Latent Inhibitors. Part 7.[1] Inhibition of Dihydro-orotate Dehydrogenase by Spirocyclopropanobarbiturates

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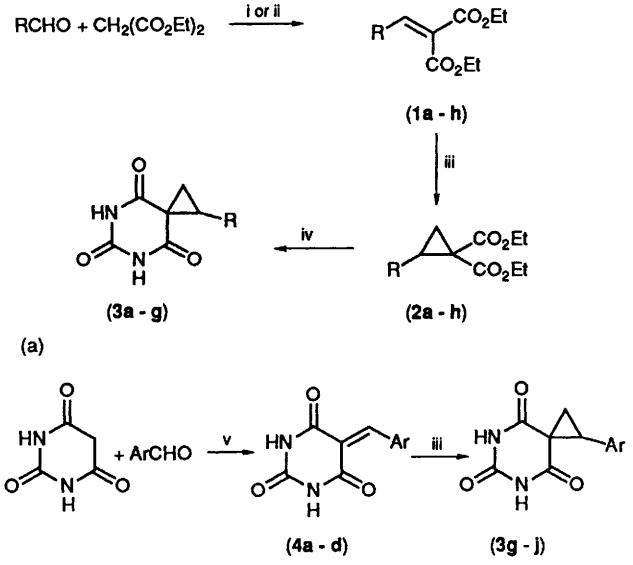
A series of 5-spirocyclopropanobarbiturates bearing alkyl and aryl substituents on the cyclopropane ring has been synthesized. Dihydro-orotate dehydrogenase from *Clostridium oroticum* was shown to be inhibited by these compounds. A related series of 5-membered-ring compounds (hydantoins and pyrazoles) was prepared but all the compounds were found to be inactive. In order to correlate these observations with previous results concerning 5-arylmethylhydantoins and 5-arylidene-hydantoins as inhibitors, 5-arylidenebarbiturates were also assessed as inhibitors and found to be the most active of the compounds investigated. The results are interpreted in the context of molecular recognition by this enzyme and the possibility of using substrate surrogates as templates for constructing latent inhibitors of enzymes.

Previous studies in this laboratory have shown that dihydro-orotate dehydrogenase (DHODase), which is a significant target for chemotherapy,[2] can be inhibited irreversibly by 5-arylmethylhydantoins, provided that a 1-carboxy-2-phenylethyl substituent is present at N-3.[1,3] We have also shown that a cyclopropane ring can be introduced into substrates to afford latent inhibitors of a wide variety of enzymes.[4,5] The goal of investigating such latent inhibitors is to establish design methodologies that will make it possible to obtain highly selective enzyme-activated inhibitors as lead compounds for chemotherapy. Selectivity can be generated by enzyme activation but since many naturally occurring compounds are substrates or products for more than one enzyme, substrates modified to contain latent functionalities may not attain the desired selectivity. In view of our fortuitous discovery of compounds that have a totally different molecular framework from the substrate, namely the hydantoins that inhibit DHODase,[1,3] the question arose whether substrate surrogates that differ markedly in overall structure from the normal substrate but which retain key features for enzyme-catalysed activation might provide a general design concept for highly selective enzyme inhibitors.[6] So far, such studies have been restricted largely to peptidases for which some substrate surrogate irreversible inhibitors based upon lactones have been described.[7] In this paper we describe the synthesis and evaluation of a number of heterocyclic compounds designed as potential inhibitors of DHODase to explore the potential of the concept of substrate surrogate in this case.

Design and Synthesis of Inhibitors.—A preliminary study has shown that 5-spirocyclopropanobarbituric acid was an inhibitor of DHODase[6] and in view of the hydrophobic binding effects discovered with the hydantoins as inhibitors, the synthesis of a series of alkyl- and aryl-substituted 5-spiro-cyclopropano analogues was undertaken (Scheme 1a). Condensation of aryl or alkyl aldehydes with diethyl malonate led to the α,β-unsaturated diesters (**1a–h**) which were cyclopropanated with trimethylsulphoxonium ylide to afford compounds (**2a–h**). The 1,1-cyclopropanedicarboxylate diesters were cyclised to the corresponding barbituric acids (**3a–g**) by reaction with urea in the presence of potassium t-butoxide in yields between 11 and 77%. The synthesis of heterocycles when using urea as a nucleophile is not usually a successful reaction; the acceptable yields obtained in this case are probably due to the cyclopropane ring which promotes cyclisation by restricting conformational freedom. The cyclisation, however, failed in the case of the 4-chlorophenyl-substituted diester (**2h**), and to obtain this and other aryl-substituted compounds an alternative route was used (Scheme 1b). Barbituric acid was condensed with a series of aryl aldehydes to give the well known arylidenebarbituric acids (**4a–d**). These compounds underwent cyclopropanation with trimethylsulphoxonium ylide to afford the 5-cyclopropanobarbiturates (**3g–j**).

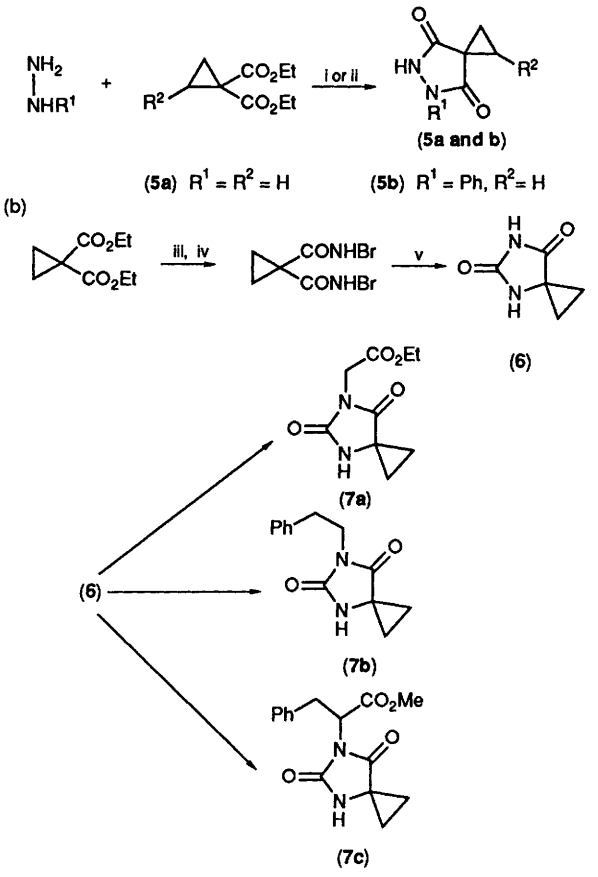


(1)–(3) **a**; R = Et **b**; R = Pr^i **c**; R = Pr **d**; R = Bu^i **e**; R = Bu **f**; R = $CHEt_2$ **g**; R/Ar = Ph **h**; R/Ar = 4-ClC_6H_4 **i**; Ar = 4-FC_6H_4 **j**; Ar = 4-MeC_6H_4

(4) **a**; Ar = Ph **b**; Ar = 4-ClC_6H_4 **c**; Ar = 4-FC_6H_4 **d**; Ar = 4-MeC_6H_4

Scheme 1. *Reagents:* i, piperidine benzoate; ii, Ac_2O; iii, $Me_3\overset{+}{S}O\ I^-$, NaH, DMF; iv, urea, Bu^{t}OK, DMSO; v, 13% aq. HCl.

The chemical reactivity of the above spirocyclopropano-barbiturates (**3a–j**) resides in the cyclopropane ring which is activated by the two adjacent carbonyl groups. A further



Scheme 2. *Reagents:* i, NaOEt; ii, KOBu^t; iii, NH_4OH; iv, Br_2, KOH; v, NaOMe.

Table. Inhibition of DHODase by 5-spirocyclopropanobarbiturates (**3**) and 5-arylidenebarbiturates (**4**).

Inhibitor	K_i/mM	$t_{\frac{1}{2}}$/min[a]	$10^4 k_2/s^{-1}$
(3; R = H)	9.5	136	5.9
(**3a**)	1.7	78	4.4
(**3b**)	4.5	62	8.3
(**3c**)	1.8	37	7.0
(**3d**)	3.1	14	16.7
(**3e**)	0.9	20	8.3
(**3f**)	4.1	17	12.5
(**3g**)	2.5	115	2.5
(**3h**)	3.2	26	13.3
(**3j**)	3.0	48	7.1
(**4a**)		<3	
(**4c**)		11	

[a] $t_{\frac{1}{2}}$-Values correspond to inhibitor concentrations of 2mM.

polarisation of one of these groups by DHODase at its active site would enhance the reactivity of the small ring towards nucleophilic addition. The same argument can reasonably be applied to analogous five-membered-ring compounds such as pyrazolones. These compounds (**5a** and **b**) were readily available by condensation of the diesters (**2**) with hydrazine or substituted hydrazines (Scheme 2a). The hydantoins studied previously were shown to have their key chemical reactivity at the 5-substituent and a further approach to probe the surrogate substrate concept was to prepare a series of 5-cyclopropanohydantoins (**7a–c**) (Scheme 2b). 5-Spirocyclopropanohydantoin (**6**) was obtained in three steps from diethyl cyclopropane-1,1-dicarboxylate.[8] Alkylation on N-3 was successful with 1-bromo-2-phenylethane in the presence of potassium t-butoxide to give compound (**7b**), but took place only in low yield with ethyl bromoacetate to give compound (**7a**) and failed completely with methyl 2-bromo-3-phenylpropanoate.

