Antitumour imidazotetrazines. Part 36.[1] Conversion of 5-amino-imidazole-4-carboxamide to imidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-ones and imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones related in structure to the antitumour agents temozolomide and mitozolomide

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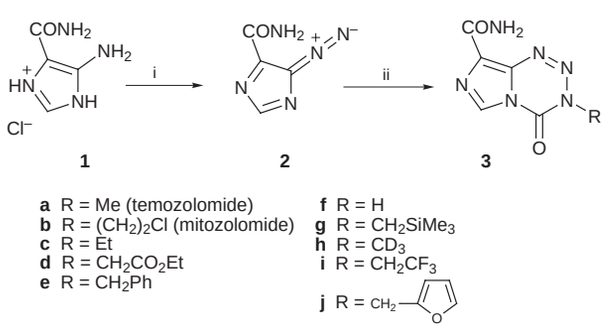
Novel 3-substituted imidazo[5,1-*d*][1,2,3,5]tetrazinones 3 have been prepared by two routes: reaction of 5-diazoimidazole-4-carboxamide 2 and isocyanates, and nitrosative cyclisation of 5-amino-1-carbamoyl-imidazole-4-carboxamides 7. The latter cyclisations do not proceed efficiently when the 1-carbamoyl group bears an electron-donating alkyl group. 5-Amino-1-carbamoylimidazole-4-carboxamides 7 cyclise with triethyl orthoformate or triethyl orthobenzoate to yield imidazo[1,5-*a*][1,3,5]triazinones 15. A ^{1}H NMR study of the decomposition of 8-carbamoyl-3-ethylimidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-one 3c in deuteriated phosphate buffer has shown that its ethylating capacity is attenuated by the unproductive generation of ethene. This observation explains why the ethylimidazotetrazine possesses weaker antitumour properties than the clinically-used congener temozolomide 3a.

Introduction

The significant molecular milestones leading to the discovery of the antitumour imidazotetrazinones temozolomide **3a** and mitozolomide **3b** can be traced back over several decades.[2] The molecules have 'evolved' from simple bicyclic polyaza-heterocycles bearing bridgehead nitrogen atoms which were synthesised in the 1970s,[3] with the first examples of the imidazo[5,1-*d*][1,2,3,5]tetrazine ring system being reported in 1984.[4] Initial interest in mitozolomide, the agent originally selected for clinical trial, was undermined by its unpredictable and life-threatening toxicity to the human bone marrow,[5] whereas the emergenge of temozolomide into the spotlight[6] was encouraged by a relatively clean toxicity profile and clear evidence of efficacy against brain tumours[7] and malignant melanoma.[8]

The original route to temozolomide started with 5-amino-imidazole-4-carboxamide (AIC), commercially available as a hydrochloride salt **1**. This was converted to 5-diazoimidazole-4-carboxamide **2** (Scheme 1) which reacted slowly with methyl, 2-chloroethyl and ethyl isocyanates in ethyl acetate at 25 °C to afford the imidazotetrazines **3a–c** in high yields.[4] In recent years we have focused our chemical efforts on developing several alternative routes for the synthesis of temozolomide, which obviate the use of methyl isocyanate. These efforts have met with mixed success: thus although the ester **3d** could be hydrolysed to the corresponding acetic acid and decarboxylated to temozolomide in poor yield using Barton radical methodology,[9] we were unable to effect removal of the benzyl group of the benzylimidazotetrazine **3e** to afford the potentially valuable synthon 'nor-temozolomide' **3f**, which might have afforded temozolomide on methylation. On the other hand, removal of the protecting silyl group of the (trimethylsilylmethyl)imidazo-tetrazine **3g** with tetrabutylammonium fluoride proceeded efficiently in acetonitrile–acetic acid to afford temozolomide in 78% yield.[9]



Scheme 1 *Reagents and conditions*: i, excess $NaNO_2$, 0–5 °C; ii, RNCO, EtOAc–DMSO, 25 °C

We now report the synthesis of three novel 3-substituted analogues of temozolomide and mitozolomide which were required to test our current hypothesis on the mode of action of this type of agent.[10] We also give full details of the scope of a new synthesis of imidazo[1,2,3,5]tetrazinones[11] and related imidazo[1,3,5]triazinones.

Results and discussion

Synthesis of imidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-ones

For the synthesis of isocyanates **5a–c** that were not commercially available we found that the best approach was to react *N*,*N*-diphenylcarbamoyl chloride with [$^2H_3$]methylamine, 2,2,2-trifluoroethylamine and furfurylamine, respectively, in base to form the diphenylureas **4a–c**. Thermolysis of the ureas above 200 °C liberated the isocyanates **5a–c** (Scheme 2). The volatile isocyanates were condensed in a cold trap and reacted immediately with 5-diazoimidazole-4-carboxamide **2** in DMSO to give the imidazotetrazines **3h–j**. Similarly, 1,3-bis(isocyanatomethyl)benzene reacted with the diazoimidazole **2** (2.5 equiv.) in dry DMSO to afford impure bis(imidazotetrazine) **6**. No improvement in purity was achieved by using alternative

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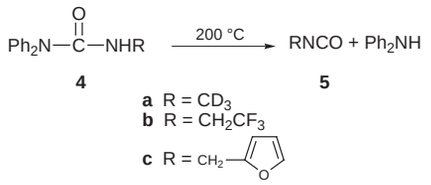
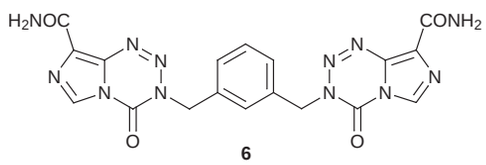


