Synthetic Studies of Bioactive Substances of 4-Hydroxybenzalhydantoin Derivatives

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Abstract. 4-hydroxybenzalhydantoin derivatives were synthesized by the condensation reaction between benzaldehydes 12-13 and substituted hydantoins 14-16 under standard conditions of reflux in glacial acetic acid, in the present of sodium acetate and a little amount of acetic anhydride as a catalyst. All compounds were identified by spectral analysis to give 4-hydroxybenzalhydantoins 17-21.

1. Introduction
Benzalhydantoin or 5-(benzylidene)imidazolidine-2,4-diones) derivatives are important and prevalent classes of biologically active natural and un-natural substances [1-10]. For example, (Z)-4-hydroxybenzalhydantoin 1 was isolated from the Red Sea sponge Laxosubrites sp but reported not to have any biological activity [11]. Recently, compound 1 was also isolated from the aqueous ethanolic extracts of the Red Sea sponge Hemimycale arabica, along with other substituted hydantoins, (R)-5-(4-hydroxybenzyl)hydantoin 2, and (Z)-5-((6-bromo-1H-indol-3-yl)methylene)hydantoin 3 [12].

Interestingly, on this occasion, compound 1 was found to have potent in vitro anti-growth and anti-invasive properties against PC-3M prostate cancer cells [12]. As a result, thirty-nine other aryl-substituted benzalhydantoins 4-11 were synthesized and examined as novel antimetastatic agents with potential to control metastatic prostate cancer [12].
Benzalhydantoins substituted with alkyl, halogen, trifluoromethyl, and alkoxy groups on the phenyl ring were found to exhibit good anticonvulsant activity [2-12]. Researchers have found benzalhydantoins active against *Mycobacterium tuberculosis* H37Rv [14], while, others have reported that benzalhydantoins and 5-alkyl- and 5-arylmethyl-hydantoins, and their 2-thio analogues, have fungicidal and bacterial activity [8,15]. Many benzalhydantoins have been used for aromatic amino acid synthesis. Spectroscopic properties [16] and crystal structures of selected benzalhydantoin derivatives have been studied in order to understand the structure-activity relationships better, especially since *E/Z* geometrical isomers are possible because of the restricted rotation of the exocyclic methylidene double bond [17-18].

Our group has prepared a wide range of new [8-10], substituted benzalhydantoins 14-16 as part of a synthetic study toward the tandem approach to the molecules. It occurred to us that these can be viewed as spatial mimics of benzalhydantoin 1.

2. Experimental

2.1. General

Melting points were measured on a Reichert hotstage microscope and are uncorrected. Ultraviolet spectra were measured on a Hitachi U-3200 spectrophotometer and refer to solutions in absolute MeOH, and data reported as wavelength ($\lambda_{\text{max}}$) in nm and absorption coefficient ($\varepsilon_{\text{max}}$) in dm$^3$.mol$^{-1}$.cm$^{-1}$. Infrared spectra were recorded on an FTIR Shimadzu 8400 spectrophotometer. The samples were prepared as neat thin films for liquids and KBr disks for solids. Proton NMR spectra were recorded in designated solvents on an Agilent Varian instrument. Proton spectra were recorded at 500 MHz and data were reported as chemical shift ($\delta$) in parts per million (ppm) downfield from tetramethylsilane (TMS), multiplicity, observed coupling constant ($J$) in Hertz (Hz), and proton assignment. Multiplicities were reported as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q) or multiplet (m). Broad signals were reported as br. C-13 NMR spectra were recorded at 125 MHz. Chemical shifts ($\delta$) were reported in ppm downfield from TMS. Mass spectra were recorded on High Resolution Time of Flight Mass Spectroscopy (HR-TOF-MS ES), Waters spectrometry.

2.2. Materials

4-hydroxybenzaldehyde 12, 3-methoxy-4-hydroxybenzaldehyde 13, imidazolidine-2,4-dione (hydantoin) 14 were obtained from commercial sources and used without purification. The remaining compounds 15-21 were synthesized.

