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# Synthesis of some novel 7-(1*H*-benzimidazol-2-ylazo)-1,3--dimethyl-6,8-disubstituted-1*H*-pyrimido[4,5-*b*][1,4]diazepine-2,4-diones as potential anti-anxiety agents

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*Abstract*: An efficient and mild method for the synthesis of some novel 7-(1*H*--benzimidazol-2-ylazo)-1,3-dimethyl-6,8-disubstituted-1*H*-pyrimido[4,5-*b*]-[1,4]diazepine-2,4-diones in water has been developed. This method is a good option to obtain the title compounds in quantitative yields in a simple and inexpensive manner. Further, the NH of the title compounds was replaced by various substituents in an ionic liquid, [bmim]$PF_6$, a recyclable and environmentally benign solvent. The synthesized compounds were characterized by analytical and spectral (IR, $^1$H-NMR, $^{13}$C-NMR and FAB mass) data and have been screened for their anti-anxiety activity in mice.

*Keywords*: 2-aminobenzimidazole, diketones, benzimidazolyl-pyrimido-diazepines, aqueous/ionic liquid mediated synthesis; anti-anxiety activity.

## INTRODUCTION

Nitrogen-containing heterocycles represent the central framework of many biologically active compounds. Benzimidazoles show antifungal,[1] anti-inflammatory,[1] analgesic,[1] CNS depressant,[2] antitubercular,[3] anticancer,[4] anti-HIV,[5] *etc*. activities. Pyrimidines exhibit varied activities, such as anticancer[6] and antibacterial.[7] 5-Fluorouracil is a well-known anticancer agent. Diazepine derivatives are used as tranquilizers,[8] anticonvulsant, anxiolytic, analgesics, sedatives, antidepressives and hypnotic agents.[9] 1,5-Benzodiazepine shows anti-anxiety,[10] analgesic,[11] anticonvulsant[11] and anti-inflammatory[11] activities.

Due to their wide range of pharmacological activity and industrial applications, the development of mild and efficient protocols for the preparation of diazepines continues to be challenging endeavor in synthetic organic chemistry. The common procedure for the synthesis of these compounds is a one-pot condensation between *o*-phenylenediamines and carbonyl compounds.[12] However, a

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large number of modified methods reported in the literature[13] suffer from several drawbacks, such as the use of a large amount of catalysts, unsatisfactory product yield and critical product isolation procedures. These disadvantages requires the development of an efficient and practically useful process of preparation.[14]

In continuation of work on bioactive heterocycles,[15–17] reported herein is for the first time a rapid efficient, clean and environmentally benign exclusive synthesis of 7-(1*H*-benzimidazol-2-ylazo)-1,3-dimethyl-6,8-disubstituted-1*H*-pyrimido[4,5-*b*][1,4]diazepine-2,4-diones **4a**–**c** by the reaction of 2-[(1*H*-benzimidazol--2-yl)hydrazono]-1,3-disubstituted-1,3-dione **3a**–**c** and 5,6-diamino-1,3-dimethyl-2,4(1*H*,3*H*)-pyrimidinedione hydrochloride in aqueous medium in excellent yields (92–97 %). The use of water as a solvent for organic transformations offers green chemistry benefits[18] and is widely used to enable and expedite the synthesis of diverse heterocycles. Compounds **3a**–**c** were prepared by coupling reaction[19] of a diazonium salt, formed by the diazotization of 2-aminobenzimidazole **1** and 1,3-diketones **2** in presence of sodium acetate and ethanol. Further, the NH of title compounds was substituted by various substituents by reacting with methyl iodide, benzyl chloride, chloroacetyl chloride, formaldehyde and secondary amines, trifluoroacetic anhydride in [bmim]$PF_6$, an ionic liquid to give the *N*-substituted derivatives **5a**–**i** (Scheme 1). The synthesized compounds **4a**–**c** and **5a**–**i** were screened for their anti-anxiety activity in mice and they exhibited excellent results.