Scheme 2

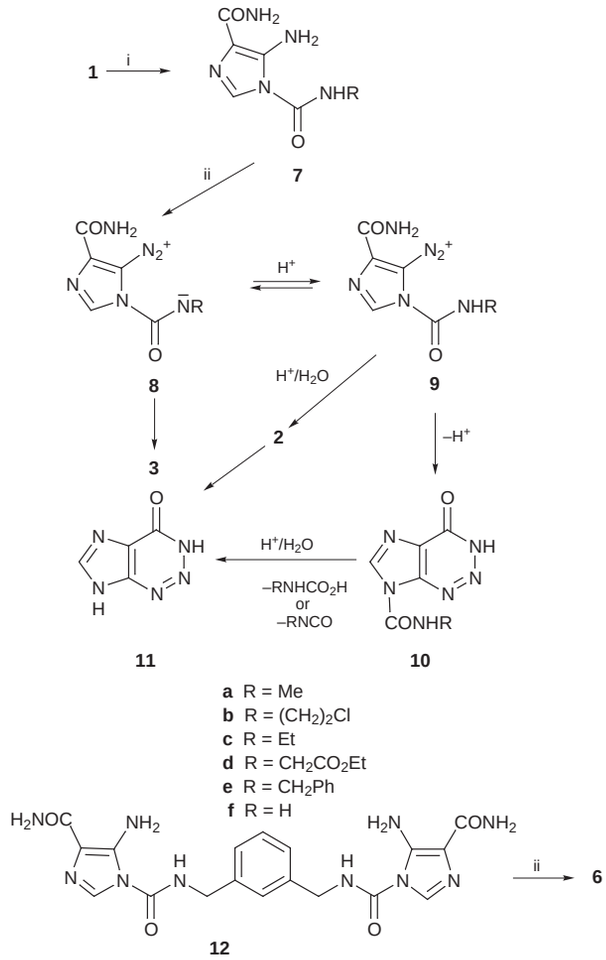


reaction solvents such as hexamethylphosphoramide or *N*-methylpyrrolidin-2(1*H*)-one. A pure sample (55%) of **6** was secured by crystallisation of the crude bis(imidazotetrazine) from formic acid. This is the first reported example of a bis(imidazotetrazine) but Ege and his colleagues have published previously on the use of diisocyanates in the synthesis of related bis(pyrazolotetrazines).[12] Whereas monofunctional aryl isocyanates cyclise efficiently with the diazoimidazole **2** to afford 3-arylimidazotetrazinones,[4] the aromatic bis(isocyanates) 1,3- and 1,4-bis(isocyanato)benzene failed to cyclise with diazoimidazole **2** and 2-azahypoxanthine **11** was the only isolated product.

Ege and colleagues also showed that alkyl and aryl isocyanates reacted regioselectively with substituted 5-amino-1*H*-pyrazoles: the products were pyrazoles carbamoylated at *N* which subsequently underwent nitrosative cyclisation to pyrazolotetrazines.[12] We have published preliminary work [11] on a new route to antitumour imidazotetrazines involving the initial conversion of AIC to ureas **7**. Although an attractive 'paper route' to prepare temozolomide **3a**, this route proved problematic in practice. The urea **7a** could be prepared in high yield by several routes but perversely the nitrosative ring closure was inefficient and, despite a comprehensive yield optimisation programme, the maximum yield of temozolomide secured in the final step was only 45%.[1]

In the present study, reaction of AIC free base and a series of isocyanates in DMSO at room temperature, or acetonitrile in the temperature range −10 to +20 °C, furnished the ureas **7b**–**e** generally in yields of >80%. Notably, no evidence for the formation of isomeric ureas in the crude products (by TLC) was adduced despite the availability of two realistic competing sites for carbamoylation. It is noteworthy that AIC reacts with methyl isothiocyanate [11] and aryl isothiocyanates [13] at the exocyclic amino group. The unsubstituted urea **7f** was formed from AIC and trimethylsilyl isocyanate.

Practicable yields of imidazotetrazines **3** from the nitrosation of ureas **7** using sodium nitrite in 2 M hydrochloric acid were not realised when the 1-carbamoyl substituent bore an electron-donating alkyl group. Thus the ethyl urea **7c** behaved like the corresponding methyl analogue **7a** [1] and afforded the 3-ethylimidazotetrazinone **3c** in only 30% yield together with the by-product 2-azahypoxanthine **11** (25%). The azahypoxanthine presumably arises from a competing cyclisation at the 4-carbamoyl group of the putative intermediate **8c** to give the 9-(*N*-ethylcarbamoyl)-2-azahypoxanthine **10c**, which then decomposed either by elimination of ethyl isocyanate or hydrolysis under the reaction conditions (2 M HCl) to afford **11** (Scheme 3). On the other hand the ureas **7b**,**d**,**e** gave cyclic products **3b**,**d**,**e** in 70, 93 and 88% recovered yields, respectively. Evidently there was a competition between two possible routes for cyclisation of the intermediates **8a**–**e** (Scheme 3), the balance of which appeared to be controlled by the pK_a of the



Scheme 3 *Reagents and conditions*: i, RNCO, DMSO or MeCN, Et_3N, 20 °C; ii, $NaNO_2$ (excess) in 2 M HCl, 0–20 °C

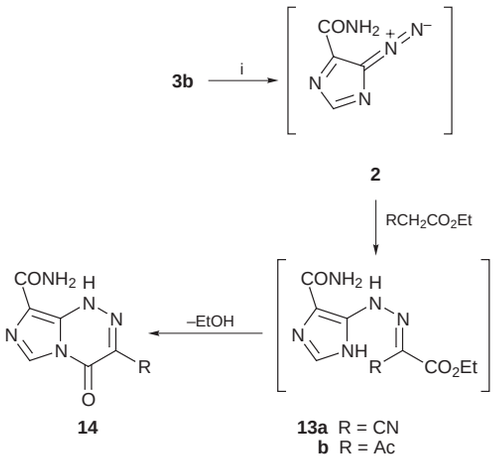
urea. When group R was a simple electron-donating Me or Et group (**8a**,**c**), partial protonation of the urea forced cyclisation to the route which yielded predominantly azahypoxanthine **11**, either *via* intermediate **2** or **10**. In the other cases (**8b**,**d**,**e**), where group R bore an electron-withdrawing (Cl or CO_2Et) or neutral (Ph) group attached to the alkyl residue, presumably the pK_a of the urea was lowered sufficiently that appreciable protonation did not occur and thus allowed efficient cyclisation to the tetrazinones **3b**,**d**,**e**, respectively, to proceed. The 'standard' (*i.e.* Scheme 1) synthesis of 3-alkylimidazotetrazines, from the interaction of diazoimidazoles and alkyl isocyanates, has been proposed to proceed by a stepwise mechanism:[4] the present observations support this proposal and argue against alternative [3 + 2] or [7 + 2] cycloaddition processes.

