Latent Inhibitors. Part 6.[1] Inhibition of Dihydro-orotate Dehydrogenase by Substituted 5-Benzylhydantoins

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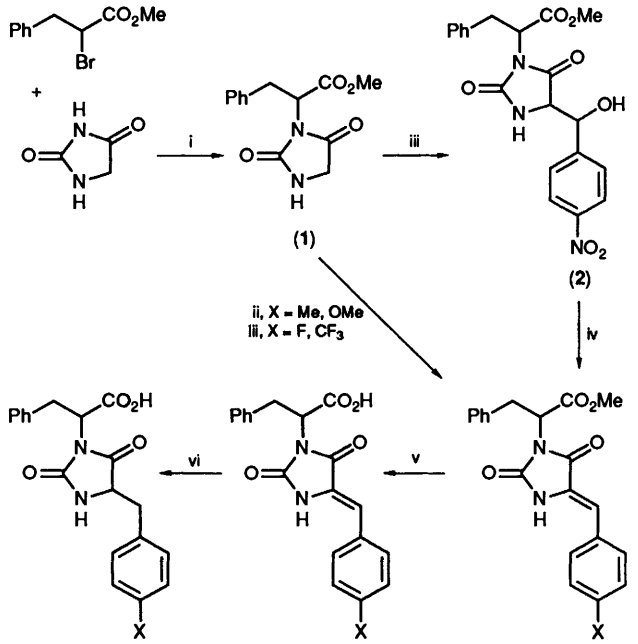
A series of substituted 5-benzyl-3-(1-carboxy-2-phenylethyl)hydantoins was prepared by condensation of aromatic aldehydes with the corresponding 5-unsubstituted hydantoin followed by reduction of the intermediate benzylidene derivative. The compounds were assessed as inhibitors of dihydro-orotate dehydrogenase from *Clostridium oroticum*. It was found that hydrophobic and electron-donating substituents in the phenyl ring of the benzyl group favoured binding and irreversible inhibition. The results for the series were correlated with standard substituent parameters from which a mechanism of inhibition was derived. This involved rapid deprotonation of the hydantoin at C-5 followed by rate-determining removal of hydride, or its equivalent.

Dihydro-orotate dehydrogenase (DHODase) is an enzyme in the biosynthetic pathway to pyrimidines and has been recognised as a potential candidate for inhibition in chemotherapy.[2] We have shown that dihydro-orotate dehydrogenase from *Clostridium oroticum* was inhibited by the parent member of the series, 5-benzyl-3-(1-carboxy-2-phenylethyl)hydantoin† **5a** in a time-dependent and irreversible manner.[3] On the basis of the intrinsic chemical properties of the inhibitor and a computer-generated comparison of the structures of substrate and inhibitor, we proposed a mechanism in which the 5-benzyl group was oxidised by the enzyme to a benzylidene group; the product would have structure **4a** and this would give rise to an α,β-unsaturated carbonyl compound to which the enzyme might be expected to add. The present work was designed to probe further the mechanism of this unexpected reaction and to obtain additional related compounds with potentially improved biological activity.

Synthesis.—The previous synthesis[3] starting from (*S*)-phenylalanine was restricted to the parent compound **5a** and it afforded a single enantiomeric product with the *S*-configuration at the two chiral centres. We also found that the *R,R*-isomer was an inhibitor of comparable potency to its enantiomer. The syntheses now investigated to prepare substituted analogues of compound **5a** were chosen to maximise flexibility in incorporating electron-withdrawing or electron-donating substituents into the benzyl group; stereospecific syntheses were not investigated at this stage. The most successful route was to alkylate hydantoin (imidazolidine-2,4-dione) with methyl 1-bromo-2-phenylpropanoate under carefully controlled conditions below 50 °C for several days (Scheme 1), leading to the intermediate **1**. If higher temperatures were used a rearrangement of compound (**1**) occurred to afford 5-benzyl-3-(methoxycarbonylmethyl)hydantoin **6**; a ring-opening–ring-closing mechanism is probable for this reaction (Scheme 2). Under all conditions investigated, some elimination to afford methyl cinnamate took place. The 3-alkyl 5-unsubstituted hydantoin **1** was resistant to alkylation by benzylic bromides but underwent condensation with aromatic aldehydes in the presence of a weak base to give the arylidenehydantoins **4** *via* the corresponding esters **3**. 4-Nitrobenzaldehyde underwent addition under these conditions to give the 5-hydroxybenzylhydantoin **2**, which was subsequently dehydrated, and the ester group hydrolysed, with hydrobromic acid to give the arylidenehydantoin **4b**.

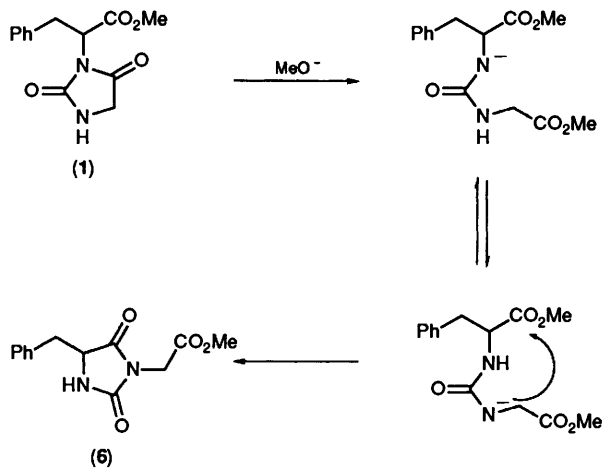


Scheme 1. *Reagents and conditions:* i, NaOMe, MeOH, 10 days, 50 °C; ii, piperidine; iii, Me_3N; iv, aq. HBr, heat; v, aq. HCl; vi, H_2, Pd–C, MeOH.

The arylidenehydantoins **4** were shown to have the *Z*-configuration by comparison of the ^1H and ^{13}C NMR spectra with those[4] of benzylidenehydantoins, the structure of one of which had been established by X-ray crystallography.[5]

Reduction of the double bond was achieved using hydrogen and palladium on charcoal in methanolic solution, giving samples of the required 5-arylmethylhydantoins **5c–g** as

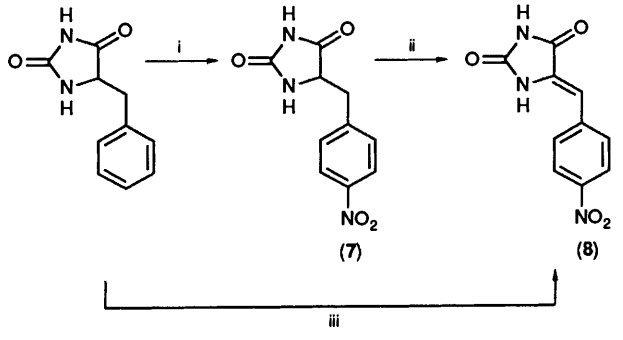
† 2-(4-Benzyl-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanoic acid.



Scheme 2.

mixtures of stereoisomers. These mixtures were separated by fractional crystallisation in some cases into samples of the two racemates. In the case of the nitro compound **4b** transfer hydrogenation[6] using sodium borohydride as hydrogen source and palladium on charcoal led to the 4-aminoarylidenehydantoin **4g**.

In the course of these syntheses, some unexpected reactions were discovered in addition to the rearrangement (Scheme 2) mentioned earlier. 5-Benzylhydantoin was nitrated with standard nitrating mixture to afford the 5-(4-nitrobenzyl)hydantoin **7**. Many attempts were made to alkylate this compound on N-3 under basic conditions. The anion was very unreactive and, invariably, dehydrogenation to give the corresponding 5-(4-nitrobenzylidene)hydantoin **8**, a strongly yellow coloured compound, occurred on admission of air (Scheme 3). The same compound was obtained by nitration of 5-benzylhydantoin under more vigorous work-up conditions. This reaction once again points to the ready oxidation of 5-arylmethylhydantoins which is a feature of the proposed mechanism of inhibition.



Scheme 3. *Reagents and conditions:* i, H_2SO_4/HNO_3, −15 °C; ii, MeO^-, air; iii, H_2SO_4/HNO_3, −15 °C, different work-up.

