

Synthesis, Characterization and Antioxidant Activity of Novel 3-(2,5-dioxo-imidazolidin-1-yl)-1,1-diethylurea Derivatives

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Abstract: A novel class of hydantoin derivatives was synthesized and their antioxidant activity was evaluated. The newly synthesized compounds were characterized using ¹H NMR, IR, Mass spectra and elemental analysis. The antioxidant activity of synthesized compounds (**3a-d**) were examined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide hydroxyl radical scavenging assay. It is revealed from the antioxidant screening results that the compounds **3a** and **3d** manifested profound antioxidant potential.

Keywords: Hydantoin, DPPH, Nitric oxide, Antioxidant, Hydroxyl radical

Introduction

Hydantoins, a class of cyclic imides, have attracted continuing interest over the years because of the use of its ring system as important core structure in many drug substances. Depending on the nature of substitution on the hydantoin ring, a wide range of other pharmacological properties, *e.g.*, antihypertensive¹, herbicidal², antitumor³, anti-HIV⁴, antibacterial⁵ and antiviral⁶ activities have also been identified.

The search for new molecules with anti-oxidant properties is a very active domain of research, since they can protect the human body from free radicals and retard the progress of many chronic diseases⁷. High levels of free radicals can cause damage to biomolecules such as lipids, proteins, enzymes and DNA in cells and tissues. This may result in many diseases such as: cancer, diabetes, cardiovascular and autoimmune diseases, and neurodegenerative disorders, aging, and other diseases through the violent reactivity of the free radicals⁸.

Based on these findings, and in view of the significant biological potential alluded by hydantoins, it was though worthwhile to design and synthesize a new series of biologically active hydantoin derivatives.

Experimental

The starting materials and reagents were obtained from commercial suppliers and were used without further purification. Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on Bruker Advance 400 MHz NMR instrument using TMS as internal standard and DMSO- d_6 as solvent. The FT-IR spectra were recorded using nujal on FT-IR Jasco 4100 infrared spectrophotometer. Column chromatography was performed using Merck 7734 silica gel (60-120 mesh) and Merck made pre-coated TLC plates were used. Elemental analysis (C, H, and N) was undertaken with Perkin-Elmer model 240C analyzer and the data showed good agreement between the experimentally determined values and the theoretically calculated values within $\pm 0.4\%$.

Synthesis

Synthesis of ethyl diethylcarbamate (1)

Diethylamine (6 mmol) was refluxed with propionyl chloride (6.2 mmol) in THF (25 mL) and in the presence of triethylamine (6.2 mmol). Then the reaction mixture was stirred at room temperature for about 8 h. After completion of the reaction (TLC), the reaction mixture was quenched in ice cold water and extracted with dichloromethane. The ether layer was washed with 5% NaHCO_3 and dried over Na_2SO_4 and concentrated *in vacuo* to give the light brown product. Yield: 92%. ^1H NMR (300 MHz, DMSO- d_6) δ : 4.13 (q, 2H, $-\text{CH}_2$), 3.72 (q, 4H, $-\text{CH}_2$), 1.34 (t, 6H, $-\text{CH}_3$), 1.26 (t, 3H, $-\text{CH}_3$). MS, m/z : 145 (M^+). Anal. calc. for ($\text{C}_7\text{H}_{15}\text{NO}_2$): C 57.90; H 10.41; N 9.65; found: C 57.95; H 10.49; N 9.72.