Nor-temozolomide **3f** was not isolated from nitrosation of the unsubstituted urea **7f.** In contrast the bis(urea) **12**, prepared (92%) from AIC **1** and 1,3-bis(isocyanatomethyl)benzene, cyclised to the crude bis(imidazotetrazine) **6** (25%) in excess sodium nitrite–aqueous 2 M hydrochloric acid.

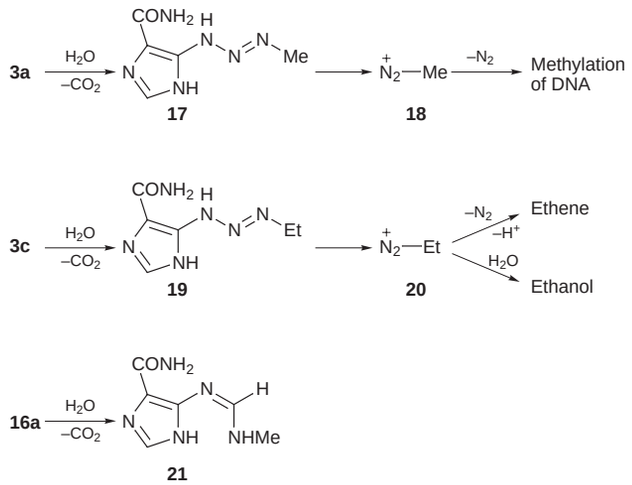
Synthesis of imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones

The imidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-one nucleus of mitozolomide **3b** (but not temozolomide) can be transformed by reactive methylenic substrates into imidazo[5,1-*c*][1,2,4]triazin-4(3)-ones in a 'one-pot' process which involves the intermediacy of 5-diazoimidazole-4-carboxamide **2**.[14] For example, degradation of mitozolomide in ethanolic pyridine containing ethyl cyanoacetate or ethyl acetoacetate afforded unstable hydrazones **13a**,**b** which cyclised *in situ* to imidazotriazinones **14a**,**b** (Scheme 4).

The ureas **7a**–**f** could be cyclised to imidazotriazinones with

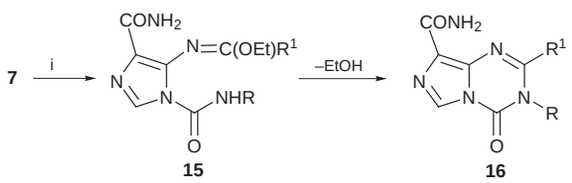


Scheme 4 *Reagents and conditions*: i, RCH_2CO_2Et, pyridine, reflux



Scheme 6

triethyl orthoformate in DMSO but the nature of the products was highly dependent on the conditions. At 50 °C for prolonged reaction times (>48 h) the main products were the imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones **16a**–**f** (Scheme 5).



	R	R^1
a	Me	H
b	$(CH_2)_2Cl$	H
c	Et	H
d	CH_2CO_2Et	H
e	CH_2Ph	H
f	H	H
g	Me	Ph
h	$(CH_2)_2Cl$	Ph
i	CH_2Ph	Ph

Scheme 5 *Reagents and conditions*: i, $R^1C(OEt)_3$, DMSO, 60 h

Imidates **15a**–**f** are presumably involved as intermediates since, when cyclisation of the urea **7a** was carried out with triethyl orthoformate in DMSO at 50 °C for 20 h, the main isolated product was an unstable compound with spectroscopic properties consistent with the imidate structure **15a**. Reaction of **7a**,**b**,**e** with triethyl orthobenzoate in DMSO at 80–100 °C furnished the 2-phenylimidazotriazinones **16g**–**i**. Yields of the imidazotriazine products were moderate (generally 40–50%) because cyclisation to imidazotriazinones competes with thermal loss of the alkylcarbamoyl fragment as an alkyl isocyanate prior to cyclisation; this latter process dominates at reaction temperatures >100 °C.

Decomposition of imidazotetrazines and imidazotriazines

The molecular events underlying the antitumour activity of temozolomide **3a** have been clarified by NMR studies of its degradation chemistry. The drug undergoes ring-opening at physiological pH to form the monomethyltriazene (MTIC; **17**). This transformation can be conducted on a preparative scale in 5% aqueous sodium carbonate.[15] MTIC is unstable: when a solution in $(CD_3)_2SO$ is maintained at 25 °C, after 5 days the single *N*-methyl resonance at δ 3.0 is replaced by a multitude of resonances in the δ 2–4 range. None of the products, the majority presumably *N*-methylated imidazoles, have been identified.[1] Under physiologically-relevant conditions[10] MTIC fragments to the diffusable methyldiazonium ion reactive species **18** ($t_{1/2}$ = 0.4 s)[16] which must then react with cellular nucleophiles (Scheme 6). In essence temozolomide is a prodrug delivery device to liberate a *methylating* agent in the vicinity of guanine sequences in the major groove of DNA.[17] The consequence of these events is illustrated dramatically in the positron emission tomography (PET) scans of brain tumours of patients treated with [^{11}C-methyl]temozolomide.[6,18]

The ethylimidazotetrazinone **3c** is devoid of antitumour activity[15] despite undergoing ring-opening to the ethyltriazene **19** and thence (presumably) the ethyldiazonium species **20**. In the present work a ^{1}H NMR study of the breakdown of **3c** in deuteriated phosphate buffer revealed a small singlet for ethene at δ 5.32 accompanied by signals for ethanol and ethyl phosphate. From the integrals the ratio of ethene to ethanol was <1 : 10. By carrying out the decomposition in a sealed tube in a two phase chloroform–aqueous system ethene was trapped and reacted with bromine to form 1,2-dibromoethane (δ 3.57) which was unambiguously identified by spiking with an authentic sample. Notably, there was no deuterium incorporation into the ethylating species derived from **3c**, which implied that its fate was to react immediately with the first nucleophile it encountered—or undergo β-elimination. This was in marked contrast to the deuterium exchange seen with the methyldiazonium species derived from **3a**.[10] Thus the difference in antitumour activity between **3a** and **3c** may be accounted for by differences in the lifetime of the derived alkyldiazonium ions. Methyldiazonium is relatively long-lived and able to locate its target in the major groove of DNA in clinically-significant amounts before decomposing: in contrast, that proportion of the ethyldiazonium species which escapes quenching by non-DNA nucleophiles will undergo β-elimination with concomitant loss of alkylating (and cytotoxic) activity. A similar contrast in biological activities is known for methyl and ethyl nitrosoureas[19] which have fragmentations closely related to those of the imidazotetrazinones.[20]

The trifluoroethylimidazotetrazine **3i** is more potently cytotoxic overall against human tumour cells *in vitro* than temozolomide and mitozolomide and displays selective actions especially against leukaemia and melanoma cell lines with IC_{50} concentrations (concentrations to inhibit cell growth by 50%) of <1 μM (*cf.* temozolomide and mitozolomide, *ca.* 100 μM).