Inhibition of DHODase.—The inhibition of DHODase was investigated using the methods previously reported[3] with an assay coupled to the production of NAD; the decrease in absorbance at 340 nm was used to determine the rate of reaction. This method was suitable for almost all of the compounds prepared. Only compounds **4b**, **f** and **g** which absorbed too strongly in this region of the spectrum, were not assessed in detail. Two assay protocols were used. As a preliminary screen, DHODase, orotate, and NADH were incubated together with inhibitor in a UV cell for a series of concentrations. A study of the time-course of reduction of orotate by DHODase in the presence of the benzylhydantoins **5c–g** and the benzylidenehydantoins **4c–e** showed that the reaction failed to reach completion and the compounds thus behaved like the parent 5-benzylhydantoin **5a** described in our earlier paper.[3] Subsequently, compounds were assayed by incubating DHODase and inhibitor together, removing samples at convenient time intervals, and initiating reaction by the addition of substrate followed by NADH. From the latter measurements, K_i-values for a binary complex were obtained together with the k_i-value for the inhibition of the enzyme. The results are shown in Table 1. It can be seen that variation of the two kinetic parameters does not occur in parallel. For the arylmethylhydantoins **5**, the most rapid inhibition was attained with the most electron-donating substituent, amino **5g**, which was approximately 12-fold better than for the case of the slowest reacting compound, the trifluoromethyl **5c**. The strongest binding was obtained with the methyl substitutent **5e**, which was in turn *ca.* 20-fold better than the weakest binding, found for the parent compound **5a**. These ranges are not large and suggest that DHODase is able to accept a wide range of substituents into a hydrophobic pocket close to the active site. The arylidenehydantoins for which data could be obtained were all found to bind substantially more strongly than the corresponding reduced compounds and to inhibit the enzyme more rapidly, thus suggesting that nucleophilic attack by an enzyme nucleophile on the α,β-unsaturated system is not rate determining in inhibition. These general observations were refined using multiple regression analysis to provide a basis for deducing more precisely the mechanism of inhibition.

Mechanism of Inhibition.—The fact that significant substituent effects were observed on the rate of inhibition when the benzyl group contained substituents is evidence supporting our initial proposal for the mechanism of action of these hydantoin-based inhibitors, namely oxidation at the benzylic site leading to a benzylidenehydantoin. It has been argued that DHODase oxidises its normal substrate through initial deprotonation at C-5, followed by removal of hydride or its equivalent from C-6.[7] A similar mechanism is likely for the inhibition reaction observed here.

The regression analysis was carried out with a wide range of standard substituent parameters for the benzylhydantoins **5a** and **c–g**. The steric bulk of the substituents was represented by E_s,[8] the hydrophobic binding potential by the molar refractivity MR,[9] and the electronic effects by standard Hammett parameters σ and σ⁺.[10] MR, instead of Hansch's π parameter, was chosen to represent hydrophobic effects because the latter refers to partition equilibria and here we are concerned with a direct intermolecular interaction better represented by MR. For the rate constant of inhibition, k_i, the best correlation (r 0.9839) was achieved with a combination of σ and σ⁺, suggesting that a significant positive charge is generated at the benzylic position in the rate-determining step but that it is less than the charge on the intermediate in the standard reference reaction for the determination of σ⁺ constants, the S_N1 substitution of cumyl halides. The regression equation was equation (1).

$$\ln [k_{i(X)}/k_{i(H)}] = 0.605 - 0.19E_s - 0.328MR + 1.47\sigma - 2.58\sigma^+ \quad (1)$$

A similar analysis of the binding of the benzylhydantoins was carried out and a regression equation that gave greater weight to the steric parameter was obtained (r 0.9965), equation (2).

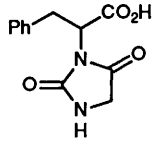
$$\ln [K_{i(X)}/K_{i(H)}] = -1.75 + 1.56E_s - 0.258MR + 13.5\sigma - 7.92\sigma^+ \quad (2)$$

Table 1. Inhibition of DHODase by 5-benzylhydantoins **5** and 5-benzylidenehydantoins **4**.

Compound	X	$10^5 k_i$* (s^{-1})	$10^3 k_i$* ($mol\ l^{-1}$)
5a	H	8.79	2.73
5c[a]	CF_3	2.23	0.52
5d	F	13.9	4.43
5e[b]	Me	4.58	0.13
5f	OMe	5.41	2.28
5g	NH_2	25.8	1.34
4c	CF_3	4.11	0.046
4d	F	9.91	0.512
4e	Me	4.19	0.016

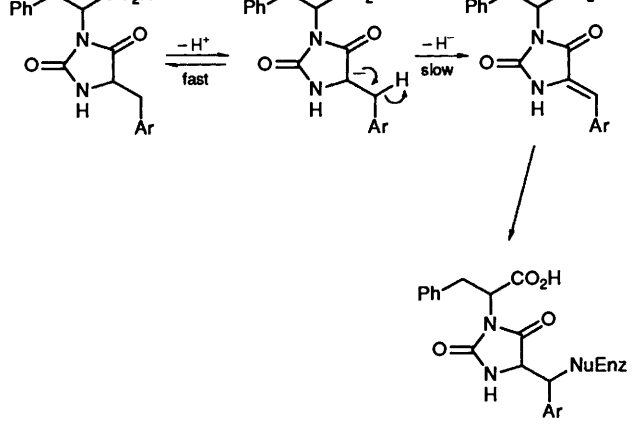
* Figures relate to the binary complex of enzyme plus inhibitor. [a] Data are for the racemate, m.p. 187–188 °C. [b] Data are for the racemate, m.p. 196–198 °C.

In both of these equations, the dependence upon *MR* indicates a significant contribution of hydrophobic binding in the action of the inhibitors. The importance of this structural feature was emphasised by the observation that a hydantoin lacking the 5-benzyl substituent but bearing the 3-alkanoate substituent, compound **9**, had no detectable action as an inhibitor of DHODase. This result is also important in helping us to understand the inhibitory actions of cyclopropane-



(9)

containing analogues of orotic acid and the hydantoins discussed in the following paper.[11] We interpret these results in terms of rapid deprotonation at C-5 followed by rate-determining hydride loss and more rapid addition of the enzymic nucleophile (Scheme 4). The dependence upon σ-values implies that a degree of positive charge is induced on the benzylic position in the rate-determining step, suggesting that carbon-to-hydrogen bond cleavage is well advanced before double-bond formation occurs; as argued above, a more fully formed cation is unlikely.



Scheme 4.

Experimental

^1H NMR spectra were recorded on Perkin-Elmer R32 (90 MHz) or Bruker WH-250 (250 MHz) spectrometers, and ^{13}C NMR spectra on the Bruker instrument (62.9 MHz). IR spectra were determined using a Perkin-Elmer 257 spectrometer, and UV spectra on a Philips PU8800 spectrophotomer. HPLC was carried out on ODS reverse-phase columns with methanol–water (60:40) as the mobile phase and a flow rate of 40 ml h^{-1}. The detector used was a Cecil Instruments CE 2012 variable-wavelength spectrophotometer. Non-systematic nomenclature is used for compounds **1**–**9**.

3-(1-*Methoxycarbonyl*-2-*phenylethyl*)*hydantoin* **1**.—A solution of sodium methoxide was prepared by reaction of sodium metal (6.9 g, 0.3 mmol) with methanol (distilled from CaH_2; 200 ml). To this solution was added hydantoin (27.7 g, 0.277 mmol) and the mixture was refluxed overnight. The temperature of the mixture was reduced to 50 °C before methyl 1-bromo-2-phenylpropanoate[12] (67.4 g, 0.277 mol) was added. The solution was stirred for 10 days at 50 °C, then cooled before hydrochloric acid (10M; 20 ml) was added. Three crops of precipitate were collected in succession by concentration of the solution. The third crop was unchanged hydantoin (16.92 g, 51% recovery). The second crop was washed successively with carbon tetrachloride, chloroform, aq. sodium hydrogen carbonate, water, hydrochloric acid, and water to yield the by-product 5-*benzyl*-3-(*methoxycarbonylmethyl*)*hydantoin* **6** (3.68 g. 6%), m.p. 142–144 °C (Found: C, 59.4; H, 5.2; N, 10.7. $C_{13}H_{14}N_2O_4$ requires C, 59.5; H, 5.4; N, 10.7%); λ_{max}(MeOH–water 60:40) 253, 258, 264 and 268 nm; ν_{max}(KCl) 3240 (NH), 3110, 3035, 2960 and 2930 (CH), 1775, 1735 and 1725 (C=O), and 1600 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.90 (1 H, dd, *J* 5.2 and 14.0 Hz, HC*H*), 3.03 (1 H, dd, *J* 5.2 and 14.0 Hz, HC*H*), 3.60 (3 H, s, Me), 4.03 (2 H, s, NCH_2), 4.50 (1 H, td, *J* 1.0 and 14.0 Hz, CH), 7.16–7.31 (5 H, m, Ph) and 8.42 (1 H, s, NH); δ_C[62.9 MHz; $(CD_3)_2SO$] 36.6 (t), 38.6 (t), 52.0 (q), 57.3 (d), 126.5 (d), 127.9 (d), 129.3 (d), 135.4 (s), 155.4 (s), 167.5 (s) and 172.8 (s); HPLC t_R 7.4 min.