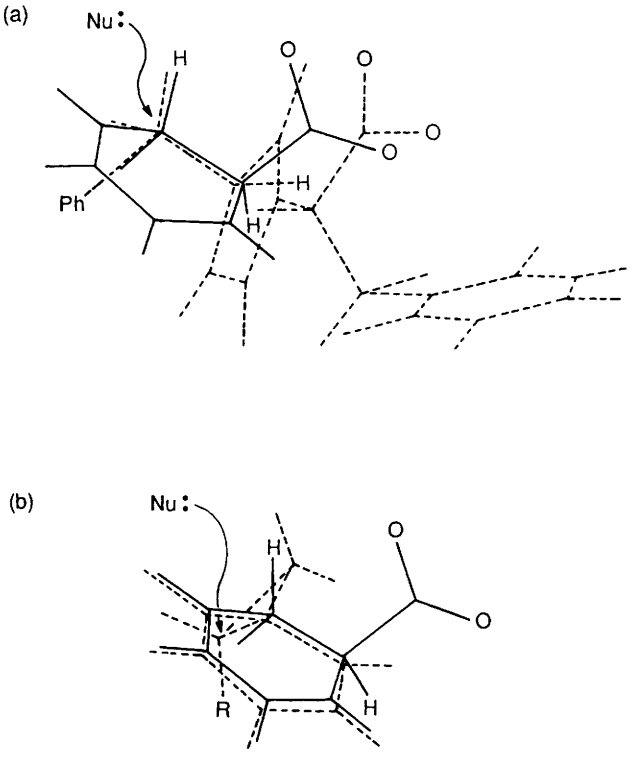
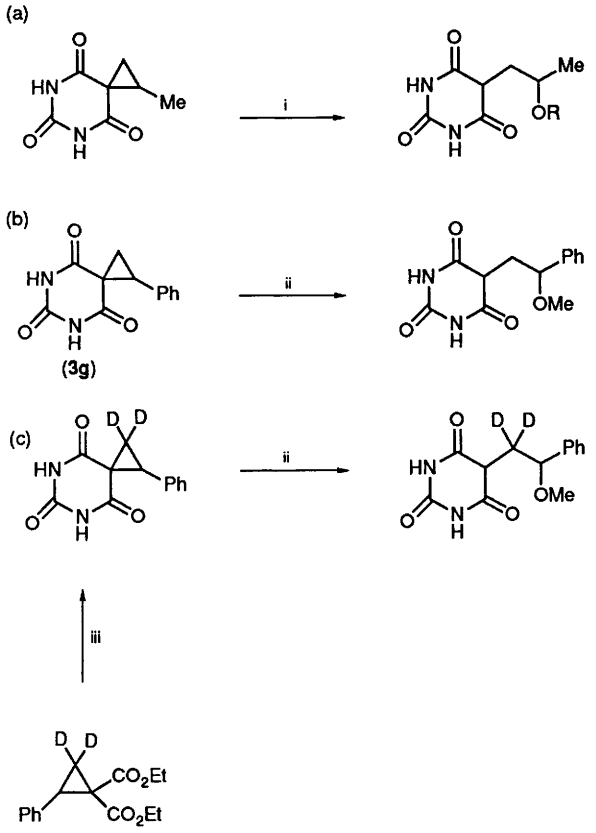


Fig. 1 Computer-derived graphical superimposition of different inhibitors (– – – –) on dihydro-orotate (——) showing possible direction of attack by an enzyme nucleophile. (a) Inhibitor = 5-arylmethylhydantoin. (b) Inhibitor = 5-spirocyclopropanobarbiturate.

Inhibition of Dihydro-orotate Dehydrogenase.—The inhibition of DHODase was studied under the standard conditions used previously[1,3] and all of the alkyl- and aryl-substituted spirocyclopropanobarbiturates (**3a–h** and **j**) were found to be irreversible inhibitors and were all bound more strongly and were more reactive than the parent unsubstituted compound (Table). The most reactive compound (**3d**), which bore an isobutyl substituent, was 3 times more reactive than the parent compound. The tightest binding was obtained with the n-butyl substituted compound (**3e**) which had *ca.* 10 times the affinity of the parent compound for the enzyme. However, the range of affinity and reactivity is not large and clearly, for the range of substituents chosen, the enzyme is not very discriminating. These results can be correlated with those from the 5-arylmethylhydantoins previously described[1,3] as shown in Figure 1. The superimposition of the hydantoin with the substrate (Figure 1a) placed the two oxidisable sites together and the phenyl group in a volume of space below the six-membered-ring plane. In a previous paper[3] we have suggested that enzymic oxidation of the benzyl group in the hydantoin leads to a benzylidene derivative which is susceptible to attack by an enzyme nucleophile as shown. When the spirocyclopropanobarbiturate structure was superimposed on that of the substrate (Figure 1b), the hydrophobic substituent can be placed in a similar relative position to the phenyl group in the hydantoin inhibitors. Attack on both activated systems by an enzymic nucleophile can then be envisaged as occurring from approximately the same direction. This model is consistent with all the results so far obtained in this series and, taken with the

model obtained from regression analysis for the hydantoins,[1] provides a basis for the design of more potent compounds with, for example, larger substituents on the benzene ring.

For the spirocyclopropanobarbiturates to have any value as inhibitors it is important to demonstrate that they have some selectivity of action. It could be argued that the cyclopropane ring, being doubly activated, would be sufficiently electrophilic to attack enzymes indiscriminately.[9] The chemical reactivity of the spirohydantoins is certainly consistent with this possibility. It has been shown[10] that the 5-spiro-(2-methyl)cyclopropanobarbiturate undergoes nucleophilic ring opening upon refluxing in solution in a range of alcohols (Scheme 3a). The



Scheme 3. *Reagents and conditions:* i, ROH, 6 h, reflux; ii, MeOH, reflux; iii, urea, Bu^{t}OK, DMSO.

location of the substitution was (surprisingly) found to be on the cyclopropane carbon bearing the substituent, the more hindered site. We have shown that similar ring opening takes place with the 2-phenylcyclopropyl derivative (Scheme 3b) and have confirmed the location of the substituent in the product by use of a deuterium-labelled derivative and by showing that no deuterium is lost (Scheme 3c). It is possible that the bond joining this tertiary carbon atom to the pyrimidine ring is weaker than the other possible cleavable bond because of unfavourable steric interactions between the ring and the substituent. Such reactivity, of course, provides a valuable insight into the probable mechanism by which these compounds act as enzyme inhibitors. Although the possibility that the inhibitors (**3**) are not selective in their action has not been extensively investigated, we previously showed that compound (**3a**) was not an inhibitor of either horse liver alcohol dehydrogenase or of lactate dehydrogenase.[3]

It was disappointing to find that none of the pyrazoles or spirocyclopropanohydantoins was found to be an irreversible inhibitor of DHODase. However, both compounds (**5b**) and (**7b**) were weak competitive inhibitors whereas the parent unsubstituted compounds (**5a**) and (**6**) were totally inactive. The favourable interaction of aryl substituents with a hydrophobic pocket at the enzyme's active site is again indicated. These results suggest that the enzyme has, not surprisingly, a preference for 6-membered-ring heterocycles and only when there are additional substituents present in 5-membered-ring compounds that can take part in ionic or adventitious hydrophobic bonding can the smaller ring form the basis for a significantly potent inhibitor. The importance of the hydrophobic group was reinforced when the 5-arylidenebarbiturates (**4**) were tested as inhibitors. These compounds were found to be the most reactive irreversible inhibitors so far investigated by us for DHODase. As a result of their intense colour and the rapidity of the inhibition reaction ($t_{\frac{1}{2}} < 3$ min under standard conditions), accurate rate constants could not be obtained by the methods available.

Computer modelling of the electrostatic potential surfaces of the basic hydantoin and barbiturate skeletons in comparison with the substrate, using an algorithm developed by Dr. P. Bladon, suggests that the enzyme would recognise the barbiturates as substrate surrogates, but not recognise the hydantoins as such. This modelling procedure automatically compares the potential surfaces of two compounds and obtains the best fit. Our studies, together with the results obtained by others on peptidases,[7] suggest that the structural limits within which substrate surrogates can be designed are likely to be narrow. This is not surprising in view of the demands inherent in the design concept expounded in the introduction. It suggests that a gradual distancing from the natural substrate is likely to be the best strategy in design, a concept that we are pursuing in our own studies of peptidases.[11]

Experimental

^{1}H NMR spectra were recorded on Perkin-Elmer R12 (90 MHz) or Bruker WM-250 (250 MHz) spectrometers, and ^{13}C NMR spectra on the Bruker instrument (63 MHz). Chemical shifts were measured relative to tetramethylsilane as internal standard. IR spectra were determined using Perkin-Elmer 397 or 257 spectrometers, and UV spectra using Philips PU 8800 and Pye-Unicam SP 800 spectrophotometers. HPLC was carried out using Waters ODS-12 reversed-phase columns, at a flow rate of 42 ml h^{-1}, and Cecil Instruments CE212 variable-wavelength monitors. GLC was carried out using Perkin-Elmer F33 Gas Chromatographs fitted with 3 ft columns and flame ionisation detectors.