The strongly electron-withdrawing trifluoroethyl group significantly affects the electronic character of the tetrazine ring. A solution of the imidazotetrazinone **3i** in $(CD_3)_2SO$, monitored by ^{1}H NMR spectroscopy, decomposed completely overnight at 25 °C to give 2-azahypoxanthine **11** and another product showing an extra coupling for the methylene group indicative of the presence of a $-CONHCH_2CF_3$ fragment. Thus **3i** must undergo 'retrocycloaddition' to the diazoimidazole carboxamide **2** (which cyclises to 2-azahypoxanthine **11**) and trifluoroethyl isocyanate **5b**. Since these conditions are similar to those employed

for synthesis of the imidazotetrazinone **3i**, there must be an equilibrium established between product **3i** and the diazoimidazole **2** plus isocyanate **5b**. In the decomposition pathway, this is perturbed by the presence of water, which traps isocyanate **5b** and converts it to a carbamic acid and/or a urea, whereas the synthetic strategy is driven by precipitation of the imidazotetrazine from a more concentrated solution under anhydrous conditions.

A preliminary study of the decomposition of **3i** in deuteriated phosphate buffer revealed a complex picture, with imidazole products including AIC **1** (minor), 2-azahypoxanthine **11** (major) and another major unidentified imidazole. The trifluoroethyl group remained intact and halogenated products included trifluoroethanol (minor) and four major products derived from trifluoroethyl isocyanate **5b**. Thus the aqueous chemistry of the 3-trifluoroethylimidazotetrazinone **3i** differs significantly from that of temozolomide (and mitozolomide) and this may account for the different biological profile against tumour cell lines. Details of the identification of the decomposition products and the biological properties of **3i** will be published separately.

Finally, the imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-one **16a** has no antitumour properties, presumably because ring-opening, if it occurs, would lead to the *N*-methylformamidine **21** which is not a methylating agent.

Experimental

Melting points are uncorrected. Thin-layer chromatography (TLC) was performed on silica gel plates (Merck: 60 F254), and UV light was used to achieve visualisation of the products. NMR spectra were acquired on Bruker ARX-250 or AC-250 spectrometers observing ^{1}H at 250.13 MHz, ^{19}F at 235.36 MHz, ^{31}P at 101.26 MHz, ^{13}C at 63 MHz, and ^{15}N at 25.36 MHz. ^{1}H and ^{13}C spectra were referenced to tetramethylsilane, ^{19}F to internal $CFCl_3$, ^{15}N to external CH_3NO_2 and ^{31}P to external 80% phosphoric acid. IR spectra were obtained with a Mattson Galaxy 2020 spectrometer. Low resolution mass spectra were recorded with a VG Quattro II spectrometer, and accurate mass measurements were obtained with a VG ZAB-E. FAB mass spectra were recorded with a VG AutoSpec High resolution spectrometer.

Synthesis of *N*,*N*-diphenylureas 4

***N*,*N*-Diphenyl-*N*′-[$^{2}H_3$]methylurea 4a.** A solution of [$^{2}H_3$]-methylamine hydrochloride (5.5 g, 78.0 mmol) in water (75 cm^3) was stirred vigorously with a solution of *N*,*N*-diphenylcarbamoyl chloride (17.8 g, 76.8 mmol) in ethyl acetate (300 cm^3) and sodium hydroxide (7.7 g, 192.5 mmol) in water (20 cm^3). The mixture was heated under reflux (4 h), cooled, and the organic layer was separated and dried (magnesium sulfate). Evaporation of solvent and trituration of the residue with diethyl ether gave the diphenylurea **4a** as a white crystalline solid (89%), mp 171–174 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3490, 1670 (C=O), 1603, 1505, 1493; $\delta_H(CDCl_3)$ 7.25 (10 H, m, 2 × Ph), 4.6 (1 H, br, NH); $\delta_C\{^{1}H\}(CDCl_3)$ 157.2 (C=O), 143.3 (C), 129.7 (CH), 127.8 (CH), 126.5 (CH), 27.1 (sept, *J* 21 Hz, CD_3); *m*/*z* 229 (M^+), 169 (100%) (Found: C, 73.4; N, 12.2; $C_{14}H_{11}D_3N_2O$ requires C, 73.3; N, 12.2%).

***N*,*N*-Diphenyl-*N*′-(2,2,2-trifluoroethyl)urea 4b.** Similarly prepared from trifluoroethylamine and *N*,*N*-diphenylcarbamoyl chloride in 79% yield, this urea **4b** had mp 150 °C, from diethyl ether; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1685, 1514, 1492; $\delta_H(CDCl_3)$ 7.5–7.2 (10 H, m, 2 × Ph), 4.88 (1 H, br t, NH), 3.91 (2 H, dq, *J* 6.5, 9.0 Hz, CH_2); $\delta_C\{^{1}H\}(CDCl_3)$ 155.2 (C=O), 142.0 (C), 129.4 (CH), 127.2 (CH), 126.6 (CH), 124.2 (q, $^{1}J_{CF}$ 278 Hz, CF_3), 41.8 (q, $^{2}J_{CF}$ 34 Hz, CH_2); $\delta_F(CDCl_3)$ 29.6 (t, $^{2}J_{HF}$ 9.0 Hz, CF_3); *m*/*z* (FAB) 317 (M+Na), 295 (M+H) (100%), 154, 136 (Found: C, 61.2; H, 4.4; N, 9.4. $C_{15}H_{13}F_3N_2O$ requires C, 61.2; H, 4.4; N, 9.5%).