The first precipitate was washed successively with aq. sodium hydrogen carbonate, water, hydrochloric acid, water, carbon tetrachloride, and chloroform to yield 3-(1-*methoxycarbonyl*-2-*phenylethyl*)*hydantoin* **1** (24.38 g, 34%), m.p. 191–192 °C (Found: C, 59.3; H, 5.2; N, 10.7. $C_{13}H_{14}N_2O_4$ requires C, 59.5; H, 5.4; N, 10.7%); λ_{max}(MeOH–water 60:40) 253, 258, 264 and 273 nm; ν_{max}(KCl) 3260 (NH), 3110, 3035, 2960 and 2935 (CH), 1780, 1735 and 1700 (C=O) and 1600 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_{32}SO$] 3.2 (1 H, dd, *J* 10.8 and 14.1 Hz, HC*H*), 3.3 (1 H, dd, *J* 5.1 and 14.0 Hz, HC*H*), 3.7 (3 H, s, Me), 3.83 (2 H, q, *J* 17 Hz, CH_2), 4.9 (1 H, dd, *J* 5.2 and 10.8 Hz, CH), 7.1–7.3 (5 H, m, Ph) and 8.1 (1 H, s, NH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.3 (t), 38.8 (d), 45.4 (t), 52.4 (q), 126.4 (d), 128.1 (d), 128.7 (d), 136.8 (s), 156.2 (s), 168.9 (s) and 171.1 (s); HPLC t_R 8.5 min.

5-*Benzyl*-3-(*methoxycarbonylmethyl*)*hydantoin* **6**.—3-(1-Methoxycarbonyl-2-phenylethyl)hydantoin **1** (1.31 g, 5 mmol) was placed in a solution of sodium methoxide [prepared by reaction of sodium metal (0.12 g, 5 mmol) with methanol (distilled from CaH_2, 30 ml]. The mixture was refluxed for 3 days, then cooled, and acidified with hydrochloric acid (10 M; 0.5 ml). The crystals which formed were collected, washed with water, and dried to yield 5-benzyl-3-(methoxycarbonylmethyl)hydantoin (**6**) (0.69 g, 53%), m.p. 144–146 °C, identical with the by-product described above.

5-(α-*Hydroxy*-4-*nitrobenzyl*)-3-(1-*methoxycarbonyl*-2-*phenylethyl*)*hydantoin* **2**.—3-(1-Methoxycarbonyl-2-phenylethyl)hydantoin **1** (17.03 g, 65 mmol), trimethylamine (15% in water; 15.5 g, 65 mmol), water (50 ml), methanol (100 ml), and 4-nitrobenzaldehyde (9.82 g, 65 mmol) were refluxed for 6 h, then

cooled to room temperature and stirred overnight. An orange precipitate was collected and dried to yield 5-(α-*hydroxy*-4-*nitrobenzyl*)-3-(1-*methoxycarbonyl*-2-*phenylethyl*)*hydantoin* **2** (20.21 g, 75%), m.p. 228–231 °C (Found: C, 58.2; H, 4.6; N, 10.3. $C_{20}H_{19}N_3O_7$ requires C, 58.1; H, 4.6; N, 10.2%); λ_{max}(MeOH–water 60:40) 273 and 325 nm; ν_{max}(KCl) 3280 (NH), 3070, 3035, 2945 and 2915 (CH), 1745, 1730 and 1715 (C=O) and 1600 cm^{-1} (arom. C=C); δ_H[90 MHz; $(CD_2)_3SO$] 3.15–3.50 (2 H, m, CH_2), 3.72 (3 H, s, Me), 4.37 (1 H, d, *J* 5 Hz, CH), 5.13 (1 H, dd, *J* 5 and 11 Hz, CH_2CH), 5.95 [1 H, m, CH(OH)], 6.5 (1 H, s, OH), 7.23 (5 H, s, Ph), 7.55 (1 N, s, NH), 7.71 (2 H, d, *J* 9 Hz, 2 × ArH) and 8.24 (2 H, d, *J* 9 Hz, 2 × ArH); HPLC t_R 4.55 min.

(Z)-3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*nitrobenzylidene*)-*hydantoin* **4b**.—5-(α-Hydroxy-4-nitrobenzyl)-3-(1-methoxycarbonyl-2-phenylethyl)hydantoin **2** (20.1 g, 48 mmol) was added to hydrobromic acid (48% in water; 100 ml) and the mixture was refluxed for 6 h before being cooled and stirred at room temperature overnight. The solvent was then evaporated off to leave a brown solid, which was washed successively with water (6 × 20 ml) and chloroform (6 × 10 ml). The yellow solid was recrystallised from methanol to yield (Z)-3-(1-*carboxy*-2-*phenylethyl*)-5-(4-*nitrobenzylidene*)*hydantoin* **4b** (11.24 g, 71%), m.p. 227–229 °C (Found: C, 59.3; H, 3.6; N, 10.6. $C_{19}H_{15}N_3O_6$ requires C, 59.8; H, 3.9; N, 11.0%); λ_{max}(MeOH–water 60:40) 273 and 355 nm; ν_{max}(KCl) 3370 (NH), 3065, 3025 and 2915 (CH), 1755, 1730 and 1715 (C=O), 1660 (C=C) and 1590 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 3.28–3.48 (2 H, m, CH_2), 5.02 (1 H, dd, *J* 5.4 and 11.2 Hz, CH_2CH), 6.58 (1 H, s, =CH), 7.15–7.26 (5 H, m, Ph), 7.82 (2 H, d, *J* 8.8 Hz, 2 × ArH), 8.18 (2 H, d, *J* 9.0 Hz, 2 × ArH) and 11.2 (1 H, s, NH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.4 (t), 53.4 (d), 76.1 (s), 107.3 (d), 123.7 (d), 126.6 (d), 128.3 (d), 128.7 (d), 130.4 (d), 137.1 (s), 139.3 (s), 146.5 (s), 154.3 (s), 163.1 (s) and 169.6 (s); HPLC t_R 3.1 min.

(Z)-3-(1-*Methoxycarbonyl*-2-*phenylethyl*)-5-(4-*trifluoromethylbenzylidene*)*hydantoin* **3c**.—3-(1)-Methoxycarbonyl-2-phenylethyl)hydantoin **1** (13.1 g, 50 mmol), trimethylamine (25–30% in water; 14 ml, 50 mmol), water (60 ml), methanol 80 ml), and α,α,α-trifluoro-*p*-tolualdehyde (8.71 g, 50 mmol) were refluxed for 22 h and the mixture was then stirred at room temperature for 24 h before being evaporated to dryness. The residue was recrystallised from methanol to yield (Z)-3-(1-*methoxycarbonyl*-2-*phenylethyl*)-5-(4-*trifluoromethylbenzylidene*)*hydantoin* **3c** (6.51 g, 31%), m.p. 158–160 °C (Found: C, 60.5; H, 4.1; N, 6.7. $C_{21}H_{17}F_3N_2O_4$ requires C, 60.3; H, 4.1; N, 6.7%); λ_{max}(MeOH–water 60:40) 315 nm; ν_{max}(KCl) 3380 and 3250 (NH), 3030, 2960 and 2930 (CH), 1765, 1740 and 1715 (C=O), 1660 (C=C) and 1610 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 3.31 (1 H, dd, *J* 11.3 and 13.8 Hz, HCH), 3.44 (1 H, dd, *J* 5.0 and 14 Hz, HCH), 3.71 (3 H, s, Me), 5.15 (1 H, dd, *J* 5.0 and 11.3 Hz, CH_2CH), 6.56 (1 H, s, CH), 7.15–7.27 (5 H, m, Ph), 7.72 (2 H, d, *J* 8.5 Hz, 2 × ArH), 7.79 (2 H, d, *J* 8.5 Hz, 2 × ArH) and 11.08 (1 H, s, NH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.4 (t), 52.7 (d), 53.0 (q), 108.4 (d), 121.8 (s), 125.3 (d), 126.1 (d), 127.3 (d), 128.1 (d), 128.7 (d), 130.0 (s), 136.4 (s), 136.6 (s), 154.0 (s), 163.0 (s), 168.7 (s) and 169.6 (s); HPLC t_R 3.6 min.