Diethyl Propylidenemalonate (**1a**).—A mixture of propanal (67 ml, 0.94 mol), diethyl malonate (71 ml, 0.47 mol), and acetic anhydride (69 ml, 0.47 mol) was refluxed at 130 °C for 8 h. Fractional distillation gave the title product (16.3 g, 17%) as a clear liquid, b.p. 118–120 °C/18 mmHg (lit.,[12] 119–120 °C/15 mmHg); ν_{max}(liq. film) 2 980 (CH), 1 720br (C=O) and 1 645 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 1.10 (3 H, t, *J* 8 Hz, Me), 1.29 and 1.32 (6 H, 2 t, *J* 7 Hz, 2 × *Me*CH_2O), 2.31 (2 H, septet, *J* 7 Hz, CH_2), 4.23 and 4.30 (4 H, 2 q, *J* 7 Hz, 2 × MeCH_2O) and 6.96 (1 H, t, *J* 7 Hz, CH); GLC on Carbowax at 120 °C t_R 23.0 min; at 150 °C, t_R 6.7 min.

Diethyl Isobutylidenemalonate (**1b**).—A mixture of 2-methylpropanal (67.7 g, 0.97 mol), diethyl malonate (75.0 g, 0.47 mol), piperidine (2.4 ml, 24 mmol), benzoic acid (2.0 g, 16 mmol), and toluene (100 ml) was stirred under reflux, in a Dean–Stark apparatus, until no more water collected (10 h). To the cool product solution was added toluene (100 ml) and the resulting solution was washed successively with water (2 × 100 ml), 1M-HCl (2 × 100 ml), and finally with saturated aq. Na_2CO_3 (100 ml). The organic phase was dried (Na_2SO_4), and the

solvent was evaporated off. Fractional distillation of the residue gave the title product (92 g, 92%) as a clear liquid, b.p. 82–86 °C/1 mmHg (lit.,[12] 135–137 °C/27 mmHg); ν_{max}(liq. film) 2 890 (CH), 1 720 (C=O) and 1 645 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 1.05 (6 H, d, *J* 7 Hz, 2 × Me), 1.23 and 1.30 (6 H, 2 t, *J* 8 Hz, 2 × *Me*CH_2O), 2.70 (1 H, m, CH), 4.21 and 4.27 (4 H, 2 q, *J* 8 Hz, 2 × MeC*H*$_2$O) and 6.74 (1 H, d, *J* 10 Hz, vinylic CH); GLC on Apiezon at 175 °C, t_R 2.7 min (100%).

Similarly prepared were:

Diethyl butylidenemalonate (**1c**). From butanal (86 ml, 0.96 mol), diethyl malonate (72 ml, 0.48 mol), piperidine (1.5 ml, 15 mmol), benzoic acid (2.0 g, 16 mmol), and toluene (100 ml). Fractional distillation gave the title product (69 g, 67%) as a clear liquid, b.p. 100 °C/4 mmHg (lit.,[12] 122–124 °C/10 mmHg); ν_{max}(liq. film) 2 970 (CH), 1 750–1 690 (C=O) and 1 640 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 0.95 (3 H, dt, Me), 1.29 and 1.34 (6 H, 2 t, *J* 7 Hz, 2 × *Me*CH_2O), 1.55 (2 H, m, MeC*H*$_2$), 2.30 (2 H, m, CH_2C*H*$_2$), 4.20 and 4.23 (4 H, 2 q, *J* 7 Hz, 2 × MeC*H*$_2$O) and 6.98 (1 H, t, *J* 8 Hz, CH); GLC on Apiezon at 175 °C, t_R 3.3 min.

Diethyl isopentylidenemalonate (**1d**). From 3-methylbutanal (63 ml, 0.58 mol), diethyl malonate (70 ml, 0.48 mol), piperidine (2.4 ml, 24 mmol), benzoic acid (2.0 g, 16 mmol), and toluene (100 ml). Fractional distillation gave the title product (61 g, 50%) as a clear liquid, b.p. 70 °C/4 mmHg (lit.,[12] 144–150 °C/26 mmHg); ν_{max}(liq. film) 2 980 (CH), 1 760–1 700 (C=O) and 1 645 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 0.95 (6 H, d, *J* 8 Hz, 2 × Me), 1.29 and 1.31 (6 H, 2 t, *J* 8 Hz, 2 × *Me*CH_2O), 1.55–2.05 (1 H, m, CH), 2.20 (2 H, t, CH_2), 4.24 and 4.30 (4 H, 2 q, *J* 8 Hz, 2 × MeC*H*$_2$O) and 7.00 (1 H, t, *J* 8 Hz, vinylic CH); GLC on Apiezon at 175 °C, t_R 4.0 min.

Diethyl pentylidenemalonate (**1e**). From pentanal (18 ml, 0.17 mol), diethyl malonate (30 ml, 0.20 mol), piperidine (1 ml, 10 mmol), benzoic acid (2.0 g, 16 mmol), and toluene (100 ml). Fractional distillation gave the title product (19.8 g, 51%) as a clear liquid, b.p. 80–86 °C/0.7 mmHg (lit.,[12] 146–147 °C/23 mmHg) (Found: C, 63.6; H, 8.7. Calc. for $C_{12}H_{20}O_4$: C, 63.2; H, 8.9%); ν_{max}(liq. film) 2 970 (CH), 1 730br (C=O) and 1 640 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 0.91 (3 H, t, *J* 7 Hz, Me), 1.15–1.90 (10 H, m, MeC*H*$_2$C*H*$_2$ and 2 × *Me*CH_2O), 2.32 (2 H, q, *J* 8 Hz, C*H*$_2$CH), 4.05–4.50 (4 H, m, 2 × MeC*H*$_2$O) and 6.99 (1 H, t, *J* 8 Hz, CH); GLC on Carbowax at 150 °C, t_R 14.8 min.

Diethyl 2-ethylbutylidenemalonate (**1f**). From 2-ethylbutanal (21 ml, 0.17 mol), diethyl malonate (30 ml, 0.20 mol), piperidine (1 ml, 10 mmol), benzoic acid (1 g, 8 mmol), and toluene (100 ml). Fractional distillation gave the title product (5.3 g, 13%) as a clear liquid, b.p. 74–82 °C/0.3 mmHg (lit.,[12] 146–148 °C/21 mmHg) (Found: C, 64.0; H, 8.7. Calc. for $C_{13}H_{22}O_4$: C, 64.5; H, 9.1%); ν_{max}(liq. film) 2 960 (CH), 1 725br (C=O) and 1 645 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 0.85 (6 H, t, *J* 7 Hz, 2 × Me), 1.30 and 1.32 (6 H, each t, *J* 7 Hz, 2 × *Me*CH_2O), 1.50 (4 H, m, 2 × CH_2), 2.30 (1 H, m, CH), 4.22 and 4.30 (4 H, 2 q, *J* 7 Hz, 2 × MeC*H*$_2$O) and 6.70 (1 H, d, *J* 11 Hz, vinylic CH); GLC on Carbowax at 150 °C, t_R 12.4 min (100%).

Diethyl benzylidenemalonate (**1g**). From benzaldehyde (72 ml, 0.71 mol), diethyl malonate (95 ml, 0.63 mol), piperidine (2.5 ml, 25 mmol), benzoic acid (2 g, 16 mmol), and toluene (100 ml). Fractional distillation gave the title product (74 g, 48%) as a clear liquid, b.p. 124–128 °C/0.4 mmHg (lit.,[13] 140–142 °C/4 mmHg) (Found: C, 68.0; H, 6.3. Calc. for $C_{14}H_{16}O_4$: C, 67.7; H, 6.4%); ν_{max}(liq. film) 3 060 and 2 980 (CH), 1 740–1 700 (C=O), 1 630 and 1 600 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 1.26 and 1.31 (6 H, 2 t, 2 × Me), 4.29 and 4.31 (4 H, 2 q, 2 × CH_2), 7.35 (5 H, m, Ph) and 7.70 (1 H, s, CH); GLC on Carbowax at 200 °C, t_R 24.4 min.

Diethyl 4-chlorobenzylidenemalonate (**1h**). From 4-chlorobenzaldehyde (20.8 g, 0.15 mol), diethyl malonate (25.0 ml, 0.15 mol), piperidine (1 ml, 10 mmol), benzoic acid (0.8 g, 7 mmol), and toluene (100 ml). Fractional distillation gave the title product (23 g, 54%) as a clear liquid, b.p. 158–160 °C/1.5 mmHg (lit.,[14] 156–158 °C/1.5 mmHg) (Found: C, 59.4; H, 4.8; Cl, 12.3. Calc. for $C_{14}H_{15}ClO_4$: C, 59.5; H, 5.3; Cl, 12.6%); ν_{max}(liq. film) 3 060 and 2 980 (CH), 1 745–1 700 (C=O), 1635 and 1 595 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 1.30 and 1.32 (6 H, 2 t, *J* 7 Hz, 2 × Me), 4.30 and 4.35 (4 H, 2 q, *J* 7 Hz, 2 × MeC*H*$_2$O), 7.39 (4 H, s, C_6H_4Cl) and 7.69 (1 H, s, CH).