***N*,*N*-Diphenyl-*N*′-furfurylurea 4c.** Similarly prepared from furfurylamine and *N*,*N*-diphenylcarbamoyl chloride in 82% yield, this urea **4c** had mp 171–174 °C, from diethyl ether–ethyl acetate; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3682, 3441, 1674, 1596, 1493; $\delta_H(CDCl_3)$ 7.30 (11 H, m, 2 × Ph, 5′-H), 6.33 (1 H, dd, *J* 1.7, 3.2 Hz, 4′-H), 6.22 (1 H, d, *J* 3.2 Hz, 3′-H), 4.9 (1 H, br, NH), 4.46 (2 H, d, *J* 5.8 Hz, CH_2); $\delta_C(CDCl_3)$ 156.1 (CO), 152.6 (C), 143.0 (C), 142.3 (CH), 129.8 (CH), 127.8 (CH), 126.6 (CH), 110.8 (CH), 107.4 (CH), 38.2 (CH_2); *m*/*z* 292 (M^+), 169 (100%), 81 (Found: C, 73.6; H, 5.4; N, 9.6. $C_{18}H_{13}N_2O$ requires C, 73.95; H, 5.5; N, 9.6%).

Synthesis of isocyanates 5: general method [21]

The ureas **4a**,**b** (5.0 g) were placed under a nitrogen atmosphere, **4c** under vacuum, in an Aldrich short-path length distillation kit with the collection vial cooled in dry ice–acetone slurry. The flask was heated to 200 °C over 1.5 h and maintained at 200–230 °C for 3 h, during which the following colourless isocyanates were distilled into the collection vial.

[$^{2}H_3$]Methyl isocyanate 5a. The urea **4a** (5.0 g, 22 mmol) was thermolysed to give a colourless liquid (1.03 g, 81%), bp 36 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2298, 2238; $\delta_C\{^{1}H\}(CDCl_3)$ 121.4 (br, $\omega_{1/2}$ = 30 Hz, C=O), 26.9 (sept, *J* 21 Hz, CD_3); *m*/*z* 60.0403 (C_2D_3NO requires M^+, 60.0405).

2,2,2-Trifluoroethyl isocyanate 5b. From the urea **4b** (5.0 g, 15 mmol), this isocyanate (1.75 g, 93%) was an unstable colourless liquid which was stored at −25 °C and used within 24 h; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2276; $\delta_H(CDCl_3)$ 3.85 (q, $^{3}J_{HF}$ 8.2 Hz); $\delta_C\{^{1}H\}(CDCl_3)$ 125.2 (br, C=O), 123.4 (q, $^{1}J_{CF}$ 278 Hz, CF_3), 44.8 (q, $^{2}J_{CF}$ 37 Hz, CH_2); δ_F 30.82 (br, $\omega_{1/2}$ 30 Hz, CF_3); *m*/*z* 125.0077, 106, 56 (100%) ($C_3H_2F_3NO$ requires M^+, 125.0088).

Furfuryl isocyanate 5c. Thermolysis of the diphenylurea **4c** (6.0 g, 20.5 mmol) gave this isocyanate (0.85 g, 34%), bp 30–35 °C/12 mbar; ν_{max}(film)/cm^{-1} 2255; $\delta_H(CDCl_3)$ 7.42 (1 H, dd, *J* 1.9, 0.8 Hz, H-5), 6.37 (1 H, dd, *J* 1.9, 3.2 Hz, H-4), 6.22 (1 H, d, *J* 3.2 Hz, H-3), 4.42 (2 H, s, CH_2); $\delta_C(CDCl_3)$, 150.5, 143.5 (CH), 125.0 (br, C=O), 111.2 (CH), 108.6 (CH), 40.4 (CH_2); *m*/*z* 123.0320, 81 (100%) ($C_6H_5NO_2$ requires M^+, 123.0320).

Synthesis of 5-amino-1-carbamoylimidazole-4-carboxamides 7: general method

5-Aminoimidazole-4-carboxamide hydrochloric acid **1** (0.5 g, 3 mmol) and dry triethylamine (1 cm^3) were mixed with dry DMSO (7 cm^3) or dry acetonitrile (10 cm^3) at 20 °C. After stirring for 5 min, a clear solution was formed when DMSO was the solvent, or a suspension when acetonitrile was the solvent. To the resultant mixture was added dropwise the isocyanate (1.2 equiv) at 10 °C (in DMSO) and at −10 °C (acetonitrile) and the reaction solution or suspension was stirred overnight at room temperature. The mixture was quenched with ice (20 g) and the precipitated solid product was collected, washed with ethyl acetate (2 × 10 cm^3) and dried under high vacuum to give the urea **7**. The following compounds were prepared by this method.

5-Amino-1-(*N*-methylcarbamoyl)imidazole-4-carboxamide 7a. From methyl isocyanate in DMSO, the imidazole **7a** (90%) had mp 170 °C (decomp.); $\nu_{max}(KBr)/cm^{-1}$ 3409, 1750 (C=O), 1661 (C=O), 1535, 1453, 1311, 1241, 949; $\delta_H[(CD_3)_2SO]$ 8.46 (1 H, q, NH), 7.62 (1 H, s, H-2), 6.83 (1 H, br s, NH), 6.39 (2 H, br s, NH_2), 2.78 (3 H, d, CH_3); $\delta_C[(CD_3)_2SO]$ 167.2, 151.6, 144.3, 127.0 (CH), 112.1, 27.5 (CH_3). The product was identical to a sample previously formed by several routes.[1]

5-Amino-1-[*N*-(2-chloroethyl)carbamoyl]imidazole-4-carboxamide 7b. From 2-chloroethyl isocyanate in acetonitrile, the imidazole **7b** (85%) had mp 152 °C (decomp.); $\nu_{max}(KBr)/cm^{-1}$ 3432, 3364, 3257, 1720 (C=O), 1651 (C=O), 1550, 1502, 1325; $\delta_H[(CD_3)_2SO]$ 8.79 (1 H, t, NH), 7.68 (1 H, s, H-2), 6.92 (1 H, br s, NH), 6.84 (1 H, br s, NH), 6.40 (2 H, br s, NH_2), 3.78 (2 H, t, CH_2), 3.59 (3 H, t, CH_2); $\delta_C[(CD_3)_2SO]$ 170.3, 154.4, 147.6, 130.1 (CH), 115.3, 46.9 (CH_2), 45.9 (CH_2); *m*/*z* 231 (M^+), 126

(100%) (Found: C, 36.5; H, 4.7. $C_7H_{10}ClN_5O_2$ requires C, 36.4; H, 4.3%).