Synthesis of 3-(2,5-dioxoimidazolidin-1-yl)-1,1-diethylurea (2)

To a solution of amine (1 equiv.) in THF at 0 °C was slowly added dimethylaluminium chloride (1 equiv.). After the addition was completed, the ice-bath was removed. The resulting solution was stirred for 30 min and then compound 1 (1 equiv.) was added. The mixture was further stirred for 6 h at r.t. Aqueous potassium tartrate tetrahydrate was added slowly, the organic layer was separated, and the aqueous phase washed with 25 mL of CHCl_3 (3x). The organic extracts were combined and washed with brine, dried (anhydrous Na_2SO_4) and concentrated *in vacuo*. Recrystallization from absolute EtOH gave product 2. Yield: 90%. ^1H NMR (300 MHz, DMSO- d_6) δ : 8.43 (s, 1H, NH), 8.12 (s, 1H, NH), 3.89 (q, 4H, $-\text{CH}_2$), 3.62 (s, 2H, $-\text{CH}_2$), 1.34 (t, 6H, $-\text{CH}_3$). MS, m/z : 145 (M^+). Anal. calc. for ($\text{C}_8\text{H}_{14}\text{N}_4\text{O}_3$): C 44.85; H 6.59; N 26.15; found: C 44.81; H 6.55; N 26.23.

Synthesis of (3a-d)

A solution of compound 2 (0.1 mmol) in dimethylformamide (1 mL) was taken and cooled to 0-5 °C in an ice bath. Triethylamine (0.12 mmol) was added to cold reaction mixture and stirred for 30 min. To the mixture different substituted phenyl thiocyanates (0.1 mmol) was added and allowed to stir at room temperature for 4 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulfate. The filtrate was concentrated *in vacuo* to get the crude product which was purified by column chromatography over silica gel (60-120 mesh) using hexane: ethyl acetate (9:1) as eluent to afford the compound 3a-d in good yields.

3-(3,3-Diethylureido)-N-(4-hydroxyphenyl)-2,4-dioxoimidazolidine-1-carbothioamide (3a)

Yield: 89%. ¹H NMR (300 MHz, DMSO-d₆) δ: 10.1 (s, 1H, Ar-OH), 8.83 (s, 1H, CONH), 8.41 (s, 1H, CSNH), 7.42-7.12 (m, 4H, Ar-H), 4.01 (s, 2H, -CH₂), 3.89 (q, 4H, -CH₂), 1.34 (t, 6H, -CH₃). IR (nujol, cm⁻¹): 3439 (Ar-OH), 3235, 3215 (N-H), 1708, 1675, 1639 (C=O). MS, m/z: 366 (M⁺). Anal. Calc. For (C₁₅H₁₉N₃O₄S): C 49.30; H 5.24; N 19.17; found: C 49.37; H 5.21; N 19.23.

3-(3,3-Diethylureido)-2,4-dioxo-N-(p-tolyl)imidazolidine-1-carbothioamide (3b)

Yield: 87%. ¹H NMR (300 MHz, DMSO-d₆) δ: 8.81 (s, 1H, CONH), 8.45 (s, 1H, CSNH), 7.45-7.12 (m, 4H, Ar-H), 4.02 (s, 2H, -CH₂), 3.81 (q, 4H, -CH₂), 2.32 (s, 3H, Ar-CH₃), 1.32 (t, 6H, -CH₃). IR (nujol, cm⁻¹): 3235, 3210 (N-H), 1708, 1670, 1635 (C=O). MS, m/z: 364 (M⁺). Anal. Calc. For (C₁₆H₂₁N₃O₃S): C 52.88; H 5.82; N 19.27; found: C 52.81; H 5.89; N 19.35.

3-(3,3-Diethylureido)-N-(4-methoxyphenyl)-2,4-dioxoimidazolidine-1-carbothioamide (3c)

Yield: 83%. ¹H NMR (300 MHz, DMSO-d₆) δ: 8.83 (s, 1H, CONH), 8.43 (s, 1H, CSNH), 7.41-7.13 (m, 4H, Ar-H, 5), 4.14 (s, 2H, -CH₂), 3.97 (q, 4H, -CH₂), 3.79 (s, 3H, -OCH₃), 1.32 (t, 6H, -CH₃). IR (nujol, cm⁻¹): 3235, 3215 (N-H), 1706, 1675, 1632 (C=O). MS, m/z: 380 (M⁺). Anal. Calc. For (C₁₆H₂₁N₃O₄S): C 50.65; H 5.58; N 18.46; found: C 50.61; H 5.63; N 18.54.