2.3. Preparation of Hydantoin Derivatives as precursor

2.3.1. Synthesis 3-methylhydantoin 15.

Following the method of Orazi et al [22], Hydantoin 14 (10.0 g, 0.10 mol) and potassium hydroxide (5.6 g, 0.1 mol) in ethanol (150 mL) and the mixture was stirred until gas evolution ceased (*ca.*15 min). Dimethyl sulphate (10.5mL) was added drop by drop and stirring was continued and the
mixtures were refluxed for 5 h. The mixture was then cooled r.t and the precipitate was filtered and the filtrate was evaporated under reduced pressure till 20 mL and then extracted with ethyl acetate (6x20 mL), the combined ethyl acetate extracts were evaporated under reduced pressure and the residue were recrystallized from EtOAc to give 3-methylhydantoin 15 as white needles 44 %, m.p. 152 ºC (lit. [22] m.p. 155 ºC). Ultraviolet spectra have $\lambda_{\text{max}}$ (MeOH) ($\varepsilon_{\text{max}}$): 220.4 (2.5x10), nm. $^1$H NMR (DMSO-d$_6$, 500 MHz): $\delta$(ppm) 10.6 (s, 1H, N1); 3.84 (s, 2H, C5); 2.8 (s, 1H, C3); $^{13}$C-n.m.r (DMSO-d6)(125 MHz): $\delta$ (ppm) 158.3 (C2); 157.8 (C4); 46 (C5); 24 (C6).

2.3.2. Synthesis 1-acetylhydantoin 16.
Hydantoin 14 (1 g, 10 mmol) and acetic anhydride (1 mL, 10 mmol) and the mixture were stirred and refluxed for 5 h. The mixture was then cooled r.t and the precipitate was filtered and washed with ethanol. The residue was recrystallized from EtOAc to give 3-acetylhydantoin as white needles 44%, m.p. 152 ºC (lit. [22] m.p. 155 ºC). HR-TOF-MS ES $m/z$: 203.0461 (Found: C$_{10}$H$_8$N$_2$O$_3$). Ultraviolet spectra have $\lambda_{\text{max}}$ (MeOH) ($\varepsilon_{\text{max}}$): 203 (1.94 x 10$^5$) nm. $^1$H NMR (DMSO-d$_6$, 500MHZ): $\delta$(ppm) 11.1 (s, br, 1H, N3); 10.3 (s, br,1H, N1); 9.8 (s, 1H, OH); 7.4 (dd, 2H, C5' and C3'); 6.7 (dd, 2H; H6' and C2'); 6.3 (s, 1H, H7'). $^{13}$C-NMR (DMSO-d$_6$, 125 MHz): $\delta$ (ppm) 165.6 (C2); 158.0 (C4); 155.6 (C4'); 131.2 (C2' and C6'); 125.3 (C5); 123.8 (C1'); 115.7 (C3' and C5'); 109.3 (C7').

2.4. Preparative Experiments towards Benzalhydantoins 17-21

2.4.1. General procedure. Following the method of Hoffman and Wheeler [23], hydantoins 14-16 (50 mmol) and benzaldehydes 12-13 (50-150 mmol), anhydrous NaOAc (50-150 mmol), Ac$_2$O (50 µL, 0.49 µmol) were dissolved together in glacial AcOH (3 mL) at r.t. and the mixture warmed to reflux (140 ºC). Reactions were monitored by thin layer chromatography until all the aldehyde had reacted. Upon cooling, the mixture gave a precipitate that was collected and recrystallized.