5-Amino-1-(*N*-ethylcarbamoyl)imidazole-4-carboxamide 7c. From ethyl isocyanate in acetonitrile, the imidazole **7c** (45%) had mp 152 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3476, 3360, 3276, 1718 (C=O), 1656 (C=O), 1532, 1294, 847; δ_H[$(CD_3)_2SO$] 8.51 (1 H, m, NH), 7.64 (1 H, s, H-2), 6.92 (1 H, br s, NH), 6.81 (1 H, br s, NH), 6.37 (2 H, br s, NH_2), 3.22 (2 H, m, CH_2), 1.11 (3 H, q, CH_3); δ_C[$(CD_3)_2SO$] 167.2, 150.9, 144.3, 127.1 (CH), 112.1, 35.8 (CH_2), 15.3 (CH_3) (Found: C, 42.9; H, 5.9. $C_7H_{11}N_5O_2$ requires C, 42.6; H, 5.6%).

***N*-(5-Amino-4-carbamoylimidazol-1-ylcarbonyl)glycine ethyl ester 7d.** From ethyl isocyanatoacetate in DMSO, the imidazole **7d** (85%) had mp 183–185 °C (decomp.) and spectroscopic and analytical properties identical to an authentic sample.[9]

5-Amino-1-(*N*-benzylcarbamoyl)imidazole-4-carboxamide 7e. From benzyl isocyanate in DMSO, the imidazole **7e** (88%) had mp 166–168 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3323, 3202, 1735 (C=O), 1637, 1532, 1492, 1316, 1250; δ_H[$(CD_3)_2SO$] 9.09 (1 H, t, NH), 7.74 (1 H, s, H-2), 7.34 (5 H, m, Ph), 6.92 (1 H, br s, NH), 6.84 (1 H, br s, NH), 6.42 (2 H, br s, NH_2), 4.45 (2 H, d, CH_2); δ_C[$(CD_3)_2SO$] 168.2, 152.3, 145.5, 140.2 (CH), 130.3, 129.2, 129.0, 128.0, 113.1, 45.2 (CH_2) (Found: C, 56.0; H, 4.8. $C_{12}H_{13}N_5O_2$ requires C, 55.6; H, 5.0%).

5-Amino-1-carbamoylimidazole-4-carboxamide 7f. From trimethylsilyl isocyanate in DMSO, the imidazole **7f** (80%) had mp 168 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3444, 3363, 3187, 1750 (C=O), 1673 (C=O), 1592, 1385; δ_H[$(CD_3)_2SO$] 7.95 (2 H, br s, NH_2), 7.65 (1 H, s, H-2), 6.93 (1 H, br s, NH), 6.82 (1 H, br s, NH), 6.43 (2 H, br s, NH_2); δ_C[$(CD_3)_2SO$] 171.87, 156.85, 149.23, 132.07, 116.65 (Found: C, 35.8; H, 4.5. $C_5H_7N_5O_2$ requires C, 35.5; H, 4.1%).

1,3-Bis(5-amino-4-carbamoylimidazolecarboxamidomethyl)-benzene 12. From 1,3-bis(isocyanatomethyl)benzene in acetonitrile for 60 h, imidazole **12** was recovered as a grey solid (92%), mp 140–143 °C; ν_{max}(KBr)/cm^{-1} 3462, 3345, 3264, 1723 (C=O), 1640 (C=O), 1532, 1310; δ_H[$(CD_3)_2SO$] 9.09 (2 H, br, 2 × NH), 7.71 (2 H, s, 2 × H-2), 7.31 (4 H, m, Ar-H), 6.90 (2 H, br, 2 × NH), 6.82 (2 H, br, 2 × NH), 6.39 (4 H, br, 2 × NH_2), 4.43 (4 H, s, 2 × CH_2).

Synthesis of imidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-ones: (i) From 5-diazoimidazole-4-carboxamide and isocyanates

8-Carbamoyl-3-[$^2H_3$]methylimidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-one 3h. 5-Diazoimidazole-4-carboxamide **2** (1.6 g, 11.8 mmol) and [$^2H_3$]methyl isocyanate **5a** (1.0 g, 8.0 mmol) were stirred in DMSO (10 cm^3) at 25 °C for 24 h. Dilution of the mixture with ethyl acetate (300 cm^3) precipitated the imidazotetrazinone **3h** (0.47 g, 20%) as a pink solid, mp 200 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3381, 3125, 1744, 1680, 1618, 1456, 1402, 1354; δ_H[$(CD_3)_2SO$] 8.84 (1 H, s, 6-H), 7.83 (1 H, br, NH), 7.70 (1 H, br, NH); δ_C\{^{1}H\}[$(CD_3)_2SO$] 165.1 ($CONH_2$), 140.1 (C-4), 135.5 (C-8a), 131.4 (C-8), 129.4 (C-6), 36.4 (sept, *J* 22 Hz, CD_3); *m*/*z* (FAB) 198.0819 (M+H), 154 (100%), 137, 107, 89, 77.0 ($C_6H_4D_3N_6O_2$ requires M+H, 198.0819).

8-Carbamoyl-3-(2,2,2-trifluoroethyl)imidazo[5,1-*d*][1,2,3,5]-tetrazin-4(3*H*)-one 3i. Similarly prepared from the diazoimidazole **2** and 2,2,2-trifluoroethyl isocyanate **5b**, the white imidazotetrazinone **3i** (66%) had mp >300 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3389, 3159, 1745 (C=O), 1691 (C=O), 1610, 1323, 1258, 1194, 1020; δ_H($CDCl_3$) 8.92 (1 H, s, 6-H), 7.90 (1 H, br, NH), 7.76 (1 H, br, NH), 5.27 (2 H, q, $^3J_{HF}$ 8.7 Hz, CH_2); δ_C\{^{1}H\}($CDCl_3$) 162.1 ($CONH_2$), 140.2 (C-4), 134.6 (C-8a), 132.9 (C-8), 130.7 (C-6), 124.5 (q, $^1J_{CF}$ 280 Hz, CF_3), 49.0 (q, $^2J_{CF}$ 35 Hz, CH_2); δ_F($CDCl_3$) −69.5 (t, $^3J_{HF}$ 9 Hz, CF_3) (Found: C, 31.9; H, 1.9; N, 32.4. $C_7H_5F_3N_6O_2$ requires, C, 32.1; H, 1.9; N, 32.1%).