N-(4-aminophenyl)-3-(3,3-diethylureido)-2,4-dioxoimidazolidine-1-carbothioamide (3d)

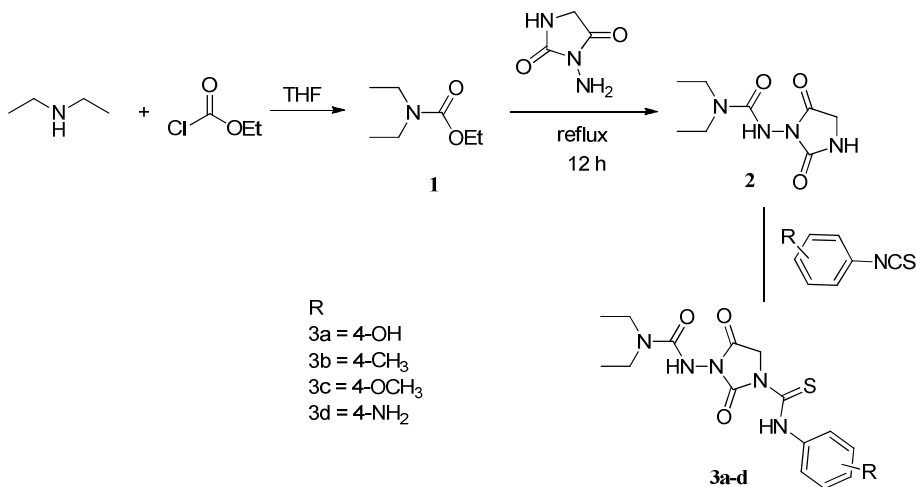
Yield: 85%. ¹H NMR (300 MHz, DMSO-d₆) δ: 8.81 (s, 1H, CONH), 8.46 (s, 1H, CSNH), 7.44-7.12 (m, 4H, Ar-H, 5), 5.71 (s, 2H, -NH₂), 4.12 (s, 2H, -CH₂), 3.97 (q, 4H, -CH₂), 1.32 (t, 6H, -CH₃). IR (nujol, cm⁻¹): 3379 (NH₂), 3235, 3213 (N-H), 1710, 1671, 1640 (C=O). MS, m/z: 365 (M⁺). Anal. Calc. For (C₁₅H₂₀N₆O₃S): C 49.44; H 5.53; N 23.06; found: C 49.48; H 5.59; N 23.15.

Antioxidant activity

All synthesized compounds were tested for antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO) and hydroxyl radical scavenging methods. Standard procedures were followed for the determination of free radical scavenging activity

Results and discussion*Synthesis*

The syntheses of the compounds **3a-d** were accomplished according to the reaction sequence illustrated in Scheme 1. The synthesis begins from condensation of diethyl amine with propionyl chloride in tetrahydrofuran (THF) afford the compound **1**. The key intermediate compound **2** was obtained by the reacting compound **1** with 3-aminoimidazolidine-2,4-dione under reflux for 12 h. Finally the compound **2** was coupled with different substituted isothiocyanatobenzene to afford the desired compounds **3a-d** with good yields (84-91%). The structures of the synthesized compounds were deduced on the basis of ¹H NMR, IR and mass spectra. The composition of all the compounds was obtained by elemental analysis. In addition, the chemical shift and multiplicity patterns correlated well with the proposed structures. The elemental analysis data showed good agreement between the experimentally determined values and the theoretically calculated values.



Scheme 1. Reaction scheme of hydantoin derivatives (**3a-d**)

Antioxidant activity

Radical scavenging potency of the synthesized compounds **3a-d** was assessed *in vitro* by the DPPH⁹, hydroxyl¹⁰ and nitric oxide radical scavenging¹¹ methods in comparison with the standards, namely ascorbic acid and BHA. The results are presented in Table 1.