Diethyl 2-Ethylcyclopropane-1,1-dicarboxylate (**2a**).—To a stirred suspension of NaH (1.2 g, 50 mmol) in dimethylformamide (DMF) (100 ml) under nitrogen was added trimethylsulphoxonium iodide (TMSI) (11.4 g, 52 mmol) in a single portion.[15,16] Vigorous evolution of hydrogen lasted *ca.* 5 min. After a further 15 min a solution of diethyl propylidenemalonate (**1a**) (10.0 g, 50 mmol) in DMF (50 ml) was added in a single portion and the mixture was stirred for 1 h. The product solution was poured into HCl–ice (100 ml; 10%), and the resultant acidic solution (pH 1–2) was extracted with diethyl ether (4 × 50 ml). The extract was washed with water (4 × 50 ml), dried (Na_2SO_4), and evaporated. Fractional distillation of the residue gave the title product (7.2 g, 72%) as a clear liquid, b.p. 70 °C/0.2 mmHg (lit.,[17] 102–104 °C/3 mmHg) (Found: C, 62.0; H, 8.6. Calc. for $C_{11}H_{18}O_4$: C, 61.7; H, 8.4%); ν_{max}(liq. film) 2 970 (CH) and 1 720br cm^{-1} (C=O); δ_H(90 MHz; $CDCl_3$) 0.85 (1 H, overlapping m, CH), 1.08 (3 H, overlapping t, *J* 5 Hz, Me), 1.25 and 1.29 (6 H, 2 t, *J* 7 Hz, 2 × *Me*CH_2O), 1.40 (2 H, overlapping m, MeC*H*$_2$), 1.79 (2 H, septet, *J* 8 Hz, CH_2) and 4.18 and 4.24 (4 H, 2 q, *J* 7 Hz, 2 × MeC*H*$_2$O); GLC on Carbowax at 130 °C, t_R 9.8 min (100%).

Similarly prepared were:

Diethyl 2-isopropylcyclopropane-1,1-dicarboxylate (**2b**). From diethyl isobutylidenemalonate (**1b**) (32.1 g, 0.15 mol), TMSI (34.4 g, 0.16 mol), NaH (3.6 g, 0.15 mol), and DMF (200 ml). Fractional distillation gave the title product (28.1 g, 82%) as a clear liquid, b.p. 80–92 °C/0.1 mmHg) (lit.,[17] 110 °C/2 mmHg); ν_{max}(liq. film) 3 080 and 2 980 (CH) and 1 720 cm^{-1} (C=O); δ_H(250 MHz; $CDCl_3$) 0.95 and 0.97 (6 H, t, d, 2 × Me), 1.01 (1 H, m, CH), 1.21 and 1.24 (6 H, 2 t, *J* 7.1 Hz, 2 × *Me*CH_2O), 1.30 (1 H, dd, *H*CH), 1.32 (1 H, dd, HC*H*), 1.60–1.74 (1 H, m, cyclopropyl CH) and 4.01–4.24 (4 H, m, 2 × MeC*H*$_2$O); δ_C(63 MHz; $CDCl_3$) 14.07 (q), 14.13 (q), 20.36 (t), 21.80 (q), 22.43 (q), 28.01 (d), 34.80 (s), 36.21 (d), 61.22 (t), 61.28 (t), 168.45 (s) and 170.52 (s); GLC on Apiezon at 175 °C, t_R 2.8 min (100%).

Diethyl 2-propylcyclopropane-1,1-dicarboxylate (**2c**). From diethyl butylidenemalonate (**1c**) (10.7 g, 50 mmol), TMSI (11.0 g, 50 mmol), NaH (1.2 g, 50 mmol) and DMF (100 ml). Fractional distillation gave the title product (8.7 g, 77%) as a clear liquid, b.p. 78 °C/0.2 mmHg (lit.,[18] 104–106 °C/4 mmHg); ν_{max}(liq. film) 2 960 (CH) and 1 735 cm^{-1} (C=O); δ_H(250 MHz; $CDCl_3$) 0.92 (3 H, dd, Me), 1.01 (2 H, t, *J* 7.5 Hz), 1.26 and 1.20 (6 H, 2 t, *J* 7.1 Hz, 2 × *Me*CH_2O), 1.32–1.56 (3 H, m), 1.83–1.95 (1 H, m,), 2.05–2.14 (1 H, m) and 4.11–4.32 (4 H, m, 2 × MeC*H*$_2$O); GLC on Apiezon at 175 °C, t_R 3.6 min.

Diethyl 2-isobutylcyclopropane-1,1-dicarboxylate (**2d**). From diethyl isopentylidenemalonate (**1d**) (22.8 g, 0.1 mol), TMSI (22.1 g, 0.1 mol), NaH (2.4 g, 0.1 mol), and DMF (200 ml). Fractional distillation gave the *cyclopropyl diester* (**2d**) (17.5 g, 72%) as a clear liquid, b.p. 100 °C/1 mmHg (Found: C, 63.8; H, 9.0. $C_{13}H_{22}O_4$ requires C, 64.4; H, 9.2%); ν_{max}(liq. film) 3 090 and 2 980 (CH) and 1 720 cm^{-1} (C=O); δ_H(250 MHz; $CDCl_3$) 0.96 (6 H, dd, 2 × Me), 0.99–1.02 (1 H, m), 1.26 and 1.29 (6 H, 2 t, *J* 7.1 Hz, 2 × *Me*CH_2O), 1.34–1.54 (3 H, m), 1.63–1.79 (1 H, m), 1.85–1.97 (1 H, m) and 4.07–4.33 (4 H, m, 2 × MeC*H*$_2$O); GLC on Apiezon at 175 °C; t_R 4.3 min.

Diethyl 2-butylcyclopropane-1,1-dicarboxylate (**2e**). From diethyl pentylidenemalonate (**1e**) (12.0 g, 50 mmol), TMSI (12.3 g, 60 mmol), NaH (1.3 g, 50 mmol), and DMF (100 ml).

Fractional distillation gave the title product (8.2 g, 65%) as a clear liquid, b.p. 82–86 °C/0.05 mmHg (lit.,[19] 69–70 °C/0.08 mmHg) (Found: C, 64.0; H, 9.1. Calc. for $C_{13}H_{22}O_4$: C, 64.5; H, 9.1%); ν_{max}(liq. film) 2 980 (CH) and 1 725br cm^{-1} (C=O); δ_H(90 MHz; $CDCl_3$) 0.89 (3 H, m, Me), 1.10–2.20 (15 H, overlapping m, 3 × CH_2, cyclopropyl $CHCH_2$, and 2 × $MeCH_2O$) and 4.20 (4 H, m, 2 × $MeCH_2O$); GLC on 5% FFAP on Chromosorb G at 150 °C, t_R 5.7 min (100%).

Diethyl 2-(1-*ethylpropyl*)*cyclopropane*-1,1-*dicarboxylate* (**2f**). From diethyl 2-ethylbutylidenemalonate (**1f**) (5.17 g, 23 mmol), TMSI (5.94 g, 27 mmol), NaH (0.60 h, 25 mmol), and DMF (100 ml). Fractional distillation gave the *cyclopropyl diester* (**2f**) (4.38 g, 81%) as a clear liquid, b.p. 80 °C/0.1 mmHg (Found: C, 65.7; H, 9.6. $C_{14}H_{24}O_4$ requires C, 65.2; H, 9.4%); ν_{max}(liq. film) 2 960 (CH) and 1 720br and 1 685 cm^{-1} (C=O); δ_H(90 MHz; $CDCl_3$) 0.86 (6 H, q, *J* 7 Hz, 2 × Me), 1.25 and 1.27 (6 H, 2 t, *J* 7 Hz, 2 × $MeCH_2O$), 1.20–1.95 (7 H, overlapping m, 2 × CH_2, CH, and cyclopropyl CH_2), 2.92 (1 H, d, *J* 8 Hz, cyclopropyl CH) and 4.00–4.35 (4 H, m, 2 × $MeCH_2O$); GLC on 5% Carbowax on Chromosorb G at 150 °C, t_R 10.6 min (100%).