2.4.2. Synthesis 5-(4-hydroxybenzylidene)imidazolidine-2,4-dione 17
4-Hydroxybenzaldehyde 12 (5.3069 g, 50 mmol), hydantoin 14 (5.0184 g, 50 mmol), NaOAc (10.3583 g, 75 mmol), and Ac$_2$O (20 µL, 0.20 µmol) in AcOH (3 mL), were refluxed for 8 h, a white solid that was recrystallized from EtOH to give 5-(4-hydroxybenzylidene)imidazolidine-2,4-dione 17 as white needles (0.1506 g, 11%) m.p. 290 ºC (lit.[21] m.p 311ºC). HR-TOF-MS ES $m/z$: 203.0461 (Found: C$_{10}$H$_8$N$_2$O$_3$). Ultraviolet spectra have $\lambda_{\text{max}}$ (MeOH) ($\varepsilon_{\text{max}}$): 203 (1.94 x 10$^5$) nm. $^1$H NMR (DMSO-d$_6$)(125 MHz): $\delta$(ppm) 11.1 (s, br, 1H, N3); 10.3 (s, br,1H, N1); 9.8 (s, 1H, OH); 7.4 (dd, 2H, C5' and C3'); 6.7 (dd, 2H; H6' and C2'); 6.3 (s, 1H, H7'). $^{13}$C-NMR (DMSO-d$_6$, 125 MHz): $\delta$ (ppm) 165.6 (C2); 158.0 (C4); 155.6 (C4'); 131.2 (C2' and C6'); 125.3 (C5); 123.8 (C1'); 115.7 (C3' and C5'); 109.3 (C7').

2.4.3. Synthesis 5-(4-hydroxy-3-methoxybenzylidene)imidazolidine-2,4-dione 18
Compound 3-Methoxy-4-hydroxybenzaldehyde 13 (1.0659 g, 7 mmol), hydantoin 14 (0.7005 g, 7 mmol), NaOAc (2.0007 g, 15mmol), and Ac$_2$O (20 µL, 0.20 µmol) in AcOH (3 mL), were refluxed for 8 h, a white solid that was recrystallized from EtOH to give 5-(4-hydroxy-3-methoxybenzylidene)imidazolidine-2,4-dione 18 as white needles (0.0731 g, 4 %) m.p. 245 ºC (lit.[21]m.p 271ºC). HR-TOF-MS ES $m/z$: 233.0556 (Found: C$_{11}$H$_9$N$_2$O$_3$). Ultraviolet spectra have $\lambda_{\text{max}}$ (MeOH) ($\varepsilon_{\text{max}}$): 345.4 (3.9 x 10$^5$) nm. (DMSO-d$_6$, 500MHZ): $\delta$(ppm) 11.4 (s, br, 1H, N3); 10.4 (s, br,1H, N1); 9.4 (s, 1H, OH); 7.0-7.1 (d, 1H, H5'); 6.7 (d, 1H, H6'); 6.3 (s, 1H, H7'); 3.8 (s, 3H, OCH$_3$). $^{13}$C-NMR (DMSO-d$_6$): $\delta$(ppm) 165.7(C4); 155.7 (C2); 147.7 (C3'); 147.6 (C4'); 125.4 (C5); 124.3 (C1'); 123.5 (C6'); 115.7 (C5'); 113.2 (C2'); 109.8 (C7'); 55.8 (OCH$_3$).

2.4.4. Synthesis 5-(4-hydroxybenzylidene)-1-acetylimidazolidine-2,4-dione 19.
Compound 4-hydroxybenzaldehyde 12 (1.0050 g, 7 mmol), acetyl hydantoin 16 (0.9942 g, 7 mmol), NaOAc (2.0293 g, 15mmol), and Ac$_2$O (10 µL, 0.10µmol) in AcOH (0.5 mL), were refluxed for 8 h, a yellow solid that was recrystallized from EtOH to give 5-(4-hydroxybenzylidene)-1-acetylimidazolidine-2,4-dione 19 as yellow needles (39 %) m.p. 165.7 ºC (lit.[21] m.p 163.0 ºC). HR-
TOF-MS ES m/z: 245 (Found: C_{12}H_{12}N_{2}O_{5}). Ultraviolet spectra have υ_{max} (MeOH) (ε_{max}): 334.8 (2.9 x 10^{3}) nm. H NMR (MeOH) (ε_{max}): 10.61 (s, 1H, H1); 7.13 (s, 1H, H2); 7.08-7.10 (d, J 8.0, H, H6); 6.78-6.80 (d, J 8.0, H, H5); 6.47 (s, H, H7'); 6.4 (s, H, H6'); 6.78-6.80 (d, J 8.0 Hz, C2' and C6'); 6.77-6.79 (d, 2H, J8.0 Hz, C2' and C6'); 6.40 (s, 1H, H7'); 2.27 (s, 3H, CH3).