(Z)-3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*trifluoromethylbenzylidene*)*hydantoin* **4c**.—(*Z*)-3-(1-Methoxycarbonyl-2-phenylethyl)-5-(4-trifluoromethylbenzylidene)hydantoin **3c** (6.27 g, 15 mmol) was added to hydrobromic acid (48% in water; 40 ml) and the mixture was refluxed for 47 h, then filtered, and the solid was washed with water (2 × 10 ml) and dried *in vacuo* to yield (Z)-3-(1-*carboxy*-2-*phenylethyl*)-5-(4-*trifluoromethylbenzylidene*)*hydantoin* **4c** (6.05 g, 100%), m.p. 227–229 °C (Found: C, 59.7; H, 3.7; N, 6.9. $C_{20}H_{15}F_3N_2O_4$ requires C, 59.4; H, 3.7; N, 6.9%); λ_{max}(MeOH–water 60:40) 318 nm; ν_{max}(KCl) 3230 (NH), 3130, 3030 and 2915 (CH), 1760, 1735 and 1720 (C=O), 1665 (C=C) and 1615 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 3.30–3.45 (2 H, m, CH_2), 5.00 (1 H, dd, *J* 5.4 and 11.2 Hz, CH_2CH), 6.54 (1 H, s, CH), 7.15–7.26 (5 H, m, Ph), 7.71 (2 H, d, *J* 8.5 Hz, 2 × ArH), 7.79 (2 H, d, *J* 8.5 Hz, 2 × ArH), 11.4 (1 H, s, NH) and 13.4–13.6 (1 H, br s, OH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.4 (t), 53.3 (d), 108.0 (d), 125.3 (s), 126.5 (d), 127.5 (d), 128.0 (d), 128.3 (d), 128.6 (d), 129.9 (s), 136.5 (s), 137.1 (s), 154.2 (s), 163.2 (s), 168.7 (s) and 169.6 (s); HPLC t_R 3.8 min

(Z)-3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*fluorobenzylidene*)-*hydantoin* **4d**.—3-(1-Methoxycarbonyl-2-phenylethyl)hydantoin **1** (10.48 g, 40 mmol), trimethylamine (45% in water; 5.4 g, 40 mmol), water (30 ml), methanol (60 ml), and 4-fluorobenzaldehyde (4.96 g, 40 mmol) were refluxed for 24 h and then stirred at room temperature for 17 h. The solution was evaporated to leave a yellow residue. To this was added hydrobromic acid (48% in water; 100 ml) and the mixture was refluxed for 24 h, then stirred at room temperature for 17 h. The solution was evaporated to leave a yellow solid, which was taken up in ethyl acetate and dried (Na_2SO_4) before being recrystallised from ethanol to yield (Z)-3-(1-*carboxy*-2-*phenylethyl*)-5-(4-*fluorobenzylidene*)*hydantoin* (3.42 g, 24%), m.p. 242–244 °C (Found: C, 64.1; H, 4.2; F, 5.3; N, 7.8. $C_{19}H_{15}FN_2O_4$ requires C, 64.4; H, 4.2; F, 5.4; N, 7.9%); λ_{max}(MeOH–water 60:40) 321 nm; ν_{max}(KCl) 3220 (NH), 3090, 3025, 2960 and 2910 (CH), 1760, 1725 and 1715 (C=O), 1665 (C=C) and 1600 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 3.36 (2 H, m, CH_2), 4.95 (1 H, dd, *J* 5.5 and 10.2 Hz, CH_2CH), 6.48 (1 H, s, =CH), 7.15–7.25 (5 H, m, Ph), 7.63 (2 H, d, *J* 8.3 Hz, 2 × ArH), 7.66 (2 H, d, *J* 8.3 Hz, 2 × ArH) and 10.8 (1 H, s, NH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.4 (t), 53.2 (d), 109.1 (d), 115.6 (d), 125.4 (s), 126.5 (d), 128.2 (d), 128.6 (d), 129.0 (s), 131.7 (d), 137.2 (s), 154 (s), 160.0 (s), 163.4 (s) and 169.7 (s); HPLC t_R 3.2 min.

(Z)-3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*methylbenzylidene*)-*hydantoin* **4e**.—3-(1-Methoxycarbonyl-2-phenylethyl)hydantoin **1** (13.1 g, 50 mmol) and piperidine (distilled from CaH_2; 10 ml, 100 mmol) were placed in a flask with 4-tolualdehyde (6.0 g, 50 mmol) and the mixture was heated slowly to 140 °C during 3 h. The mixture was heated at 140 °C for 2 h before being cooled to 60 °C and treated with water (200 ml). Hydrochloric acid (10 M; 12 ml) was added to acidify the mixture before it was left overnight. A white solid was filtered off, washed with water (6 × 50 ml), and then recrystallised from methanol to yield (Z)-3-(1-*carboxy*-2-*phenylethyl*)-5-(4-*methylbenzylidene*)*hydantoin* **4e** (4.17 g, 21%), m.p. 243–245 °C (Found: C, 68.5; H, 5.0; N, 8.0. $C_{20}H_{18}N_2O_4$ requires C, 68.6; H, 5.1; N, 8.0%); λ_{max}(MeOH–water 60:40) 322 nm; ν_{max}(KCl) 3240 (NH), 3030, 2970 and 2920 (CH), 1755, 1735 and 1715 (C=O), 1660 (C=C) and 1605 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.30 (3 H, s, Me), 3.30 (2 H, m, CH_2), 4.96 (1 H, dd, *J* 5.6 and 10.8 Hz, CH_2CH), 6.43 (1 H, s, =CH), 7.08–7.24 (5 H, m, Ph), 7.24 (2 H, d, *J* 8.05 Hz, 2 × ArH), 7.49 (2 H, d, *J* 8.05 Hz, 2 × ArH), 10.72 (1 H, s, NH) and 13.2–13.4 (1 H, br s, OH); δ_C[62.9 MHz; $(CD_3)_2SO$] 20.8 (q), 33.4 (t), 53.1 (d), 110.3 (d), 124.8 (s), 126.5 (d), 128.2 (d), 128.6 (d), 129.3 (d), 129.5 (d), 129.6 (s), 137.2 (s), 138.6 (s), 154.2 (s), 163.5 (s) and 169.8 (s); HPLC t_R 3.3 min.

(Z)-3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*methoxybenzylidene*)-*hydantoin* **4f**.—4-Methoxybenzaldehyde (8.17 g, 60 mmol) and piperidine (distilled from CaH_2; 25 ml, 0.25 mol) were placed in a flask containing 3-(1-methoxycarbonyl-2-phenylethyl)-hydantoin (**1**) (15.72 g, 60 mmol). The mixture was heated slowly for 3 h from 15 to 130 °C and was then kept at that temperature for 2 h before being cooled to 60 °C and placed

in water (200 ml). The mixture was cooled, acidified with hydrochloric acid (10 M; 30 ml), and left overnight. A brown oil settled out and was left behind by decantation of the light green liquid. This liquid was evaporated and ethyl acetate was added to the residue. A white solid was filtered off. The remaining mixture was filtered to give more white solid. The second precipitate was recrystallised from chloroform to yield the by-product 5-*benzyl*-3-(*piperidinocarbonylmethyl*)*hydantoin* (0.47 g, 3%), m.p. 205–208 °C (Found: C, 64.3; H, 7.0; N, 13.0. $C_{17}H_{21}N_3O_3$ requires C, 64.7; H, 6.7; N, 13.3%); λ_{max}(MeOH–water 60:40) 252, 258, 264, 268 and 337 nm; ν_{max}(KCl) 3240 (NH), 3110, 3025, 2945 and 2910 (CH), 1750, 1715 and 1700 (C=O) and 1600 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 1.50 (10 H, m, 5 × CH_2), 2.86 (1 H, dd, *J* 6.6 and 14.0 Hz, HC*H*), 3.03 (1 H, dd, *J* 4.8 and 14.0 Hz, HC*H*), 4.07 (2 H, s, NCH_2), 4.43 (1 H, dd, *J* 4.8 and 6.5 Hz, CH), 7.23 (5 H, m, Ph) and 8.03 (1 H, s, NH); HPLC t_R 2.95 min (100%).