Diethyl 2-*phenylcyclopropane*-1,1-*dicarboxylate* (**2g**). From diethyl benzylidenemalonate (**1g**) (24.8 g, 0.10 mol), TMSI (22.1 g, 0.10 mol), NaH (2.4 g, 0.10 mol), and DMF (100 ml). Fractional distillation gave the title product (18 g, 60%) as a clear liquid, b.p. 135–136 °C/1 mmHg (lit.,[20] 141 °C/0.7 mmHg) (Found: C, 68.8; H, 7.0. Calc. for $C_{15}H_{18}O_4$: C, 68.7; H, 6.9%); ν_{max}(liq. film) 3 060 and 2 980 (CH), 1 735–1 720br (C=O) and 1 605 cm^{-1} (C=C); δ_H(250 MHz; $CDCl_3$) 0.85 and 1.29 (6 H, 2 t, *J* 7.1 Hz, 2 × $MeCH_2O$), 1.67–1.73 (1 H, dd, *H*CH), 2.15–2.20 (1 H, dd, HC*H*), 3.22 (1 H, t, *J* 8.6 Hz, CH), 3.84 and 4.24 (4 H, m, 2 × $MeCH_2O$) and 7.17–7.30 (5 H, m, Ph); δ_C(63 MHz; $CDCl_3$) 13.65 (q), 14.08 (q), 18.68 (dd, cyclopropyl CH_2), 32.12 (d), 37.47 (s), 61.07 (t), 61.63 (t), 127.26 (d), 128.07 (d), 128.57 (d), 134.74 (s), 166.59 (s) and 169.82 (s); GLC on Carbowax at 200 °C, t_R 22.3 (100%), on Apiezon at 150 °C, t_R 15.5 min (100%).

Diethyl 2,2-*dideuterio*-3-*phenylcyclopropane*-1,1-*dicarboxylate* [$^2H_2$]-(**2g**). From diethyl benzylidenemalonate (**1g**) (7.9 g, 32 mmol), fully deuteriated TMSI[21] (7.2 g, 33 mmol), NaH (0.9 g, 39 mmol), and DMF (100 ml). Fractional distillation gave the *cyclopropyl diester* [$^2H_2$]-(**2g**) (5.2 g, 61%) as a clear liquid, b.p. 122–124 °C/0.5 mmHg (Found: C, 68.4; H + D, 6.9. $C_{15}H_{16}D_2O_4$ requires C, 68.2, H + D, 6.8%); ν_{max}(liq. film) 3 060 and 2 980 (CH), 1 730br (C=O) and 1 600 cm^{-1} (C=C); δ_H(250 MHz; $CDCl_3$) 0.85 and 1.29 (6 H, 2 t, *J* 7.1 Hz, 2 × $MeCH_2O$), 3.21 (1 H, s, CH), 3.81 and 4.24 (4 H, 2 q, *J* 7.1 Hz, 2 × $MeCH_2O$), and 7.18–7.30 (5 H, m, Ph); δ_C(63 MHz; $CDCl_3$) 13.66 (q), 14.07 (q), 18.29 (m, CD_2), 32.03 (d), 37.36 (s), 61.04 (t), 61.61 (t), 127.27 (d), 128.09 (d), 128.63 (d), 134.81 (s), 166.58 (s) and 169.80 (s); GLC on Carbowax at 200 °C, t_R 14.8 min (100%).

Diethyl 2-(4-*chlorophenyl*)*cyclopropane*-1,1-*dicarboxylate* (**2h**). From diethyl 4-chlorobenzylidenemalonate (**1h**) (10.5 g, 37 mmol), TMSI (8.4 g, 38 mmol), NaH (0.9 g, 38 mmol), and DMF (100 ml). Fractional distillation gave the title product (22.7 g, 54%) as a clear liquid, b.p. 200 °C/1.0 mmHg (lit.,[22] 134 °C/0.3 mmHg); ν_{max}(liq. film) 3 060 and 2 980 (CH), 1 720br (C=O), and 1 635 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 0.87 and 1.23 (6 H, 2 t, *J* 7 Hz, 2 × $MeCH_2O$), 1.65 (1 H, dd, *H*CH), 2.16 (1 H, dd, HC*H*), 3.11 (1 H, t, *J* 10 Hz, CH), 3.82 and 4.10 (4 H, 2 q, *J* 7 Hz, 2 × $MeCH_2O$) and 7.15 (4 H, m, C_6H_4Cl).

Pyrimidine-5-*spirocyclopropane*-2,4,6(1H,3H,5H)-*trione* (**3**; R = H).—To a stirred suspension of urea (1.91 g, 32 mmol) in a mixture of diethyl cyclopropane-1,1-dicarboxylate (5.00 g, 27 mmol) and dry ethanol (20 ml) at 0 °C was added, dropwise during 30 min, a solution of sodium ethoxide [from sodium (1.81 g, 79 mmol) in ethanol (32 ml)]. The mixture was stirred for 2 h at 0 °C and at room temperature for a further 3 h. The partially solid product mixture was poured into HCl–ice (50 ml; 10%) and the precipitate was filtered off and dried. Soxhlet extraction of the solid with acetone, followed by recrystallisation of the acetone-soluble material from ethanol, gave the title product (115 mg, 3%) as crystals, m.p. > 300 °C (decomp.) [lit.,[23] 320 °C (decomp.)] (Found: C, 46.8; H, 3.7; N, 17.8. Calc. for $C_6H_6N_2O_3$: C, 46.8; H, 3.7; N, 18.2%); ν_{max}(KCl) 3 190 and 3 140 (NH), 3 060 and 2 920 (CH), and 1 750, 1 715, and 1 670 cm^{-1} (C=O); δ_H[90 MHz; $(CD_3)_2SO$] 1.65 (4 H, s, 2 × CH_2) and 11.30 (2 H, s, 2 × NH).

2′-Ethylpyrimidine-5-*spirocyclopropane*-2,4,6(1H,3H,5H)-*trione* (**3a**).—To a stirred solution of diethyl 2-ethylcyclopropane-1,1-dicarboxylate (**2a**) (2.0 g, 10 mmol), urea (3.0 g, 50 mmol), and dimethyl sulphoxide (DMSO) (25 ml) at room temperature was added dropwise, during 30 min, a solution of Bu^{t}OK (2.2 g, 20 mmol) in DMSO (20 ml). After being stirred for 24 h the partially solid product mixture was poured into HCl–ice (100 ml; 10%), and the acidic aq. solution (pH 1–2) was extracted with diethyl ether (4 × 50 ml). The extract was dried (Na_2SO_4) and evaporated. Recrystallisation of the residue from ethyl acetate gave the *spiropyrimidinetrione* (**3a**) (0.34 g, 18%) as a solid, m.p. 179–182 °C (Found: C, 52.6; H, 5.3; N, 15.2. $C_8H_{10}N_2O_3$ requires C, 52.7; H, 5.5; N, 15.3%); ν_{max}(KCl) 3 180 and 3 060 (NH), 2 960 (CH) and 1 745, 1 720 and 1 680 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 0.85 (3 H, t, *J* 7.3 Hz, Me), 1.47–1.77 (2 H, m, CH_2), 1.97–2.07 (1 H, m, CH), 1.65 (1 H, dd, *H*CH), 1.86 (1 H, dd, HC*H*) and 11.23 (2 H, 2 × NH); δ_C[63 MHz; $(CD_3)_2SO$] 13.16 (q), 18.99 (t), 25.44 (t), 31.67 (s), 42.76 (d), 150.77 (s), 168.30 (s), and 170.18 (s).

Similarly prepared were:

2′-Isopropylpyrimidine-5-*spirocyclopropane*-2,4,6(1H,3H,5H)-*trione* (**3b**). From diethyl 2-isopropylcyclopropane-1,1-dicarboxylate (**2b**) (1.0 g, 4 mmol), urea (1.3 g, 22 mmol), DMSO (25 ml), and Bu^{t}OK (1.0 g, 9 mmol). Recrystallisation from acetone–hexane gave the *spiropyrimidinetrione* (**3b**) (1.82 g, 71%) as a solid, m.p. 197–198 °C (Found: C, 54.8; H, 6.1; N, 14.2. $C_9H_{12}N_2O_3$ requires C, 55.1; H, 6.1; N, 14.3%); ν_{max}(mull) 3 200 and 3 060 (NH), and 1 745, 1 720 and 1 670 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 0.78 (3 H, d, *J* 5.8 Hz, Me), 0.99 (3 H, d, *J* 6.0 Hz, Me), 1.66–1.87 (4 H, m, CH and cyclopropyl $CHCH_2$) and 11.31 (2 H, s, 2 × NH, D_2O exchangeable); δ_C[63 MHz; $(CD_3)_2SO$] 21.33 (q), 21.92 (q), 24.93 (t), 25.25 (d), 32.15 (s), 48.56 (d), 150.78 (s), 169.41 (s) and 170.12 (s); HPLC λ 275 nm; aq. sodium phosphate buffer–methanol (4:1) (pH 3), t_R 10.3 min (100%).

2′-Propylpyrimidine-5-*spirocyclopropane*-2,4,6(1H,3H,5H)-*trione* (**3c**). From diethyl 2-propylcyclopropane-1,1-dicarboxylate (**2c**) (2.4 g, 10 mmol), urea (3.0 g, 50 mmol), DMSO (25 ml), and Bu^{t}OK (2.2 g, 19 mmol). Recrystallisation from acetone–hexane gave the *spiropyrimidinetrione* (**3c**) (0.23 g, 11%) as a solid, m.p. 184–187 °C (decomp.) (Found: C, 55.0; H, 6.0; N, 14.4%); ν_{max}(mull) 3 190 and 3 050 (NH) and 1 750, 1 725, and 1 680 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 0.82 (3 H, t, *J* 7.3 Hz, Me), 1.18–1.39 (2 H, m), 1.46–1.66 (3 H, m), 1.84–2.07 (2 H, m), and 11.29 (2 H, s, 2 × NH, D_2O exchangeable); δ_C[63 MHz; $(CD_3)_2SO$] 13.23 (q), 21.55 (t), 25.21 (t), 27.30 (dt, cyclopropyl CH_2), 31.57 (s), 40.79 (d), 150.70 (s), 168.26 (s) and 170.10 (s); HPLC λ 275 nm; aq. sodium phosphate buffer–methanol (4:1) (pH 3), t_R 11.7 min (100%).