Table 1. Antioxidant activity of the synthesized compounds

Compounds	IC ₅₀ , µg/mL		
	DPPH	Nitric oxide	Hydroxyl
3a	12.7±0.06	13.4±0.64	10.6±0.03
3b	46.5±0.69	59.1±0.78	48.9±0.51 10.6±0.03
3c	38.6±0.55	55.9±0.67	48.1±0.71
3d	13.1±0.14	14.1±0.53	11.7±0.18
AA ^a	12.6±0.43	n.t ^c	n.t ^c
BHA ^b	n.t ^c	14.3±0.18	15.3±0.47

^aascorbic acid, ^bbutylatedhydroxyanisole, ^cnot tested

The stable free radical DPPH is a useful reagent to investigate the scavenger properties of phenols, catechols, and anilines. This assay evaluates the ability of the substance under examination to donate H atoms and quench the visible absorption at 515 nm of the nitrogen radical of DPPH¹². There are many reports describing the antiradical effect of phenolic compounds by DPPH assay. On other hand, it is well-established that organic molecules incorporating an electron donating group (amine, hydroxyl and methoxy) moiety can act as free radical trapping agents and are capable of opposing oxidative challenges.

All the compounds (**3a-d**) showed comparable or slightly less activity to the standards (ascorbic acid and BHA). The antioxidant activity was expressed as the 50% inhibitory concentration (IC₅₀) based on the amount of compound required for a 50% decrease in the initial DPPH radical concentration. It was observed that all the compounds notably reduced the concentration of DPPH free radical.

The difference in radical scavenging activity of the synthesized hydantoin compounds (**3a-d**) were due to the difference in the stability of the oxygen centred radical formed in

these compounds. The better activity of compound **3a** (IC_{50} 12.7 $\mu\text{g/mL}$) having hydroxyl group at p- position in the aromatic ring is due to high electron-releasing properties (positive mesomeric effect is higher than negative inductive effect) and this activates the aromatic ring. The compound **3d** (IC_{50} 13.1 $\mu\text{g/mL}$) bearing an electron donating amine group at para position showed better DPPH radical scavenging activity compared to **3b** and **3c**.

Hydroxyl radical scavenging capacity of synthesized compounds (**3a-d**) is directly related to its antioxidant activity and hydroxyl radicals are the major active oxygen species causing lipid oxidation and enormous biological damage¹³. IC_{50} values (Table 1) of all tested compounds through hydroxyl radical scavenging activity test were ranging from 10.6 to 48.9 $\mu\text{g/mL}$. In this study, compound **3a** exhibited the strongest antioxidant activity (10.6 $\mu\text{g/mL}$), followed by the compound **3d** (11.7 $\mu\text{g/mL}$) and the compound **3b** (48.9 $\mu\text{g/mL}$) which showed the weakest activity.

In addition to reactive oxygen species, nitric oxide has a key role in various types of inflammatory processes and is a potent pleiotropic inhibitor of physiological processes. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities¹⁴. The nitric oxide radical scavenging activity indicates that the OH and NH_2 substitution in the phenyl group is the main factor responsible for the scavenging effect of this reactive nitrogen species. Compounds **3a** and **3d** were the most effective ones with IC_{50} value 13.4 and 14.1 $\mu\text{g/mL}$, respectively.

Conclusion

The synthesis and electrochemical investigation of novel hydantoin derivatives may draw the following conclusions: (i) A series of hydantoin derivatives was prepared and tested for their antioxidant activity. These compounds offer the advantage of a facile synthesis. (ii) Further, the compounds **3a** and **3d** displayed pronounced antioxidant activity as interpreted by the results of DPPH, superoxide and hydroxyl radical scavenging assays. On the basis of above observations, we will further modify to improve the antidepressant activity of piperamides.

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