The first precipitate was also recrystallised from methanol to yield (*Z*)-3-(1-*carboxy*-2-*phenylethyl*)-5-(4-*methoxybenzylidene*)*hydantoin* **4f** (0.96 g, 5%), m.p. 245–248 °C (Found: C, 65.2; H, 4.7; N, 7.5. $C_{20}H_{18}N_2O_5$ requires C, 65.6; H, 4.9; N, 7.6%); λ_{max}(MeOH–water 60:40) 338 nm; ν_{max}(KCl) 3240 (NH), 3060, 3030, 3005, 2930, 2920 and 2835 (CH), 1745, 1730 and 1710 (C=O), 1655 (C=C) and 1600 cm^{-1} (arom C=C); δ_H[250 MHz; $(CD_3)_2SO$] 3.39 (2 H, d, CH_2), 3.77 (3 H, s, Me), 4.85 (1 H, dd, *J* 6.2 and 10.1 Hz, CH_2CH), 6.41 (1 H, s, =CH), 6.94 (2 H, d, *J* 8.8 Hz, 2 × ArH), 7.18 (5 H, m, Ph), 7.56 (2 H, d, *J* 8.5 Hz, 2 × ArH), 10.64 (1 H, s, NH) and 13.27 (1 H, s, OH); HPLC t_R 2.75 min.

(*Z*)-5-(4-*Aminobenzylidene*)-3-(1-*carboxy*-2-*phenylethyl*)-*hydantoin* **4g**.—(*Z*)-3-(1-Carboxy-2-phenylethyl)-5-(4-nitrobenzylidene)hydantoin **4b** (1.143 g, 3 mmol) was added to a solution of sodium hydroxide (1.68 g, 30 mmol) in water (20 ml). A suspension of palladium on charcoal (10%; 20 mg, 0.2 mmol Pd) in water (10 ml) containing sodium borohydride (0.24 g, 6 mmol) under nitrogen was then treated with the first solution dropwise during 5 min. The mixture was stirred for 30 min at room temperature. Formic acid was added to destroy the sodium borohydride, and the aq. solution was repeatedly extracted with ethyl acetate (15 × 100 ml). The extracts were dried (Na_2SO_4) and evaporated to yield (*Z*)-5-(4-*aminobenzylidene*)-3-(1-*carboxy*-2-*phenylethyl*)*hydantoin* **4g** (1.00 g, 95%), m.p. > 320 °C (decomp. 220 °C) (Found: C, 64.7; H, 4.7; N, 11.9. $C_{19}H_{17}N_3O_4$ requires C, 65.0; H, 4.8; N, 12.0%); λ_{max}(MeOH–water 60:40) 363 nm; ν_{max}(KCl) 3550 (OH), 3380 and 3270 (NH), 3080, 3060, 3030, 2960 and 2930 (CH), 2855, 2790 and 2690 (NH_2), 1735, 1690 and 1590 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 3.43 (2 H, m, CH_2), 4.36 (1 H, dd, *J* 5.3 and 11.0 Hz, CH_2CH), 5.57 (2 H, s, NH_2), 6.19 (1 H, s, =CH), 6.52 (2 H, d, *J* 8.6 Hz, 2 × ArH), 7.26 (2 H, d, *J* 8.6 Hz, 2 × ArH), 7.08–7.22 (5 H, m, Ph) and 10.05 (1 H, s, NH); δ_C[62.9 MHz; $(CD_3)_2SO$] 34.4 (t), 57.4 (d), 110.0 (d), 113.7 (s), 120.3 (d), 122.4 (d), 125.5 (d), 128.0 (d), 128.3 (d), 130.8 (s), 140.7 (s), 149.3 (s), 149.5 (s), 155.5 (s) and 164.6 (s); HPLC t_R 2.85 min.

When a solution of (*Z*)-3-(1-carboxy-2-phenylethyl)-5-(4-nitrobenzylidene)hydantoin (**4b**) (38.1 mg, 0.1 mmol) in ethanol (60 ml) was stirred with tris(triphenylphosphine)rhodium chloride (3.272 mg, 0.0035 mmol) under hydrogen for 4 weeks, partial isomerisation to the (*E*)-isomer occurred: δ_H[250 MHz; $(CD_3)_2SO$] 3.35–3.39 (2 H, m, CH_2), 4.95 (1 H, dd, *J* 6 and 11 Hz, CH_2CH), 6.46 (1 H, s, CH), 7.12–7.22 (5 H, m, Ph), 7.56 (2 H, s, *J* 9.0 Hz, 2 × ArH), 8.03 (2 H, d, *J* 8.7 Hz, 2 × ArH) and 10.63 (1 H, s, NH).

5-*Benzyl*-3-(1-*methoxycarbonyl*-2-*phenylethyl*)*hydantoin*.—A solution of sodium methoxide was prepared by reaction of sodium metal (3.7 g, 0.16 mol) with methanol (distilled from CaH_2; 300 ml). This solution was added to 5-benzylhydantoin [13] (27.5 g, 0.15 mol) and the mixture was refluxed for 2 days, then cooled to 50 °C before methyl 1-bromo-2-phenylpropanoate (36.45 g, 0.15 mol) was added. The mixture was stirred for 10 days at 50 °C and the solution was then cooled to room temperature. Fractional crystallisation gave 5-benzyl-3-(1-methoxycarbonyl-2-phenylethyl)hydantoin (9.86 g, 19%), m.p. 192–195 °C (lit.,[14] 193–196 °C) (Found: C, 68.3; H, 5.7; N, 8.1. Calc. for $C_{20}H_{20}N_2O_4$: C, 68.2; H, 5.7; N, 8.0%); λ_{max}(MeOH–water 60:40) 252, 258, 264 and 268 nm; ν_{max}(KCl) 3340 (NH), 3035, 2970 and 2955 (CH), 1760, 1740 and 1705 (C=O) and 1605 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.44 (1 H, dd, *J* 7.5 and 14.2 Hz, HC*H*), 2.72 (1 H, dd, *J* 5.1 and 14.2 Hz, HC*H*), 3.23 (1 H, dd, *J* 11.1 and 13.9 Hz, HC*H*), 3.35 (1 H, dd, *J* 5.6 and 13.9 Hz, HC*H*), 3.60 (3 H, s, Me), 4.30 (1 H, dd, *J* 5.1 and 7.5 Hz, CH), 4.84 (1 H, dd, *J* 5.6 and 11.1 Hz, CH), 7.07–7.18 (5 H, m, Ph), 7.20–7.30 (5 H, m, Ph) and 8.26 (1 H, s, NH); HPLC t_R 3.7 min.

5-*Benzyl*-3-(1-*carboxy*-2-*phenylethyl*)*hydantoin* **5a**.—A mixture of 5-benzyl-3-(1-methoxycarbonyl-2-phenylethyl)hydantoin (1.76 g, 5 mmol), methanol (distilled from CaH_2; 30 ml), and potassium hydroxide (crushed, 0.28 g, 5 mmol) was stirred for 22 h at room temperature. The solvent was evaporated off, water was added, and the solution was washed with ethyl acetate (6 × 20 ml). The aq. layer was acidified and the white precipitate was filtered off, and dried at reduced pressure, to yield 5-benzyl-3-(1-carboxy-2-phenylethyl)hydantoin **5a** (0.25 g, 15%) as a mixture of stereoisomers, m.p. 199–202 °C (lit.,[3] 203–207 °C for the *S,S*-isomer); λ_{max}(MeOH–water 60:40) 253, 259, 265 and 268 nm; ν_{max}(KCl) 3330 (NH), 3030 and 2925 (CH), 1765, 1740, 1715 and 1695 (C=O) and 1600 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.37 (1 H, dd, *J* 7.7 and 14.2 Hz, HC*H*), 2.71 (1 H, dd, *J* 5.1 and 14.2 Hz, HC*H*), 2.92 (0.5 H, dd, *J* 11.1 and 13.9 Hz, HC*H*), 3.10 (0.5 H, dd, *J* 10.7 and 13.8 Hz, HC*H*), 3.12 (0.5 H, dd, *J* 4.8 and 13.9 Hz, HC*H*), 3.29 (0.5 H, dd, *J* 5.2 and 13.8 Hz, HC*H*) 4.25 (1 H, dd, *J* 5.1 and 7.7 Hz, CH), 4.64 (0.5 H, dd, *J* 4.8 and 11.1 Hz, CH), 4.73 (0.5 H, dd, *J* 5.1 and 10.7 Hz, CH), 7.19–7.30 (10 H, m, 2 × Ph), 8.17 (0.5 H, s, NH) and 8.22 (0.5 H, s, NH); HPLC t_R 3.5 min; no separation of the diastereoisomers was observed.