2′-Isobutylpyrimidine-5-*spirocyclopropane*-2,4,6(1H,3H,5H)-*trione* (**3d**). From diethyl 2-isobutylcyclopropane-1,1-dicarboxylate (**2d**) (2.4 g, 10 mmol), urea (3.1 g, 52 mmol), DMSO (25 ml) and Bu^{t}OK (2.1 g, 19 mmol). Recrystallisation from acetone–hexane gave the *spiropyrimidinetrione* (**3d**) (0.85 g, 41%) as a solid, m.p. 206–209 °C (Found: C, 56.6; H, 6.1; N, 13.2. $C_{10}H_{14}N_2O_3$ requires C, 57.1; H, 6.7; N, 13.3%); ν_{max}(mull) 3 190 and 3 030 (NH) and 1 755, 1 725 and 1 680 cm^{-1} (C=O);

δ_H[250 MHz; $(CD_3)_2SO$] 0.85 (6 H, dt, 2 × Me), 1.45–1.70 (4 H, m), 1.95 (2 H, s), and 11.25 (2 H, s, 2 × NH, D_2O exchangeable); δ_C[63 MHz; $(CD_3)_2SO$] 21.77 (q), 21.96 (q), 25.14 (t), 27.53 (d), 31.38 (s), 33.89 (dt, cyclopropyl CH_2), 39.56 (d), 150.68 (s), 168.32 (s), and 170.13 (s); HPLC λ 275 nm; aq. sodium phosphate buffer–methanol (4:1) (pH 3), t_R 21.5 min (100%).

2′-Butylpyrimidine-5-spirocyclopropane-2,4,6(1H,3H,5H)-*trione* (**3e**). From diethyl 2-butylcyclopropane-1,1-dicarboxylate (**2e**) (2.0 g, 9 mmol), urea (2.7 g, 45 mmol), DMSO (25 ml), and Bu^{t}OK (2.2 g, 19 mmol). Recrystallisation from acetone–hexane gave the *spiropyrimidinetrione* (**3e**) (0.5 g, 26%) as a solid, m.p. 188–192 °C (decomp.) (Found: C, 57.5; H, 6.6; N, 13.4%); ν_{max}(KCl) 3 180 and 3 050 (NH), 2 950 (CH) and 1 745, 1 720, and 1 675 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 0.82 (3 H, t, *J* 7.3 Hz, Me), 1.22 (2 H, overlapping q, CH_2), 1.13–1.32 (2 H, m, CH_2), 1.56–1.66 (2 H, overlapping m, CH_2), 1.86 (1 H, dd, *H*CH), 1.96 (1 H, dd, HC*H*), 1.91–2.05 (1 H, m, CH), and 11.28 (2 H, s, 2 × NH); δ_C[63 MHz; $(CD_3)_2SO$] 13.70 (q), 21.58 (t), 25.08 (t), 25.34 (t), 30.65 (t), 31.71 (s), 41.16 (d), 150.74 (s), 168.26 (s) and 170.13 (s).

[*2′-(1-Ethylpropyl)*]*pyrimidine-5-spirocyclopropane*-2,4,6-(1H,3H,5H)-*trione* (**3f**). From diethyl 2-(1-ethylpropyl)-cyclopropane-1,1-dicarboxylate (**2f**) (2.0 g, 9 mmol), urea (2.2 g, 36 mmol), DMSO (25 ml), and Bu^{t}OK (2.0 g, 18 mmol). Recrystallisation from acetone–hexane gave the *spiropyrimidinetrione* (**3f**) (0.5 g, 25%) as a solid, m.p. 169–174 °C (Found: C, 58.9; H, 6.7; N, 12.3. $C_{11}H_{16}N_2O_3$ requires C, 58.9; H, 7.1; N, 12.5%); ν_{max}(KCl) 3 190 and 3 060 (NH), 2 960 (CH) and 1 745, 1 720 and 1 680 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 0.70 and 0.89 (6 H, 2 t, *J* 7.5 Hz, 2 × Me), 1.09–1.46 (4 H, overlapping m, 2 × CH_2), 1.50 (1 H, overlapping m, CH), 1.70 (1 H, dd, CH), 1.74 (1 H, m, *H*CH), 1.92 (1 H, dd, HC*H*), and 11.32 (2 H, s, 2 × NH); δ_C[63 MHz; $(CD_3)_2SO$] 10.43 (q), 11.08 (q), 24.78 (t), 25.84 (t), 26.33 (t), 31.37 (s), 36.52 (d), 45.95 (d), 150.68 (s), 168.59 (s) and 170.28 (s).

2′-Phenylpyrimidine-5-spirocyclopropane-2,4,6(1H,3H,5H)-*trione* (**3g**). From diethyl 2-phenylcyclopropane-1,1-dicarboxylate (**2g**) (2.8 g, 12 mmol), urea (2.8 g, 47 mmol), DMSO (25 ml) and Bu^{t}OK (1.7 g, 15 mmol). Recrystallisation from acetone–hexane gave the *spiropyrimidinetrione* (**3g**) (0.94 g, 54%) as a solid, m.p. > 300 °C (decomp.) (Found: C, 62.6; H, 4.3; N, 12.1. $C_{12}H_{10}N_2O_3$ requires C, 62.6; H, 4.4; N, 12.1%); ν_{max}(mull) 3 180 and 3 060 (NH), and 1 740–1 680 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 2.15 (1 H, dd, *H*CH), 2.35 (1 H, dd, HC*H*), 3.27 (1 H, t, CH), 7.21–7.34 (5 H, m, Ph), 11.04 and 11.38 (2 H, 2 s, 2 × NH, D_2O exchangeable); δ_C[63 MHz; $(CD_3)_2SO$] 21.27 (t), 34.93 (s), 93.26 (d), 127.39 (d), 127.63 (d), 129.80 (d), 133.29 (s), 150.80 (s), 166.06 (s) and 169.45 (s); HPLC λ 275 nm; aq. sodium phosphate buffer–methanol (4:1) (pH 3), t_R 14.8 min (100%).

2′,2′-Dideuterio-3′-phenylpyrimidine-5-spirocyclopropane-2,4,6(1H,3H,5H)-*trione*[$^2H_2$]-(**3g**). From diethyl 2,2-dideuterio-3-phenylcyclopropane-1,1-dicarboxylate [$^2H_2$]-(**2g**) (2.2 g, 8 mmol), urea (2.5 g, 42 mmol), DMSO (25 ml), and Bu^{t}OK (1.9 g, 17 mmol). Recrystallisation from acetone–hexane gave the *spiropyrimidinetrione* [$^2H_2$]-(**3g**) (1.4 g, 74%) as crystals, m.p. > 300 °C (decomp.) (Found: C, 62.7; H + D, 4.2; N, 12.1. $C_{12}H_8D_2N_2O_3$ requires C, 62.6; H + D, 4.3; N, 12.1%); ν_{max}(KCl) 3 180 (NH), 3 000 (CH) and 1 705, 1 720 and 1 680 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 3.27 (1 H, s, CH), 7.19–7.41 (5 H, m, Ph) and 11.04 and 11.38 (2 H, 2 s, 2 × NH, D_2O exchangeable); δ_C[63 MHz; $(CD_3)_2SO$] 20.86 (m, CD_2), 34.73 (s), 43.10 (d), 127.30 (d), 127.55 (d), 129.73 (d), 133.20 (s), 150.68 (s), 165.96 (s) and 169.35 (s); HPLC λ 275 nm; 9:1 pH 4.7 aq. sodium phosphate buffer–methanol (9:1) (pH 4.7), t_R 16.3 min (100%).

5-Benzylidenepyrimidine-2,4,6(1H,3H,5H)-*trione* (**4a**).—To a stirred solution of barbituric acid (2 g, 16 mmol) in aq. HCl (150 ml; 13%) was added benzaldehyde (1.65 g, 16 mmol) at room temperature. The precipitated product was filtered off and washed successively with hot water, ethanol, and diethyl ether. The dried title product (2.2 g, 66%) was collected as a solid, m.p. 254–260 °C (lit.,[24] 256 °C) (Found: C, 60.6; H, 3.3; N, 13.0. Calc. for $C_{11}H_8N_2O_3$: C, 61.1; H, 3.7; N, 12.9%); λ_{max}(EtOH) 233, 262 and 327 nm; ν_{max}(KCl) 3 210 and 3 080 (NH), 1 730br and 1 675br (C=O) and 1 640 cm^{-1} (C=C); δ_H[90 MHz; $(CD_3)_2SO$] 7.60 and 8.20 (5 H, m, Ph), 8.36 (1 H, s, CH) and 11.26 and 11.42 (2 H, 2 s, 2 × NH).

