.87, 123.45, 129.44, 136.11, 140.34, 143.40, 150.30 (NCON), 163.76 (C=O), 164.56 (C=O). Mass spectrum, m/z = 255 [M]+. Found, %: C 61.15, H 3.54, N 16.43; Calculated, %: C 61.18, H 3.55, N 16.46.

2-(1H-indol-3-yl)methylene)cyclohexane-1,3,5-trione (3co): Cream solid; M.F.: C_{18}H_{17}NO; Mp (C) 210-213; ¹HNR (MS-DO-d₆, 400MHz) δ (ppm): 4.18 (s, 4H, CH₂), 7.19-7.35(m, 4H, ArH), 8.30 (s, 1H, =CH), 10.01 (s, 1H, NH Indole), 13CNMR (MS-DO-d₆, 100 MHz) δ (ppm): 51.46, 110.13, 11.12, 119.56, 120.43, 126.56, 130.22, 135.58, 146.34, 196.66, 206.86. Mass spectrum, m/z = 267[M]+. Found, %: C 76.35, H 6.39, N 5.23.

5-(4-(dimethylamino)benzylidene)pyrimidinyl-2,4,6(1H,3H,5H)-trione (3dm): M.F.: C_{18}H_{17}N₂O₃S; Mp (C) 270-272; ¹HNR(MS-DO-d₆, 400MHz) δ (ppm): 3.12 (s, 6H, N-CH₃), 6.80 (d,2H, ArH), 8.15 (s,1H), 8.42 (d,2H), 10.90 (s, 1H), 11.03 (s, 1H); ¹³CNMR (MS-DO-d₆, 100 MHz) δ (ppm): 39.77, 109.56, 111.45, 119.32, 139.90, 150.57, 154.90, 155.30, 162.54, 164.47. Mass spectrum, m/z = 295 [M]+. Found, %: C 60.20, H 5.02, N 16.19. Calculated, %: C 60.22, H 5.05, N 16.21.

5-(4-(dimethylamino) benzylidene)-dihydro-2-thioxopyrimidine-4,6(1H,5H)-dione (3dn): M.F.: C_{18}H_{17}N₂O₃S; Mp (C) 250-252; ¹HNR(MS-DO-d₆, 400MHz) δ (ppm): 3.15 (s, 6H, N-CH₃), 6.81 (d, 2H, Ar-H), 8.14 (s, 1H), 8.45 (d, 2H, Ar-H), 12.02 (s, 1H), 12.12 (s, 1H); ¹³CNMR (MS-DO-d₆, 100 MHz) δ (ppm): 94.45, 109.11, 111.34, 120.21, 140.55, 155.60, 156.67, 160.43, 163.45,177.56. Mass spectrum, m/z = 275 [M]+. Found, %: C 56.69, H 4.74, N 15.24, S 11.62; Calculated, %: C 56.71, H 4.76, N 15.26, S 11.65.

2-(4-(dimethylamino)benzylidene)-5,5-dimethylcyclohexane-1,3-dione (3do): M.F.: C_{17}H_{21}N₂O₃S; Mp (C) 188-190; ¹HNR (MS-DO-d₆, 400MHz) δ (ppm): 1.33 (s, 6H, C-CH₃), 2.88 (s, 6H, N-CH₃), 3.01(s, 4H), 6.66-7.30 (m, 4H, ArH), 8.32 (s, 1H); ¹³CNMR (MS-DO-d₆, 100 MHz) δ (ppm): 26.67, 30.87, 40.45, 53.44, 114.04, 124.67, 127.56, 140.34, 148.88, 146.77, 194.81; Mass spectrum, m/z = 271 [M]+. Found, %: C 75.22, H 7.78, N 5.13; Calculated, %: C 75.25, H 7.80, N 5.16.

3.4. Antimicrobial assay

Antimicrobial activity of all synthesized compounds was determined using a modified Kirby Bauer disk diffusion method [39]. Briefly, 100μl of some human pathogenic bacteria and fungi were grown in 10 mL of fresh media until they reached a count of approximately 108 cells/mL for bacteria or 105 cells/mL for fungi [40]. One hundred microliters of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Blank filter paper disks (Whatman) with a diameter of 11 mm were impregnated with 100 μl of tested compound dissolved in 1% DMSO of the stock solutions (20 mg/mL). Disk diffusion method for bacteria and filamentous fungi were tested by using approved

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standard method developed by the national committee for Clinical Laboratory Standards [41]. Bacterial strains were incubated at 37 °C for 24 hrs. After incubation, the antimicrobial activity was measured in terms of the zone of inhibition in mm.

Microbial cultures used to test antimicrobial activities are fungus culture as (I-Candida sp.), gram positive bacteria as (II-Staphylococcus aureus, III-Staphylococcus albus, IV-Streptococcus faecalis, V-Bacillus sp.) and gram negative bacteria as (VI-Klebsiellapnuemoniae, VII-Escherichia coli, VIII-Pseudomonas sp., IX-Proteus sp.) The results of antimicrobial activity obtained for all synthesized compounds are summarized in Table 3.

4. Conclusions

The partial solubility problem of Barbituric and thiobarbituric acid in water and organic solvents are overcome by the use of 1-butyl-3-methylimidazolium hydroxide ionic liquid. However, the development of basic task specific ionic liquids offering a new possibility to develop environmentally friendly basic catalysts due to the combination of the advantages of inorganic bases, stability in water and air, easy separation and high catalytic efficiency go into the large family of the TSLs and used in some base catalyzed organic unit reactions. Thus compared to the reported methods, we are sure to state that the synthesized heteroaryl barbiturates and thiobarbiturates in presence of this basic ionic liquid catalyze the reaction very smoothly, in short reaction time with high product yield and showing good microbial activity.

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