3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*trifluoromethylbenzyl*)-*hydantoin* **5c**.—(*Z*)-3-(1-Carboxy-2-phenylethyl)-5-(trifluoromethylbenzylidene)hydantoin **4c** (2.02 g, 5 mmol) was added to a mixture of methanol (100 ml) and water (10 ml) and then solution was degassed before palladised charcoal (10%; 50 mg, 5 × 10^{-5} mol Pd) was added. The mixture was hydrogenated (1 atm) for 72 h, then the catalyst was filtered off. The solvent was evaporated off and the solid was recrystallised from methanol–chloroform to yield 3-(1-*carboxy*-2-*phenylethyl*)-5-(4-*trifluoromethylbenzyl*)*hydantoin* **5c** (1.90 g, 94%) m.p. 155–157 °C. This compound was found to be a mixture of four stereoisomers. Fractional crystallisation was used to separate one pair of isomers from the mixture, (0.28 g, 14%) m.p. 187–188 °C (Found: C, 59.6; H, 4.1; N, 6.9. $C_{20}H_{17}F_3N_2O_4$ requires C, 59.1; H, 4.2; N, 6.9%); λ_{max}(MeOH–water 60:40) 252, 258, 264 and 268 nm; ν_{max}(KCl) 3330 (NH), 3115, 3070, 3030 and 2915 (CH), 1760, 1715 and 1695 (C=O) and 1615 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.5 (1 H, dd, *J* 6.8 and 14.0 Hz, HC*H*), 2.80 (1 H, dd, *J* 5.1 and 14.0 Hz, HC*H*), 3.18 (1 H, dd, *J* 11.3 and 14.0 Hz, HC*H*), 3.32 (1 H, dd, *J* 5.25 and 14.0 Hz, HC*H*), 4.35 (1 H, dd, *J* 5.1 and 6.8 Hz, CH), 4.73 (1 H, dd, *J* 5.25 and 11.3 Hz, CH), 7.12 (2 H, d, *J* 8.3 Hz, 2 × ArH), 7.23–7.30 (5 H, m, Ph), 7.58 (2 H, d, *J* 8.1 Hz, 2 × ArH), 8.20 (1 H, s, NH) and 13.0–13.2 (1 H, br s, OH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.4 (t), 37.0 (t), 52.8 (d), 56.4 (d), 124.9 (d), 126.3 (d), 127.5 (q), 128.2 (d), 128.6 (d), 130.2 (s), 137.4 (s), 140.6 (s), 155.6 (s), 169.9 (s) and 172.7 (s); HPLC t_R 3.3 min.

The other pair of isomers, with m.p. 176–178 °C was obtained from the mother liquors (Found: C, 59.6; H, 4.1; N, 6.9%); λ_{max}(MeOH–water 60:40) 252, 258, 264 and 268 nm; ν_{max}(KCl) 3330 (NH), 3115, 3065, 3030 and 2920 (CH), 1760, 1720 and 1700 (C=O), and 1620 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.5 (1 H, dd, *J* 6.7 and 14.0 Hz, HC*H*), 2.74 (1 H, dd, *J* 5.1 and 14.0 Hz, HC*H*), 3.01 (1 H, dd, *J* 11.3 and 14.0 Hz, HC*H*), 3.32 (1 H, dd, *J* 5.2 and 14.0 Hz, HC*H*), 4.35 (1 H, dd, *J* 5.1 and 6.7 Hz, CH), 4.67 (1 H, dd, *J* 5.2 and 11.3 Hz, CH), 7.00 (2 H, d, *J* 8.8 Hz, 2 × ArH), 7.17–7.38 (5 H, m, Ph), 7.61 (2 H, d, *J* 8.6 Hz, 2 × ArH), 8.25 (1 H, s, NH) and 13.0–13.2 (1 H, br s, OH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.3 (t), 36.6 (t), 52.4 (d), 56.3 (d), 124.9 (d), 126.3 (d), 127.5 (q), 128.1 (d), 128.6 (d), 128.8 (d), 130.1 (s), 137.3 (s), 140.5 (s), 155.5 (s), 169.8 (s) and 172.7 (s); HPLC t_R 4.0 min.

Similarly prepared were:

3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*fluorobenzyl*)*hydantoin* **5d**. (*Z*)-3-(1-Carboxy-2-phenylethyl)-5-(4-fluorobenzylidene)-hydantoin **4d** (1.068 g, 3 mmol) gave the *hydantoin* **5d** (0.41 g, 38%) as a single racemate, m.p. 205–206 °C (Found: C, 63.9; H, 4.8; F, 5.3; N, 7.8. $C_{19}H_{17}FN_2O_4$ requires C, 64.0; H, 4.8; F, 5.3; N, 7.9%); λ_{max}(MeOH–water 60:40) 254, 259, 265 and 271 nm; ν_{max}(KCl) 3325 (NH), 3035, 2960 and 2930 (CH), 1760, 1725 and 1700 (C=O) and 1600 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.41 (1 H, dd, *J* 6.7 and 13.8 Hz, HC*H*), 2.71 (1 H, dd, *J* 5.0 and 13.8 Hz, HC*H*), 3.15 (1 H, dd, *J* 11.2 and 13.8 Hz, HC*H*), 3.29 (1 H, dd, *J* 5.2 and 13.8 Hz, HC*H*), 4.26 (1 H, dd, *J* 5.0 and 6.7 Hz, CH), 4.72 (1 H, dd, *J* 5.2 and 11.2 Hz, CH), 7.05 (2 H, d, *J* 8.8 Hz, 2 × ArH), 7.11 (2 H, d, *J* 8.8 Hz, 2 × ArH), 7.07–7.28 (5 H, m, Ph), 8.16 (1 H, s, NH) and 12.8–13.3 (1 H, br s, OH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.4, 36.3, 52.4, 56.8, 114.6, 114.9, 126.3, 128.1, 128.8, 131.1, 131.7, 137.3, 155.5, 169.9 and 172.7; HPLC t_R 2.9 min.

3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*methylbenzyl*)*hydantoin* **5e**. (*Z*)-3-(1-Carboxy-2-phenylethyl)-5-(4-methylbenzylidene) hydantoin **4e** (1.40 g, 4 mmol) yielded the *title product* **5e** (0.32 g, 22%) as a pair of isomers, m.p. 196–198 °C (from EtOAc) (Found: C, 68.2; H, 5.6; N, 8.0. $C_{20}H_{20}N_2O_4$ requires C, 68.2; H, 5.7; N, 8.0%); λ_{max}(MeOH–water 60:40) 252, 258, 264 and 268 nm; ν_{max}(KCl) 3320 (NH), 3125, 3060, 3030, 2955 and 2920 (CH), 1760, 1720 and 1695 (C=O) and 1605 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.23 (3 H, s, Me), 2.33 (1 H, dd, *J* 7.7 and 14.0 Hz, HC*H*), 2.56 (1 H, dd, *J* 4.9 and 14.0 Hz, HC*H*), 3.16 (1 H, dd, *J* 11.0 and 13.9 Hz, HC*H*), 3.29 (1 H, dd, *J* 5.1 and 13.9 Hz, HC*H*), 4.22 (1 H, dd, *J* 4.9 and 7.7 Hz, CH), 4.73 (1 H, dd, *J* 5.1 and 11.0 Hz, CH), 6.96 (2 H, d, *J* 8.0 Hz, 2 × ArH), 7.03 (2 H, d, *J* 7.8 Hz, 2 × ArH), 7.11–7.29 (5 H, m, Ph), 8.15 (1 H, s, NH) and 12.6–13.0 (1 H, br s, OH); δ_C[62.9 MHz; $(CD_3)_2SO$] 20.6 (q), 33.5 (t), 37.1 (t), 52.3 (d), 57.0 (d), 126.3 (d), 128.2 (d), 128.7 (d), 128.8 (d), 129.1 (d), 132.7 (s), 135.4 (s), 137.4 (s), 155.6 (s), 169.9 (s) and 172.9 (s); HPLC t_R 3.4 min.