2.4.5. Synthesis 5-(4'-hydroxybenzylidene)-3-methylimidazolidine-2,4-dione 20.

Compound 4-hydroxybenzaldehyde 12 (0.7179 g, 5 mmol), methyl hydantoin 15 (0.7102 g, 5 mmol), NaOAc (2.0293 g, 15 mmol), and Ac₂O (10 μL, 0.10 μmol) in AcOH (0.5 mL), were refluxed for 8 h, a white solid that was recrystallized from EtOH to give 5-(4'-hydroxybenzylidene)-3-methylimidazolidine-2,4-dione 20 as white needles (0.128 g, 12%) m.p. 302 °C (lit.[21] m.p. 320°C). HR-TOF-MS ES m/z: 217.0639 (Found: C_{11}H_{10}N_{2}O_{3}). Ultraviolet spectra have υ_{max} (MeOH) (ε_{max}): 340.4 (1.3 x 10^{3}) nm.

2.4.6. Synthesis 5-(4'-hydroxy-5'-methoxybenzylidene)-3-methylimidazolidine-2,4-dione 21

4-Hydroxy-5-methoxybenzaldehyde 13 (0.76 g, 5 mmol), methyl hydantoin 15 (0.57 g, 5 mmol), NaOAc (2.0293 g, 15 mmol), and Ac₂O (10 μL, 0.10 μmol) in AcOH (0.5 mL), were refluxed for 8 h, a white solid that was recrystallized from EtOH to give 5-(4'-hydroxy-5'-methoxybenzylidene)-3-methylimidazolidine-2,4-dione 21 as white needles (0.6267 g, 51%) m.p. 222.7-222.9 °C (lit.[21] m.p. 320°C). HR-TOF-MS ES m/z: 245 (Found: C_{12}H_{12}N_{2}O_{5}). Ultraviolet spectra have υ_{max} (MeOH) (ε_{max}): 346.5 (4.3 x 10^{3}) nm.

3. Result and Discussion

3.1. Synthesis of Benzalhydantoin Precursors

At the outset of this study, it was decided to adopt the potassium acetate modification [19-21] to synthesize a somewhat wider range of benzalhydantoins derived from hydantoin 14, N(3)-methylhydantoin 15 and N(1)-acetyl 16 to more thoroughly explore the influence of substituents on the reaction. It was also decided to restrict the study to commercially available aldehydes 12-13.

Hydantoin 14 is commercially available and very inexpensive, making it an attractive partner in this reaction. 3-Methylhydantoin 15 and 1-acetylhydantoin 16 are also well known [24], but not commercially available, and were synthesized. Structures were confirmed by comparative melting point and by infrared, H NMR and C-NMR spectroscopy.

In hydantoin chemistry, it is known that the N-3 position of hydantoin is reactive towards electrophiles, presumably due to the most acidic position between the two activating carbonyl groups [24]. According to Pinner [24], hydantoin could be alkylated in the N-3 position by treatment with alkyl halides in alkaline solution. The reaction usually proceeds smoothly, especially for methylation reactions, while substitution at N-1 could not be achieved through direct alkylation [25]. Treatment of hydantoin 14 with dimethyl sulphate in the presence of potassium hydroxide in ethanol gave 3-methylhydantoin 15 as white needles in 44% yield. High resolution mass spectrometric analysis supported the molecular formula C_{12}H_{12}N_{2}O_{5} and H and C-NMR spectroscopy confirmed the presence of the N3-methyl group. Similar treatment of hydantoin 14 with acetic anhydride gave the...
corresponding \(\text{N1-acetylhydantoin 16 as white needles in 64 \% yield, again confirmed by high}
resolution mass spectrometry and appropriate \(^1\text{H} \) and \(^{13}\text{C} \) NMR spectroscopic signals.