Similarly prepared were:

5-(4-Chlorobenzylidene)pyrimidine-2,4,6(1H,3H,5H)-*trione* (**4b**). From barbituric acid (2 g, 16 mmol), aq. HCl (150 ml; 13%), and 4-chlorobenzaldehyde (2.81 g, 20 mmol). The title product (3.5 g, 88%) was collected as a solid, m.p. 273–275 °C (decomp.) (lit.,[25] 271 °C); ν_{max}(KCl) 3 200 and 3 090 (NH), 1 750, 1 700 and 1 675 (C=O) and 1 590 cm^{-1} (C=C); δ_H[90 MHz; $(CD_3)_2SO$] 7.52 and 8.10 (4 H, 2 d, C_6H_4Cl), 8.28 (1 H, s, CH) and 11.25 and 11.40 (2 H, 2 s, 2 × NH, D_2O exchangeable).

5-(4-Fluorobenzylidene)pyrimidine-2,4,6(1H,3H,5H)-*trione* (**4c**). From barbituric acid (0.5 g, 4 mmol), aq. HCl (50 ml; 13%), and 4-fluorobenzaldehyde (0.48 g, 4 mmol). The *pyrimidinetrione* (**4c**) (0.65 g, 72%) was collected as a cream coloured solid, m.p. > 300 °C (decomp.) (Found: C, 56.0; H, 2.7; N, 11.8. $C_{11}H_7FN_2O_3$ requires C, 56.4; H, 3.0; N, 12.0%); ν_{max}(KCl) 3 205 and 3 080 (NH), 1 740, 1 695 and 1 670 (C=O) and 1 590 cm^{-1} (C=C); δ_H[90 MHz; $(CD_3)_2SO$] 7.30 and 8.22 (4 H, 2 t, C_6H_4F), 8.30 (1 H, s, CH) and 11.23 and 11.38 (2 H, 2 s, 2 × NH).

5-(4-Methylbenzylidene)pyrimidine-2,4,6(1H,3H,5H)-*trione* (**4d**). From barbituric acid (2 g, 16 mmol), aq. HCl (100 ml; 13%), and 4-methylbenzaldehyde (2.4 g, 20 mmol). The title product (3.37 g, 94%) was collected as a cream coloured solid, m.p. 268–273 °C (lit.,[26] 275 °C) (Found: C, 62.4; H, 3.9; N, 21.1. Calc. for $C_{12}H_{10}N_2O_3$: C, 62.6; H, 4.3; N, 12.2%); λ_{max}(EtOH) 232, 262 and 343 nm; ν_{max}(KCl) 3 200 and 3 080 (NH), 1 745, 1 700 and 1 670 (C=O) and 1 580 cm^{-1} (C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.36 (3 H, s, Me), 7.28 (2 H, d, *J* 8.1 Hz, 2 × CH), 8.07 (2 H, d, *J* 7.9 Hz, 2 × CH), 8.23 (1 H, s, vinylic CH) and 11.21 and 11.36 (2 H, 2 s, 2 × NH).

2′-(4-Chlorophenyl)pyrimidine-5-spirocyclopropane-2,4,6-(1H,3H,5H)-*trione* (**3h**).—To a stirred solution of NaH (1.32 g, 55 mmol) in DMF (100 ml) was added, under nitrogen at room temperature, TMSI (12.32 g, 56 mmol) in a single portion. Vigorous evolution of hydrogen lasted *ca.* 5 min. After a further 15 min, a solution of 5-(4-chlorobenzylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**4b**) (1.60 g, 6 mmol) in DMF (50 ml) was added in a single portion, and the mixture was stirred for 1 h. The product solution was poured into HCl–ice (100 ml; 10%) and the resultant acidic solution (pH 3) was extracted with diethyl ether (3 × 100 ml). The extract was dried (Na_2SO_4) and evaporated. Recrystallisation of the residue from acetone–hexane gave the *spiropyrimidinetrione* (**3h**) (0.28 g, 17%) as a solid, m.p. 198–201 °C (Found: C, 54.4; H, 3.1; Cl, 13.7; N, 10.4. $C_{12}H_9ClN_2O_3$ requires C, 54.4; H, 3.4; Cl, 13.4; N, 10.6%); ν_{max}(KCl) 3 200 and 3 050 (NH) and 1 740, 1 690 and 1 640 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 2.13 (1 H, dd, *H*CH), 2.32 (1 H, dd, HC*H*), 3.26 (1 H, dd, CH), 7.33 (4 H, dd, C_6H_4Cl) and 11.07 and 11.39 (2 H, 2 s, 2 × NH); δ_C[63 MHz; $(CD_3)_2SO$] 21.61 (t), 34.81 (s), 42.00 (d), 127.57 (d), 131.74 (d), 132.10 (s), 132.52 (s), 150.71 (s), 166.09 (s) and 169.22 (s).

Similarly prepared were:

2′-(4-Fluorophenyl)pyrimidine-5-spirocyclopropane-2,4,6-(1H,3H,5H)-*trione* (**3i**). From 5-(4-fluorobenzylidine)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**4c**) (0.45 g, 0.2 mmol), TMSI

(0.66 g, 0.3 mmol), NaH (0.20 g, 8.3 mmol), and DMF (100 ml). Recrystallisation from acetone–hexane gave the *spiropyrimidinetrione* (**3i**) (51 mg, 1%) as a cream coloured solid, m.p. > 300 °C (decomp.) (Found: C, 57.5; H, 3.5; N, 11.2. $C_{12}H_9FN_2O_3$ requires C, 58.0; H, 3.6; N, 11.3%); ν_{max}(KCl) 3 190 and 3 060 (NH), 1 745, 1 720 and 1 680 (C=O) and 1 605 cm^{-1} (C=C); δ_H[250 MHz; $(CD_3)_2SO$] 2.14 (1 H, dd, *H*CH), 2.33 (1 H, dd, HC*H*), 3.26 (1 H, dd, CH), 7.08 and 7.37 (4 H, 2 dd, C_6H_4F) and 11.06 and 11.38 (2 H, 2 s, 2 × NH).

2′(4-*Methylphenyl*)*pyrimidine*-5-*spirocyclopropane*-2,4,6-(1H,3H,5H)-*trione* (**3j**). From 5-(4- methylbenzylidene)-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**4d**) (2 g, 8 mmol), TMSI (4 g, 18 mmol), NaH (0.38 g, 16 mmol), and DMF (50 ml). Recrystallisation from acetone gave the *spiropyrimidinetrione* (**3j**) (1.1 g, 56%) as a solid, m.p. >300 °C (decomp.) (Found: C, 63.6; H, 4.6; N, 11.3. $C_{13}H_{12}N_2O_3$ requires C, 63.9; H, 4.9; N, 11.5%); ν_{max}(KCl) 3 180 and 3 100–3 030 (NH), 2 920 (CH) and 1 745, 1 720 and 1 690 cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 2.13 (1 H, dd, *H*CH), 2.33 (1 H, dd, HC*H*), 2.24 (3 H, s, Me), 3.22 (1 H, t, CH), 7.06 (2 H, d, *J* 7.9 Hz, 2 × CH), 7.20 (2 H, d, *J* 8.1 Hz, 2 × CH) and 11.01 and 11.37 (2 H, 2 s, 2 × NH); δ_C[63 MHz; $(CD_3)_2SO$] 20.68 (q), 21.08 (t), 35.14 (s), 43.47 (d), 128.24 (d), 129.70 (d), 130.13 (s), 136.70 (s), 150.74 (s), 165.87 (s) and 169.35 (s).

Pyrazolidine-4-*spirocyclopropane*-3,5-*dione* (**5a**).—A mixture of hydrazine hydrate (2 ml, 0.04 mol) and diethyl cyclopropane-1,1-dicarboxylate (3.72 g, 0.02 mol) was heated at 110 °C for 10 min. Ethanolic sodium ethoxide [from sodium (0.46 g, 0.01 mol) in ethanol (10 ml)] was then added dropwise to the mixture, which was then heated for 6 h. The product solution was cooled, then filtered, and the filtrate was evaporated. The residue was dissolved in water and acidified to give the product as a precipitate. Recrystallisation from ethanol gave the *spiropyrazolidinetrione* (**5a**) (0.4 g, 16%) as a solid, m.p. > 300 °C (decomp.) (Found: C, 47.8; H, 4.7; N, 22.9. $C_5H_6N_2O_2$ requires C, 47.6; H, 4.8; N, 22.2%); ν_{max}(KCl) 3 150br (NH) and 1 650br cm^{-1} (C=O); δ_H[90 MHz; $(CD_3)_2SO$] 1.42 (4 H, s, 2 × CH_2) and 10.41 (2 H, br s, 2 × NH).