The other pair of isomers was obtained from the mother liquors and had m.p. 187–189 °C, δ_H([250 MHz; $(CD_3)_2SO$] 2.23 (3 H, s, Me), 2.33 (1 H, dd, *J* 7.7 and 14.0 Hz, HC*H*), 2.67 (1 H, dd, *J* 4.9 and 14.0 Hz, HC*H*), 2.87 (1 H, dd, *J* 4.4 and 13.9 Hz, HC*H*), 3.16 (1 H, dd, *J* 11.0 and 13.9 Hz, HC*H*), 4.22 (1 H, dd, *J* 5.0 and 7.7 Hz, CH), 4.64 (1 H, dd, *J* 4.4 and 11.0 Hz, CH), 6.96 (2 H, d, *J* 8.0 Hz, 2 × ArH), 7.03 (2 H, d, *J* 7.8 Hz, 2 × ArH), 7.11–7.29 (5 H, m, Ph), 8.21 (1 H, s, NH) and 12.6–13.0 (1 H, br s, OH); HPLC t_R 3.6 min.

3-(1-*Carboxy*-2-*phenylethyl*)-5-(4-*methoxybenzyl*)*hydantoin* **5f**. (*Z*)-3-(1-Carboxy-2-phenylethyl)-5-(4-methoxybenzylidene)hydantoin **4f** (183 mg, 0.5 mmol) gave the *title hydantoin* (**5f**) (70 mg, 38%) as a single racemate, m.p. 208–210 °C (Found: C, 65.2; H, 5.2; N, 7.6. $C_{20}H_{20}N_2O_5$ requires C, 65.2; H, 5.4; N, 7.6%); λ_{max}(MeOH–water 60:40) 270, 276 and 282 nm; ν_{max}(KCl) 3305 (NH), 3140, 3055, 3020, 2965 and 2920 (CH), 1760, 1720, 1705 and 1690 (C=O) and 1610 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.35 (1 H, dd, *J* 7.3 and 13.9 Hz, HC*H*), 2.65 (1 H, dd, *J* 5.1 and 13.9 Hz, HC*H*), 3.12 (1 H, dd, *J* 11.2 and 14.0 Hz, HC*H*), 3.30 (1 H, dd, *J* 5.4 and 14.0 Hz, HC*H*), 3.68 (3 H, s, Me), 4.19 (1 H, dd, *J* 5.1 and 7.3 Hz, CH), 4.70 (1 H, dd, *J* 5.4 and 11.2 Hz, CH), 6.78 (2 H, d, *J* 8.3 Hz, 2 × ArH), 6.99 (2 H, d, *J* 8.3 Hz, 2 × ArH), 7.10–7.29 (5 H, m, Ph), 8.12 (1 H, s, NH) and 12.8–13.2 (1 H, br s, OH); HPLC t_R 3.3 min.

5-(4-*Aminobenzyl*)-3-(1-*carboxy*-2-*phenylethyl*)*hydantoin hydrochloride* **5g**·*HCl*. *Reaction of* (*Z*)-3-(1-carboxy-2-phenylethyl)-5-(4-nitrobenzylidene)hydantoin **4b** (0.706 g, 2 mmol) in methanol (150 ml)–water (50 ml) with palladium on charcoal (10%; 30 mg, 0.03 mmol Pd) and hydrochloric acid (1 ml, 10 mmol) yielded 5-(4-*aminobenzyl*)-3-(1-*carboxy*-2-*phenylethyl*)-*hydantoin hydrochloride* **5g**·HCl (0.642 g, 100%) as a mixture of stereoisomers, m.p. 150–155 °C (Found: C, 58.2; H, 5.1; Cl, 8.8; N, 10.6. $C_{19}H_{20}ClN_3O_4$ requires C, 58.5; H, 5.1; Cl, 9.1; N, 10.8%); λ_{max}(MeOH–water 60:40) 282 and 368 nm; ν_{max}(KCl) 3360 (NH), 3120, 3030 and 2930 (CH), 2850, 2580 and 2370 (NH_3^+), 1765, 1720 and 1700 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 2.72 (1 H, dd, *J* 7.1 and 14.5 Hz, CH*H*), 2.91 (1 H, dd, *J* 5.0 and 14.5 Hz, HC*H*), 3.18 (1 H, dd, *J* 11.3 and 14.0 Hz, CH*H*), 3.29 (1 H, dd, *J* 5.0 and 14.0 Hz, HC*H*), 4.18 (1 H, dd, *J* 5.0 and 7.1 Hz, CH), 4.67 (0.5 H, dd, *J* 5.0 and 11.3 Hz, CH), 4.73 (0.5 H, dd, *J* 5.0 and 11.3 Hz, CH), 7.03 (2 H, d, *J* 8.7 Hz, 2 × ArH), 7.14 (2 H, d, *J* 8.8 Hz, 2 × ArH), 7.19–7.27 (5 H, m, Ph), 8.22 (0.3 H, s, NH), 8.27 (0.3 H, s, NH), 8.30 (0.2 H, s, NH) and 8.35 (0.2 H, s, NH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.3 (t), 36.5 (t), 52.4 (d), 56.5 (d), 122.6 (d), 126.4 (d), 128.2 (d), 128.7 (d), 130.5 (d), 130.7 (s), 135.3 (s), 136.7 (s), 155.5 (s), 169.8 (s) and 172.8 (s); HPLC t_R 2.5 min; no separation of the diastereoisomers was observed.

5-(4-*Aminobenzyl*)-3-(1-*carboxy*-2-*phenylethyl*)*hydantoin* **5g**. (*Z*)-3-(1-Carboxy-2-phenylethyl)-5-(4-nitrobenzylidene)-hydantoin **4b** (0.706 g, 2 mmol) yielded the *title product* **5g** (0.65 g, 100%) as a mixture of two racemates, m.p. 287–289 °C (Found: C, 64.7; H, 5.3; N, 11.8. $C_{19}H_{19}N_3O_4$ requires C, 64.6; H, 5.4; N, 11.9%); λ_{max}(MeOH–water 60:40) 282 nm; ν_{max}(KCl) 3400–3330 (NH), 3030 and 2935 (CH), 2855, 2815, 2790 and 2690 (NH_2), 1750, 1700 and 1600 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 2.22 (0.5 H, dd, *J* 7.8 and 13.8 Hz, HC*H*), 2.42 (0.5 H, dd, *J* 7.8 and 14.1 Hz, HC*H*), 2.65 (0.5 H, dd, *J* 5.0 and 11.6 Hz, HC*H*), 2.77 (0.5 H, dd, *J* 5.0 and 11.6 Hz, HC*H*), 3.1–3.5 (2 H, m, CH_2), 3.90 (1 H, dd, *J* 5.0 and 7.8 Hz, CH), 4.06 (0.5 H, dd, *J* 4.2 and 9.6 Hz, CH), 4.19 (0.5 H, dd, *J* 4.6 and 11.2 Hz, CH), 4.88 (1 H, s, NH_2), 4.90 (1 H, s, NH_2), 6.43 (1 H, d, *J* 8.3 Hz, ArH), 6.44 (0.5 H, d, *J* 8.3 Hz, 0.5 × ArH), 6.75 (0.5 H, d, *J* 8.3 Hz, 0.5 × ArH), 6.79 (1 H, d, *J* 8.3 Hz, ArH), 6.91 (1 H, d, *J* 6.9 Hz, ArH), 7.02–7.24 (5 H, m, Ph), 7.70 (0.5 H, s, NH), 7.77 (0.5 H, s, NH) and 8.50 (1 H, s, CH); δ_C[62.9 MHz; $(CD_3)_2SO$] 34.3 (t), 36.7 (t), 56.3 (d), 57.1 (d), 113.8 (s), 123.2 (d), 123.4 (d), 125.4 (d), 127.9 (d), 128.4 (d), 129.4 (s), 140.6 (s), 157.2 (s), 170.8 (s) and 173.7 (s); HPLC t_R 2.85 min; no separation of the diastereoisomers was observed.