## EXPERIMENTAL

Melting points are uncorrected and were taken in open glass capillaries using a Gallenkamp melting point apparatus. The IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer in KBr pellets and band positions are recorded in wavenumbers ($cm^{-1}$). The $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker DRX-300 instrument at 300.15 and 75 MHz, respectively. $CDCl_3$ was used as the solvent and TMS as internal reference. The mass spectra were recorded on a JEOL, SX 102 (FAB) mass spectrometer. The mass spectra and elemental analyses were performed at the Central Drug Research Institute, Lucknow, India. All employed chemicals were of analytical reagent grade, purchased from Acros and used without further purification.

*General method for the synthesis of compounds **3a**–**c***

2-Aminobenzimidazole (0.02 mol) was dissolved in a mixture of conc. HCl and water (20 mL; 1:1), then cooled to 0 °C and a cold aqueous solution of sodium nitrite (0.02 mol; 1.3 g in 10 mL water) was added slowly maintaining the temperature between 0–2 °C. The cold diazotized solution was added dropwise to a cooled mixture of 1,3-diketone (0.02 mol) and sodium acetate (10 g) in 20 mL of 50 % ethanol. The stirring was continued for 1 h and the crystals that separated were filtered, washed with water, dried and crystallized from ethanol to yield **3a**–**c**.

*Water mediated synthesis of compounds **4a**–**c***

A solution of 5,6-diamino-1,3-dimethyl-2,4(1*H*,3*H*)-pyrimidinedione hydrochloride (0.01 mol) in water (200 mL) was neutralized with ammonia to pH 7 then **3a**–**c** (0.01 mol),



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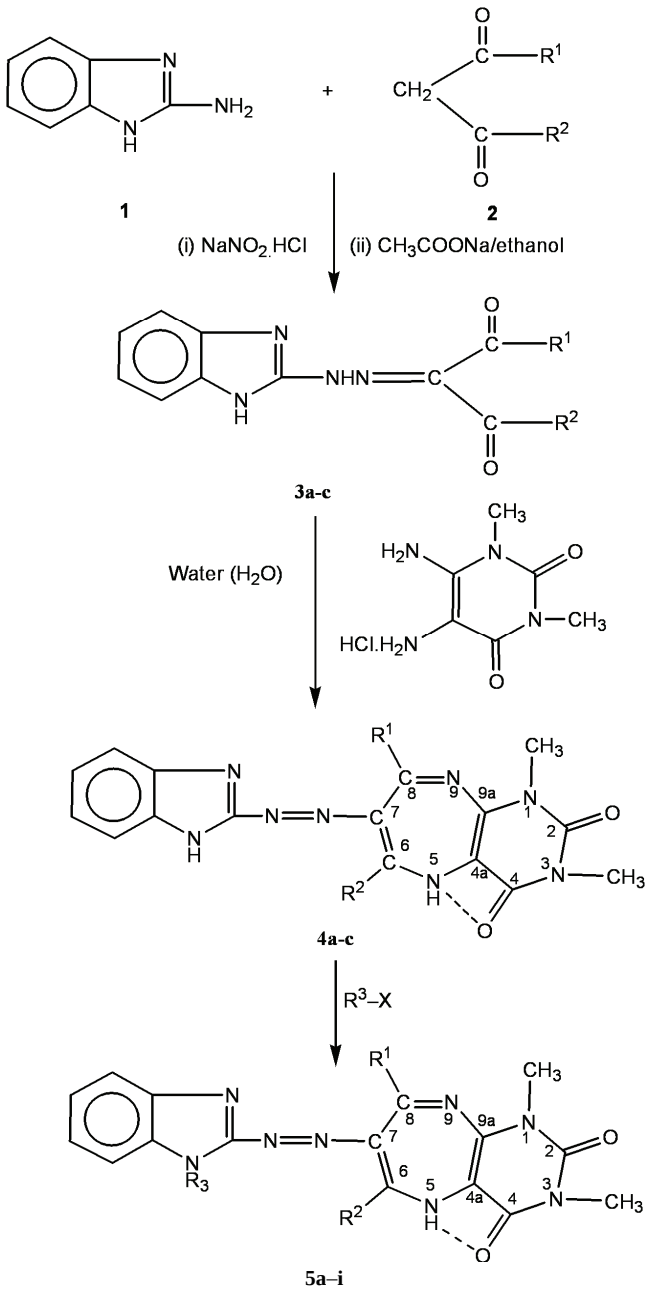
was added and the mixture heated under reflux for 30 min. The crystalline product, which started to separate out just after cooling the reaction mixture, was washed with water and found to be pure by TLC with no need for further purification. All compounds were synthesized similarly to yield **4a–c**.