8-Carbamoyl-3-furfurylimidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-one 3j. Prepared as above from the diazoimidazole **2** and furfuryl isocyanate **5c** (73%), the pink imidazotetrazinone **3j** had mp 160 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3443, 1735 (C=O), 1689 (C=O), 1606, 1455, 1369, 725; δ_H[$(CD_3)_2SO$] 8.76 (1 H, s, 6-H), 7.75 (1 H, br, NH), 7.62 (1 H, br, NH), 7.62 (1 H, dd, *J* 1.8, 0.8 Hz, 5′-H), 6.48 (1 H, d, *J* 3.2 Hz, 3′-H), 6.39 (1 H, dd, *J* 3.2, 1.8 Hz, 4′-H), 5.42 (2 H, s, CH_2); δ_C[$(CD_3)_2SO$] 162.3 ($CONH_2$), 149.2 (C-1′), 144.2 (CH), 139.7 (C-4), 135.1 (C-8a), 132.0 (C-8), 129.9 (C-6), 111.6 (CH), 110.7 (CH), 46.0 (CH_2); *m*/*z* (FAB) 261 [M + H], 154, 137 (Found: C, 45.8; H, 3.0; N, 31.9. $C_{10}H_8N_6O_3$ requires C, 46.15; H, 3.1; N, 32.3%).

1,3-Bis(8-carbamoyl-3,4-dihydro-4-oxoimidazo[5,1-*d*][1,2,3,5]-tetrazin-3-ylmethyl)benzene 6. Prepared, as above, from diazoimidazole **2** (1.0 g) and 1,3-bis(isocyanatomethyl)benzene (0.46 cm^3), the crude product was crystallised from formic acid to give the pure bis(imidazotetrazinone) **6** (55%), mp 200 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3457, 3102, 1736 (C=O), 1676 (C=O), 1591, 1454, 1366, 1074, 918; δ_H[$(CD_3)_2SO$] 8.85 (2 H, s, 2 × 6-H), 7.84 (2 H, br, 2 × NH), 7.70 (2 H, s, 2 × NH), 7.53–7.37 (4 H, m, Ar-H), 5.50 (4 H, s, 2 × CH_2); δ_C[$(CD_3)_2SO$] 161.6 (2 × $CONH_2$), 139.4 (C-4, C-4′), 136.2 (2 × C), 134.6 (C-8a, C-8a′), 131.2 (C-8, C-8′), 129.2 (C-6, C-6′), 129.0 (2 × CH), 127.5 (2 × CH), 51.8 (2 × CH_2) (Found: M$^+$ [EI], 462. $C_{18}H_{14}N_{12}O_4$ requires M, 462).

(ii) From nitrosation of 5-amino-1-carbamoylimidazole-4-carboxamides 7: general method

To a solution of a substituted 5-amino-1-carbamoylimidazole-4-carboxamide **7** (1 mmol) in 2 M hydrochloric acid (10 cm^3) at 0 °C was added a solution of sodium nitrite (1.1 equiv) in water (1 cm^3). The reaction solution was stirred for 15 min at 0 °C and allowed to warm to room temperature over 1 h. The reaction solution was concentrated to dryness by vacuum evaporation. To the residue was added ice–water (20 cm^3) to precipitate the product which was collected, washed with ethyl acetate (2 × 20 cm^3), then dried under high vacuum. The following imidazotetrazinones were prepared by this method.

.8-Carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*][1,2,3,5]-tetrazin-4(3*H*)-one (mitozolomide) 3b. Prepared from **7b**, in 70% yield the imidazotetrazine, mp 157–158 °C (decomp.), had mp and spectroscopic properties identical to those of an authentic sample prepared from the reaction between 5-diazoimidazole-4-carboxamide and 2-chloroethyl isocyanate.[4]

8-Carbamoyl-3-ethylimidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-one 3c. Prepared from **7c**, this imidazotetrazine (30%) had mp 188–190 °C (decomp.) and had mp and spectroscopic properties identical to those of an authentic sample of **3c** prepared from the reaction between 5-diazoimidazole-4-carboxamide and ethyl isocyanate.[15] Also isolated from the reaction mixture was 2-azahypoxanthine (25%).[9]

3-Benzyl-8-carbamoylimidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-one 3e. Prepared from **7e**, this imidazotetrazine (88% yield) had mp 184–186 °C (decomp.) and had mp and spectroscopic properties identical to those of an authentic sample of **3e** prepared from the reaction between 5-diazoimidazole-4-carboxamide and benzyl isocyanate.[9]

1,3-Bis(8-carbamoyl-3,4-dihydro-4-oxoimidazo[5,1-*d*][1,2,3,5]tetrazin-3-ylmethyl)benzene 6. Prepared from **12** in 25% yield, this imidazotetrazine was identical (IR and ^{1}H NMR) to the sample prepared from 5-diazoimidazole-4-carboxamide **2** and 1,3-bis(isocyanatomethyl)benzene.

The synthesis of 8-carbamoyl-3-methylimidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-one, temozolomide **3a** (from **7a**)[1] and of the imidazotetrazinone **3d** (from **7d**)[9] by this nitrosation route have been described separately.

Synthesis of 3-substituted imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones: general method

A mixture of a substituted 5-amino-1-carbamoylimidazole-4-carboxamide **7** (1 mmol), triethyl orthoformate or triethyl orthobenzoate (10 mol. equiv.) and dry DMSO or acetonitrile (1 cm^3) was maintained at 40–50 °C (or at the stated temper-

ature) for 60 h. The reaction solution was cooled to 25 °C and excess solvent removed by vacuum evaporation. The solid residue was triturated with ice–water (10 cm^3) to precipitate the products which were washed with ethyl acetate (2 × 20 cm^3). The following compounds were prepared.