### 3.2. Synthesis of Benzalhydantoins

With the necessary partner reactants in hand, the relative ease with which they reacted together was examined through the preparation of the compounds 17-21. In this part of the research, reaction progress was monitored by comparative t.l.c. analysis. Products were isolated simply by allowing the reaction mixtures to cool, filtering off the desired crystalline material, and washing the crystals with cold ethanol. Examination of the isolated products by \(^1\text{H} \) NMR spectroscopy indicated that all the products were impure. Pleasingly, they comprised at least 90\% the derived benzalhydantoin. This was measured by integration of the olefinic methylenide signal compared with others. Notably, within the methylide region, in some cases \(E/Z \) isomers were evident. When this was observed, the highest chemical shift signal dominated and was assigned by literature analogy to the \(Z \) isomer while the minor singlet appeared at higher field and was assigned to the \(E\)-isomer.

Crude product yields were recorded at this stage, but in all cases the compounds were subsequently recrystallized to analytical purity for final characterization. The reaction times and yields, product melting points after recrystallization, and characteristic olefinic and \(N-H \) \(^1\text{H} \) NMR chemical shifts were collected in Table 1.

![Chemical diagram](image)

Table 1. An initial study of the condensation of hydantoins 14-16 with aromatic aldehydes 12-13 in acetic acid in the presence of NaOAc and Ac\(_2\)O.

| Entry | Structure No. | Reflux time (h) | Yields (%) | M.p. (°C) | \(\text{Chemical shift (ppm)}\) | \(\text{Integration ratio}\) |
|-------|---------------|----------------|------------|----------|-------------------------------|-----------------------------|
|       | \(17\) 11     | 8              | 11         | 290      | 10.3; 11.1                    | 6.3; \(>99:1\)            |
|       | 12            | 12             | 8          | 245      | 10.4; 11.4                    | 6.3; \(>99:1\)            |
|       | 13            | 12             | 39         | 165.7    | 11.1                          | 6.4; \(>99:1\)            |
|       | 14            | 12             | 12         | 302      | 10.5                          | 6.4; \(>99:1\)            |
|       | 15            | 12             | 51         | 222.8    | 10.6                          | 6.5; \(>99:1\)            |

All \(^1\text{H} \) NMR spectra were recorded in DMSO-\(d_6\) due to the low solubilities of the hydantoins in most non-polar solvents [26], including deuterchloroform. As mentioned earlier, geometrical isomers (\(Z \) and \(E \) isomers) were detected through the appearance of distinct signals for the olefinic proton on the exocyclic C=C double bond of the benzalhydantoins 17-21 [15].

Chui [13] reported that the olefinic proton signal, \(H1'\), in benzalhydantoins was diagnostic. It is deshielded in the \(Z \) form and the signal appears at \(\delta 6.40-7.00\) while in the \(E \) form the effect is less so and the signal appears at \(\delta 6.20-6.30\). The anisotropic effect exerted by the nearby C4 carbonyl group
in the former is believed to be responsible [15]. This literature information was used in the assignment of geometric isomers to compounds listed in Table 1.

In keeping with earlier findings, the results presented in Table 1 indicated that the yields were dependent on both the identity of the aldehyde and the substituent at position N1 and N3 of the hydantoin. Compounds 17-18 resulted in (E)-isomer with low yields. Electron donating group (-OMe) on the aldehyde retard the initial addition process, while the same groups speed the dehydration reaction up by stabilizing the incipient cation charge at the benzylic center [21]. The lower yields of 17-18 may be an indication that the first step, addition, was rate limiting. Compounds 19-21 resulted in (Z)-isomer with varyingly higher yield, the product yields were dependent upon the type of hydantoin substituent at N1 or N3 position. However, an anomaly was observed with compound 21 where weak electron withdrawing (-OH) at R^4, strong electron donating (-OMe) at R^5 and substituent (-Me) at R^2 positions resulted in the highest yield.

A catalytic amount of acetic anhydride was used to increase the addition process of reaction to yield the intermediate alcohol, then elimination reaction was occurred to give benzalhydantoin.

4. Summary
This initial exploratory study had confirmed a number of claims from earlier studies in ours research group and had provided first-hand knowledge of the physical and spectroscopic properties of the target compounds of later investigations. It had highlighted issues of product stereochemistry and possible selective crystallization during product isolation that would need to be borne in mind in later studies.

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