1-*Phenylpyrazolidine*-4-*spirocyclopropane*-3,5-*dione* (**5b**).—To a stirred solution of diethyl cyclopropane-1,1-dicarboxylate (2.0 g, 10.8 mmol) and phenylhydrazine (3.5 g, 32.4 mmol) in dry DMSO (25 ml) under nitrogen was added dropwise a solution of Bu^{t}OK (3.0 g, 27 mmol) in dry DMSO (20 ml) during 30 min. After the mixture had been stirred at 40 °C for 60 h, the gelatinous product was poured into HCl–ice (100 ml; 10%) and extracted with diethyl ether. The extract was dried (Na_2SO_4) and evaporated. Recrystallisation of the residue from acetone–hexane gave the *spiropyrazolidinedione* (**5b**) (0.34 g, 24%) as cream coloured crystals, m.p. 207–213 °C (Found: C, 65.1; H, 5.0; N, 14.0. $C_{11}H_{10}N_2O_2$ requires C, 65.3; H, 5.0; 13.9%); ν_{max}(KCl) 3 220 (NH), 3 070 and 3 010 (CH), 1 730 and 1 685 (C=O), and 1 600 cm^{-1} (C=C); δ_H[90 MHz; $(CD_3)_2SO$] 1.65 (4 H, s, 2 × CH_2), 7.10–7.80 (5 H, m, Ph), and 11.50 (1 H, br s, NH).

Hydantoin-5-*spirocyclopropane* (*Imidazolidine*-4-*spirocyclopropane*-2,5-*dione*) (**6**).—To a stirred solution of sodium methoxide [from sodium (17 g, 0.74 mol) in methanol (250 ml)] at 0 °C was added *N*,*N*′-dibromocyclopropane-1,1-dicarboxamide (53.7 g, 0.19 mol) in a single portion. The temperature was allowed to rise and the solution boiled rapidly as NaBr precipitated. After being heated for 5 min on a steam-bath the solution was neutralised (AcOH) and the solvent was evaporated off. The residue was then extracted with acetone. Recrystallisation of the acetone-soluble material from hot water gave the title product (7.59 g, 32%) as a solid, m.p. 216–221 °C (lit.,[8] 214 °C); ν_{max}(KCl) 3 370br and 3 200br (NH), 3 060 (CH) and 1 720br and 1 670 cm^{-1} (C=O); δ_H[90 MHz; $(CD_3)_2SO$] 1.15 (4 H, s, 2 × CH_2) and 7.98 and 10.70 (1 H, 2 br s, 2 × NH).

3-*Ethoxycarbonylmethylhydantoin*-5-*spirocyclopropane* [*Ethyl* (2,5-*Dioxoimidazolidine*-4-*spirocyclopropan*-1-*yl*)*acetate*] (**7a**).—To a stirred solution of sodium ethoxide [from sodium (0.23 g, 10 mmol) in ethanol (30 ml)] under nitrogen was added hydantoin-5-spirocyclopropane (**6**) (1.0 g, 7.9 mmol) in a single portion. After 1 h at room temperature, the solution was heated at 70 °C for 20 min, and ethyl bromoacetate (2.0 g, 12 mmol) was added in a single portion. After 20 h the product solution was cooled, filtered, and evaporated. The residual oil was dissolved in chloroform, washed with water, dried (Na_2SO_4), and evaporated. Trituration of the residual oil with diethyl ether gave the *title hydantoin* (**7a**) (247 mg, 15%) as a solid, m.p. 115–120 °C (Found: C, 50.9; H, 5.6; N, 13.2. $C_9H_{12}N_2O_4$ requires C, 50.9; H, 5.7; N, 13.2%); ν_{max}(KCl) 3 200br (NH), 3 100 and 2 980 (CH), 1 755, 1 745 and 1 705 cm^{-1} (C=O); δ_H(90 MHz; $CDCl_3$) 1.27 (3 H, q, *J* 7 Hz, Me), 1.32 (4 H, s, 2 × CH_2), 4.16 (2 H, q, *J* 7 Hz, MeCH_2O), 4.21 (2 H, s, NCH_2) and 8.54 (1 H, s, NH).

Similarly prepared was:

3-*Phenethylhydantoin*-5-*spirocyclopropane* (1-*Phenethylimidazolidine*-4-*spirocyclopropane*-2,5-*dione*) (**7b**). From hydantoin-5-spirocyclopropane (**6**) (0.70 g, 3.5 mmol), (2-bromoethyl)-benzene (1.11 g, 6.0 mmol), and sodium ethoxide [from sodium (0.13 g, 5 mmol) in ethanol (30 ml)]. Recrystallisation from aq. ethanol gave the *title hydantoin* (**7b**) (0.19 mg, 15%) as a yellow solid, m.p. 94–98 °C (Found: C, 67.9; H, 6.4; N, 12.0. $C_{13}H_{14}N_2O_4$ requires C, 67.8; H, 6.1; N, 12.2%); ν_{max}(KCl) 3 200 (NH), 3 100 and 2 950 (CH), 1 765 and 1 705 (C=O), and 1 600 cm^{-1} (C=C); δ_H(90 MHz; $CDCl_3$) 1.15 (4 H, s, 2 × CH_2), 2.80 and 3.60 (4 H, 2 t, *J* 8 Hz, PhCH_2CH_2), 7.19 (5 H, s, Ph) and 8.22 (1 H, s, NH).

5-(2-*Methoxy*-2-*phenylethyl*)*pyrimidine*-2,4,6(1H,3H,5H)-*trione*.—Recrystallisation of 2′-phenylpyrimidine-5-spirocyclopropane-2,4,6(1*H*,3*H*,5*H*)-trione (**3g**) (110 mg, 0.5 mmol) from acetone–methanol gave the *title pyrimidinetrione* (12 mg, 10%) as crystals, m.p. > 300 °C (decomp.) (Found: C, 59.1; H, 4.9; N, 10.5. $C_{13}H_{14}N_2O_4$ requires C, 59.1; H, 5.3; N, 10.7%); ν_{max}(KCl) 3 220 and 3 100 (NH), 3 020 and 2 940 (CH), and 1 730–1 650br cm^{-1} (C=O); δ_H[90 MHz; $(CD_3)_2SO$] 2.30 (2 H, m, CH_2), 3.02 (3 H, s, OMe), 3.60 (1 H, m, CH), 4.30 (1 H, dd, C*H*OMe), 7.3 (5 H, m, Ph), and 11.06 and 11.15 (2 H, 2 s, 2 × NH, D_2O exchangeable); δ_C[63 MHz; $(CD_3)_2SO$] 35.78 (t), 44.75 (d), 56.03 (q), 79.92 (d), 126.92 (d), 127.64 (d), 128.33 (d), 140.93 (s), 150.72 (s), 169.88 (s) and 170.35 (s).

5-(1,1-*Dideuterio*-2-*methoxy*-2-*phenylethyl*)*pyrimidine*-2,4,6(1H,3H,5H)-*trione*.—Recrystallisation of 2′,2′-dideuterio-3′-phenylpyrimidine-5-spirocyclopropane-2,4,6(1*H*,3*H*,5*H*)-trione (0.6 mg, 2.6 mmol) from methanol gave the title pyrimidinetrione (0.11 mg, 19%) as crystals, m.p. > 300 °C (decomp.) (Found: C, 59.0; H + D, 5.2; N, 10.5. $C_{13}H_{12}D_2N_2O_4$ requires C, 59.1; H + D, 5.3; N, 10.6%); ν_{max}(KCl) 3 210 and 3 100 (NH), 3 020 and 2 920 (CH), and 1 720–1 680br cm^{-1} (C=O); δ_H[250 MHz; $(CD_3)_2SO$] 2.96 (3 H, s, OMe), 3.58 (1 H, s, CH, D_2O exchangeable), 4.24 (1 H, s, C*H*OMe), 7.23–7.39 (5 H, m, Ph) and 11.09 and 11.20 (2 H, 2 s, 2 × NH, D_2O exchangeable); δ_C[63 MHz; $(CD_3)_2SO$] 35.27 (m, CD_2), 44.63 (d), 56.04 (q), 79.83 (d), 126.30 (d), 127.64 (d), 128.35 (d), 140.93 (s), 150.76 (s), 169.91 (s) and 170.38 (s); HPLC λ 275 nm; aq. sodium phosphate buffer–methanol (9:1) (pH 4.7), t_R 16.9 min (100%).

Enzyme Studies.—Studies of the inhibition of dihydroorotate dehydrogenase by the spirocyclopropanobarbiturates

(**3**) and the arylidenebarbiturates (**4**) were carried out using the procedure for time-dependent assays of inhibitors described in our previous paper.[3] The K_i-values recorded in the Table therefore correspond to the binary complex of enzyme and inhibitor. The enzyme used was from *Zymobacterium* (*Clostridium*) *oroticum* (Sigma, lot 74F-6833).

Unlike the 5-arylmethylhydantoins described in our earlier papers,[1,3] the spirocyclopropanobarbiturates did not exhibit pure first-order kinetics. Thus plots of ln a/a_0 *vs.* time (min) consisted of an initial linear part, which extended over at least one half-life, followed by a curved region. This seemed to indicate biphasic kinetics. The inhibition data given in the Table are derived from measurements corresponding to the initial phase of the reaction.

Similar methods were used to probe the possibility of time-dependent inhibition by the pyrazoles (**5**) and the spirocyclopropanohydantoins (**6**) and (**7**) but none was observed. In these cases 10% (v/v) DMSO was used as a co-solvent in the phosphate buffer solution.

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