5-(4-*Nitrobenzyl*)*hydantoin* **7**.—5-Benzylhydantoin (47.5 g, 0.25 mol) and conc. sulphuric acid (500 ml, 5 mol) were stirred and cooled to −15 °C. Nitric acid (10M; 22.2 ml) was added dropwise during 5 min while the solution was vigorously stirred. The mixture was kept between −20 and −5 °C for 1.5 h before being poured into ice (1 kg). The resulting mixture was filtered to leave a white solid, which was washed successively with water (10 × 200 ml) and chloroform (4 × 50 ml), and was then recrystallised from methanol to yield 5-(4-nitrobenzyl)-hydantoin **7** (11.92 g, 20%), m.p. 195–198 °C (lit.,[15] 238–240 °C) (Found: C, 51.0; H, 3.4; N, 17.9. Calc. for $C_{10}H_9N_3O_4$: C, 51.1; 3.8; N, 17.9%); λ_{max}(MeOH–water 60:40) 274 nm; ν_{max}(KCl) 3360 and 3230 (NH), 3140, 3110, 3070 and 2920

(CH), 1755 and 1700 (C=O), and 1595 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 3.07 (2 H, d, *J* 5 Hz, CH_2), 4.43 (1 H, t, *J* 5 Hz, CH), 7.45 (2 H, d, *J* 9 Hz, 2 × ArH), 7.93 (1 H, s, NH), 8.17 (2 H, d, *J* 9 Hz, 2 × ArH) and 10.45 (1 H, s, NH); HPLC t_R 5.6 min.

(E)- and (Z)-5-(4-*Nitrobenzylidene)hydantoin* **8**. *Method* 1.—5-Benzylhydantoin (57.0 g, 0.3 mol) and conc. sulphuric acid (600 ml, 6 mol) were stirred and cooled to −15 °C. Nitric acid (10M; 26.7 ml) was added dropwise during 5 min while the solution was stirred vigorously. The solution was kept at −15 °C for 1.5 h before being poured into cold water (1.0 l). **CAUTION**: The mixture generated a large amount of heat, boiled and released copious amounts of brown vapour (NO_2). On cooling, a yellow precipitate formed, which was filtered off, washed successively with water (12 × 200 ml) and chloroform (3 × 50 ml), and then recrystallised from methanol to yield (*Z*)- and (*E*)-5-(4-nitrobenzylidene)hydantoin **8**. m.p. 320–325 °C (lit.,[16] >300 °C); λ_{max}(MeOH–water 60:40) 348 nm; ν_{max}(KCl) 3310 and 3220 (NH), 3105, 3030, 2940 and 2920 (CH), 1770, 1745 and 1725 (C=O), 1655 (C=C) and 1585 cm^{-1} (arom. C=C); δ_H[90 MHz; $(CD_3)_2SO$] 6.50 (0.66 H s, CH), 6.54 (0.33 H, s, CH), 7.85 (2 H, d, *J* 9 Hz, 2 × ArH), 8.21 (2 H, d, *J* 9 Hz, 2 × ArH), 10.85 (1 H, s, NH) and 11.4 (1 H, s, NH); HPLC t_R 9.3 min (70%) and 9.7 min (30).

(Z)-5-(4-*Nitrobenzylidene)hydantoin* **8**. *Method* 2.—5-(4-Nitrobenzyl)hydantoin (0.86 g, 4 mmol) was kept under nitrogen for the duration of the experiment. A solution of sodium methoxide [prepared by reaction of sodium metal (0.10 g, 4.2 mmol) with methanol (distilled from CaH_2; 20 ml)] was added to the flask containing the hydantoin. The solution of 5-(4-nitrobenzyl)hydantoin in methanolic sodium methoxide was then exposed to air before the solution was cooled and acidified. The precipitate collected from this mixture was found to be (*Z*)-5-(4-nitrobenzylidene)hydantoin (**8**) by NMR spectrometry and UV spectroscopy; HPLC t_R 9.7 min.

3-(1-*Carboxy-2-phenylethyl)hydantoin* **9**.—3-(1-Methoxycarbonyl-2-phenylethyl)hydantoin **1** 1.31 g, 5 mmol) was added to hydrobromic acid (48% in water; 20 ml) and the mixture was stirred at 80 °C for 30 min before being cooled and filtered. This yielded white crystals, which were found to be 3-(1-carboxy-2-phenylethyl)hydantoin **9** (0.90 g, 73%), m.p. 183–185 °C (lit.,[17] 190–193 °C) (Found: C, 58.0; H, 4.9; N, 11.3. Calc. for $C_{12}H_{12}N_2O_4$: C, 58.1; H, 4.8; N, 11.3%); λ_{max}(MeOH–water 60:40) 252, 258, 264 and 268 nm; ν_{max}(KCl) 3360 (NH), 3110, 3080, 3020, 2990, 2975, 2950 and 2920 (CH), 1765, 1720 and 1700 (C=O), and 1600 cm^{-1} (arom. C=C); δ_H[250 MHz; $(CD_3)_2SO$] 3.3 (2 H, m, CH_2), 3.76 (1 H, d, *J* 17.1 Hz, HC*H*), 3.86 (1 H, d, *J* 17.1 Hz, HC*H*), 4.76 (1 H, dd, *J* 6.1 and 10.2 Hz, CH), 7.1–7.3 (5 H, m, Ph), 8.0 (1 H, s, NH) and 13.3–13.4 (1 H, br s, OH); δ_C[62.9 MHz; $(CD_3)_2SO$] 33.2 (t), 45.4 (t), 52.7 (d), 126.3 (d), 128.2 (d), 128.6 (d), 137.4 (s), 156.5 (s), 169.9 (s) and 171.3 (s); HPLC t_R 2.8 min.

Enzyme Studies.—Studies of the inhibition of dihydroorotate dehydrogenase by the 5-benzylhydantoins **5** and the 5-benzylidenehydantoins **4** were carried out using the procedures for initial assays and for time-dependent assays described in our previous paper.[3] The enzyme used was from *Zymobacterium* (*Clostridium*) *oroticum* (Sigma, lot 74F-6833). In all cases 10% (v/v) dimethyl sulphoxide was used as co-solvent in the phosphate buffer solutions to improve solubility. Results are reported in Table 1.

References

1 Part 5, J. Haddow, C. J. Suckling and H. C. S. Wood, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1297.
2 T. W. Kensler and D. A. Cooney in *Design of Enzyme Inhibitors as Drugs*, eds. M. Sandler and H. J. Smith, Oxford University Press, Oxford, 1989, p. 379; A. T. Hudson in *Topics in Medicinal Chemistry*, ed. P. R. Leeming, Royal Society of Chemistry, London, 1988, p. 266.
3 I. G. Buntain, C. J. Suckling and H. C. S. Wood, *J. Chem. Soc., Perkin Trans. 1*, 1988, 3175.
4 S. F. Tan, K. P. Ang and Y. E. Fong, *J. Chem. Soc., Perkin Trans 2*, 1986, 1941.
5 M. G. B. Drew, K. F. Mok, K. P. Ang and S. F. Tan, *Acta Crystallogr., Sect. C*, 1987, **43**, 743.
6 T. Neilson, H. C. S. Wood and A. G. Wylie, *J. Chem. Soc.*, 1962, 371.
7 S. A. Frees, D. P. Sawick, B. Cunningham, P. F. Heinstein, D. J. Morre and J. M. Cassady, *Biochem. Pharmacol.*, 1988, **37**, 3807.
8 R. W. Taft, *Steric Effects in Organic Chemistry*, Wiley, New York, 1956, pp. 556–675.
9 D. Agin, L. Hersh and D. Holtzman, *Proc. Natl. Acad. Sci. USA*, 1965, **53**, 952.
10 R. W. Alder, R. Baker, and J. M. Brown, *Mechanism in Organic Chemistry*, Wiley-Interscience, London, 1975, p. 30.
11 W. Fraser, C. J. Suckling and H. C. S. Wood, following paper.
12 D. N. Harpp, L. Q. Bao, C. J. Black, J. G. Gleason and R. A. Smith, *J. Org. Chem.*, 1975, **40**, 3420.
13 H. Finkbeiner, *J. Org. Chem.*, 1965, **30**, 3414.
14 K. Schlogl, A. Siegel and F. Wessely, *Hoppe-Seyler's Z. Physiol. Chem.*, 1953, **291**, 265.
15 T. B. Johnson and C. A. Brautlecht, *J. Biol. Chem.*, 1912, **12**, 175.
16 Z. Foldi, D. Heidt-Lanyi and T. Szanto, *Acta Chim. Acad. Sci. Hung.*, 1961, **29**, 373.
17 F. Wessely, K. Schlogl and W. Wawersich, *Monatsh. Chem.*, 1952, **83**, 1426.

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