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| Compound | $R^1$ | $R^2$ | $R^3$ | Compound | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|---|---|---|
| **3a** | $CH_3$ | $CH_3$ | – | **5c** | $CH_3$ | $CH_3$ | $CH_2Ph$ |
| **3b** | $CH_3$ | $C_6H_5$ | – | **5d** | $CH_3$ | $C_6H_5$ | $CH_2Ph$ |
| **3c** | $CF_3$ | $C_6H_5$ | – | **5e** | $CH_3$ | $CH_3$ | $COCH_2Cl$ |
| **4a** | $CH_3$ | $CH_3$ | – | **5f** | $CH_3$ | $C_6H_5$ | $CH_2NMe_2$ |
| **4b** | $CH_3$ | $C_6H_5$ | – | **5g** | $CH_3$ | $C_6H_5$ | $CH_2NEt_2$ |
| **4c** | $CF_3$ | $C_6H_5$ | – | **5h** | $CH_3$ | $CH_3$ | $COCF_3$ |
| **5a** | $CH_3$ | $CH_3$ | $CH_3$ | **5i** | $CF_3$ | $C_6H_5$ | $COCF_3$ |
| **5b** | $CH_3$ | $C_6H_5$ | $CH_3$ | | | | |

Scheme 1. Synthesis of compounds **3a**–**c**, **4a**–**c** and **5a**–**i**.

*Ionic liquid mediated synthesis of compounds **5a**–**i***

A mixture of **4a**–**c** (0.01 mol), methyl iodide/benzoyl chloride/chloroacetyl chloride/ /formaldehyde and secondary amine (1:1) or trifluoroacetic anhydride (0.01 mol) and ionic liquid [bmim]$PF_6$ (5 mL) were taken in a round bottom flask and heated at 60–70 °C under $N_2$ protection for 1 h. The progress of the reaction was monitored by TLC. After completion of reaction, the mixture was extracted with diethyl ether (6×10 mL). The organic extract was washed with 5 % $Na_2CO_3$ (40 mL) and water (40 mL), dried with anhydrous sodium sulfate and evaporated under vacuum. The residual product was purified by recrystallization from AcOEt/petroleum ether (60–80 °C) or by column chromatography (silica gel, 60–120 mesh, eluent petroleum ether/AcOEt = 4:1) to give **5a**–**i** (the ionic liquid layer was washed with water (3×5 mL) and kept for 2 h. at 80–85 °C under reduced pressure, the ionic liquid was recycled).

*Anti-anxiety activity in mice*

Synthesized compounds **4a**–**c** and **5a**–**i** were screened for their anti-anxiety activity in mice on a plus maze apparatus devised by Crawley and Godwin[20] modified by Kilfoil[21] using sodium pentabarbitone as the standard.

The apparatus consisted of a Plexiglas base (102 cm×53 cm×53 cm) divided into two chambers by a black Plexiglas partition. The box was placed within a layer of a soundproof box, which was equipped with a one-way observation window. The partition dividing the sub chamber had a 33 cm×13 cm opening, through which animal could easily pass. The dark chamber (36 cm×53 cm×53 cm) was made up of dark Plexiglas except for the side ferry observation window. This side was clear and covered with black plastic. The testing was performed between 12.00 noon to 6.00 p.m. in an isolated darkened laboratory.

Mice weighing 25 g were chosen and were sorted into five animals in a group. They were allowed free access to food and water *ad libitum*. Animals were given 60 min to acclimatize to the environment prior to the administration of the drug. Drugs or test samples in DMF were given at a dose of 20 mg $kg^{-1}$ body weight, after which each animal was individually placed in the centre of the light area of the apparatus and observed for 10 min. The total amount of time spent in the dark area was measured.

## RESULTS AND DISCUSSION

2-Aminobenzimidazole (**1**) was reacted with sodium nitrite and HCl at 0–5 °C. The so-formed diazonium salt was further reacted with 1,3-diketones (**2**) and sodium acetate in ethanol to afford 2-[(1*H*-benzimidazole-2-yl)hydrazono]-1,3--disubstituted 1,3-diones **3a**–**c**. The formation of compound **3** was confirmed by



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their IR spectra, in which band appeared at 3200, 3030, 1680 and 1620 $cm^{-1}$ due to –NH benzo, NH hydrazono, C=O and C=N, respectively. The $^1$H-NMR spetra showed peaks at $\delta$ 10.62 and 9.52 ppm due to the NH hydrazono and NH of benzimidazolyl moieties, respectively. The $^{13}$C-NMR spectra showed peaks at $\delta$ 178.2, 180.1 and 162.4 ppm due to two C=O and the C=N, respectively. Further, the mass spectrum shows $M^+$ at *m*/*z* 244 (**3a**).

The advantage of using water as a solvent is its cost, safety (it is non-inflammable and is devoid of any carcinogenic effects) and simple operation. It has the highest specific heat value of all substances as well as unique enthalpic and entropic properties, which have led us to its use. Water has abnormally low volatility because its molecules are associated with each other by means of hydrogen bonding.

In view of this, compounds **3a**–**c** were reacted with 5,6-diamino-1,3-dimethyl-2,4(1*H*,3*H*)-pyrimidinedione hydrochloride in aqueous medium to afford 7-(1*H*-benzimidazol-2-ylazo)-1,3-dimethyl-6,8-disubstituted-1*H*-pyrimido[4,5-*b*]-[1,4]diazepine-2,4-diones **4a**–**c**.

The formation of compounds **4** was confirmed by their IR spectra, which showed the disappearance of the peaks due to the hydrazono group and the ketonic group of the 1,3-diketone part with the appearance of a peak due to the NH of diazepine at 3340 $cm^{-1}$ and at 1720 and 1700 $cm^{-1}$ due to the C=O of the pyrimidine ring. The $^1$H-NMR spectra showed peaks at $\delta$ 9.51 and 8.46 ppm due to the NH of benzimidazole and diazepine moiety, respectively. The $NCH_3$ methyl groups present at the positions 4 and 6 appeared at $\delta$ 3.20 and 3.62 ppm, respectively. The $^{13}$C-NMR spectra showed peaks at $\delta$ 33.5 and 34.8 ppm due to the two $NCH_3$ groups of the pyrimidine ring, while two C=O groups appeared at $\delta$ 190.2 and 195.1 ppm. Further, the mass spectrum shows $M^+$ at *m*/*z* 378 (**4a**).

In recent years ionic liquids[22] (ILs) have emerged, which are organic salts the ions of which do not pack well and remain liquid at room temperature. They have unique properties, such as, for example, a wide liquid range, good solvency, tunable polarity, high thermal stability, negligible vapor pressure and recyclability. Ionic liquids could be recycled and reused as opposed to traditional solvent catalyst systems.

In view of the above, compounds **4a**–**c** were reacted with: *i*) methyl iodide, *ii*) benzyl chloride, *iii*) chloroacetyl chloride, *iv*) formaldehyde and a secondary amine and *v*) trifluoroacetic anhydride in the ionic liquid [bmim]$PF_6$ to give *N*-benzimidazolyl substituted derivatives **5a**–**i**.

Although compounds **4a**–**c** have two NH groups, one in the benzimidazolyl moiety and another in the diazepine ring, it was observed that only NH of benzimidazolyl moiety was replaced by various substituents. The NH of the diazepine moiety could not be replaced due to hydrogen bonding with the C=O of the pyrimidine ring.



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The formation of compounds **5a**–**i** were confirmed by the disappearance of peak due to the NH of the benzimidazolyl moiety and the appearance of a peak due to N-substituent in their IR, $^{1}$H-NMR and $^{13}$C-NMR spectra. The mass spectrum also shows $M^+$ at *m*/*z* 392 (**5a**).

*Anti-anxiety activity in mice*

Synthesized compounds **4a**–**c** and **5a**–**i** were screened for their anti-anxiety activity in mice on a plus maze apparatus devised by Crawley and Godwin[20] modified by Kilfoil.[21] The less time a mouse spent in the dark space, the greater was the anti-anxiety activity of the drug. The results of the anti-anxiety activity of the compounds are given in Table I. Compounds **4c**, **5b**, **5e**, **5h** and **5i** showed better activity than the standard, sodium pentabarbitone, and the other compounds were moderately active. The good activity of these compounds is due to presence of trifluoromethyl, chloro and trifluoroacetyl groups, which enhance their activity.

TABLE I. Results of anti-anxiety activity in mice of compounds **4a**–**c** and **5a**–**i**

| Compound | $R^1$ | $R^2$ | $R^3$ | Time spent in dark space, s |
|---|---|---|---|---|
| **4a** | $CH_3$ | $CH_3$ | – | 300±9.2 |
| **4b** | $CH_3$ | $C_6H_5$ | – | 298±8.4 |
| **4c** | $CF_3$ | $C_6H_5$ | – | 291±8.2 |
| **5a** | $CH_3$ | $CH_3$ | $CH_3$ | 304±10.5 |
| **5b** | $CH_3$ | $C_6H_5$ | $CH_3$ | 294±10.2 |
| **5c** | $CH_3$ | $CH_3$ | $CH_2Ph$ | 314±9.2 |
| **5d** | $CH_3$ | $C_6H_5$ | $CH_2Ph$ | 318±10.8 |
| **5e** | $CH_3$ | $CH_3$ | $COCH_2Cl$ | 294±12.0 |
| **5f** | $CH_3$ | $C_6H_5$ | $CH_2NMe_2$ | 318±11.7 |
| **5g** | $CH_3$ | $C_6H_5$ | $CH_2NEt_2$ | 321±10.5 |
| **5h** | $CH_3$ | $CH_3$ | $COCF_3$ | 292±9.20 |
| **5i** | $CF_3$ | $C_6H_5$ | $COCF_3$ | 290±9.82 |
| Control | | | | 401.6±25.0 |
| Pentabarbitone | | | | 295±11.50 |

## CONCLUSIONS

Novel 7-(1*H*-benzimidazol-2-ylazo)-1,3-dimethyl-6,8-disubstituted-1*H*-pyrimido[4,5-*b*][1,4]diazepine-2,4-diones **4a**–**c** were synthesized in aqueous medium by the reaction of 2-[(1*H*-benzimidazol-2-yl)hydrazono]-1,3-disubstituted-1,3-diones **3a**–**c** and 5,6-diamino-1,3-dimethyluracil hydrochloride. Further, the title compounds were reacted with various reagents/reactants in [bmim]$PF_6$ to determine whether all NH groups of the title compounds could be substituted. It was observed that only monosubstitution was possible yielding **5a**–**i** as the diazepine NH is involved in hydrogen bonding with the C=O of the pyrimidine ring. Compounds **4a**–**c** and **5a**–**i** were screened for their anti-anxiety activity in



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mice and the data revealed that the compounds showed good to moderate activity. Some of the compounds were more active than the standard and could act as potential anti-anxiety agents.

## SUPPLEMENTARY MATERIAL

Analytical and spectral data for the compounds **3a**–**c**, **4a**–**c** and **5a**–**i** are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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## ИЗВОД

## СИНТЕЗА НОВИХ 6,8-ДИСУПСТИТУИСАНИХ 7-(1*H*-БЕНЗИМИДАЗОЛ-2-ИЛАЗО)-1,3-ДИМЕТИЛ-1*H*-ПИРИМИДО[4,5-*b*][1,4]ДИАЗЕПИН-2,4-ДИОНА КАО ПОТЕНЦИЈАЛНИХ ЛЕКОВА ПРОТИВ АНКСИОЗНОСТИ

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Развијен је ефикасан поступак синтезе нових 6,8-дисупституисаних 7-(1*H*-бензимидазол-2-илазо)-1,3-диметил-1*H*-пиримидо[4,5-*b*][1,4]диазепин-2,4-диона, у води под благим реакционим условима. Описани поступак је добра алтернатива за синтезу ових деривата, у квантитативном приносу на једноставан и економски приступачан начин. Осим тога, NH-група производа дериватизована је различитим супституентима, реакцијом у јонској течности, [bmim]$PF_6$, растварачу који може да се рециклира и еколошки је прихватљив. Синтетисана једињења су окарактерисана аналитичким и спектроскопским методама (IC, $^1$H-NMR, $^{13}$C-NMR и FAB масена спектрометрија) и испитана је њихова антианксиозна активност на мишевима.

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