8-Carbamoyl-3-methylimidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-one 16a. From **7a** and triethyl orthoformate in DMSO (40–50 °C) for 60 h, the imidazotriazinone (40%) had mp 315–318 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3416, 3325, 3120, 1722 (C=O), 1662 (C=O), 1598, 1358, 1090; δ_H[$(CD_3)_2SO$] 8.42 (1 H, s, H-6), 8.17 (1 H, s, H-2), 7.36 (2 H, br s, NH_2), 3.48 (3 H, s, CH_3) δ_C[$(CD_3)_2SO$] 163.2, 157.5, 147.7 (CH), 138.1, 127.6 (CH), 125.4, 34.0 (CH_3); δ_N(TFA) −168.1, −191.4, −198.0, −235.3, −272.9; *m*/*z* 193 (M^+), 177 (100%) (Found: C, 43.3; H, 4.0. $C_7H_7N_5O_2$ requires C, 43.5; H, 3.6%).

When the cyclisation of 5-amino-1-(*N*-methylcarbamoyl)-imidazole-4-carboxamide **7a** in triethyl orthoformate was conducted at 50 °C for 20 h an unstable intermediate, probably 5-ethoxymethyleneimino-1-(*N*-methylcarbamoyl)imidazole-4-carboxamide **15a** (20%) was isolated; δ_H[$(CD_3)_2SO$] 8.75 (1 H, s, H-2), 8.22 (1 H, br, NH), 8.01 (1 H, s, methine CH), 7.41 (1 H, br, NH), 7.21 (1 H, br, NH), 4.32 (2 H, q, *J* 7.1 Hz, CH_2), 2.85 (3 H, d, *J* 4.6 Hz, NHCH_3), 1.33 (3 H, t, *J* 7.1 Hz, CH_3); δ_C[$(CD_3)_2SO$] 169.5, 150.9, 153.2, 143.2, 137.8, 127.1, 69.0, 32.4, 19.5.

8-Carbamoyl-3-(2-chloroethyl)imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-one 16b. From **7b** and triethyl orthoformate in acetonitrile (40–50 °C), the imidazotriazinone (45%) had mp 185–187 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3436, 3315, 1725 (C=O), 1664 (C=O), 1597, 1475, 1362, 1279; δ_H[$(CD_3)_2SO$] 8.46 (1 H, s, H-6), 8.21 (1 H, s, H-2), 7.38 (2 H, br s, NH_2), 4.31 (2 H, t, *J* 6.1 Hz, CH_2), 3.96 (2 H, t, *J* 6.1 Hz, CH_2); δ_C[$(CD_3)_2SO$] 166.4, 150.4, 147.4 (CH), 140.7, 131.4 (CH), 128.5 (CH), 51.3 (CH_2), 45.8 (CH_2); *m*/*z* 241, 243 (M^+), 225 (100%) (Found: C, 40.1; H, 3.6. $C_8H_8ClN_5O_2$ requires C, 39.8; H, 3.3%).

8-Carbamoyl-3-ethylimidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-one 16c. From **7c** and triethyl orthoformate in acetonitrile (40–50 °C), the imidazotriazinone (35%) had mp 260–263 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3445, 3390, 3282, 3178, 3071, 1737 (C=O), 1665 (C=O), 1605; δ_H[$(CD_3)_2SO$] 8.41 (1 H, s, H-6), 8.22 (1 H, s, H-2), 7.79 (1 H, br s, NH), 7.35 (1 H, br s, NH), 3.98 (2 H, q, CH_2), 1.31 (3 H, s, CH_3); δ_C[$(CD_3)_2SO$] 165.5, 149.5 (CH), 146.4, 140.2, 130.1 (CH), 127.2, 44.6 (CH_2), 17.2 (CH_3) (Found: C, 46.2; H, 4.7. $C_8H_9N_5O_2$ requires C, 46.4; H, 4.35%).

Ethyl 8-carbamoyl-3,4-dihydro-4-oxoimidazo[1,5-*a*][1,3,5]-triazin-3-ylacetate 16d. From **7d** and triethyl orthoformate in DMSO (40–50 °C), the imidazotriazinone (45%) had mp 206–208 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3448, 1732 (C=O), 1676 (C=O), 1597, 1490, 1384, 1093, 791; δ_H[$(CD_3)_2SO$] 8.48 (1 H, s, H-6), 8.17 (1 H, s, H-2), 7.38 (2 H, br s, NH_2), 4.84 (2 H, s, CH_2Ph), 4.19 (2 H, q, CH_2), 1.22 (3 H, s, CH_3); δ_C[$(CD_3)_2SO$] 172.9, 167.8, 153.2, 151.7 (CH), 148.9, 133.1 (CH), 130.1, 67.2 (CH_2), 52.4 (CH_2), 19.5 (CH_3) (Found: C, 45.3; H, 3.95. $C_{10}H_{11}N_5O_4$ requires C, 45.3; H, 4.15%).

3-Benzyl-8-carbamoylimidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-one 16e. From **7e** and triethyl orthoformate in DMSO (40–50 °C), the imidazotriazinone (48%) had mp 250 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3376, 3117, 1732 (C=O), 1661 (C=O), 1599, 1480, 1371, 1261; δ_H[$(CD_3)_2SO$] 8.42 (1 H, s, H-6), 8.37 (1 H, s, H-2), 7.4 (2 H, br s, NH_2), 7.35 (5 H, m, Ph), 5.17 (2 H, s, CH_2); δ_C[$(CD_3)_2SO$] 163.3, 147.2, 144.4 (CH), 136.6 (CH), 129.2 (CH), 128.8, 128.6 (CH), 128.2 (CH), 125.4 (CH), 49.9 (CH_2); *m*/*z* 269 (M^+), 91 (100%) (Found: C, 58.1; H, 3.9. $C_{13}H_{11}N_5O_2$ requires C, 58.0; H, 4.1%).

8-Carbamoylimidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-one 16f. This imidazotriazine was formed (40%) from **7f** and triethyl orthoformate in DMSO (40–50 °C) and had mp 290 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3446, 3324, 3082, 2805, 2642, 1756 (C=O), 1659 (C=O), 1602; δ_H[$(CD_3)_2SO$] 12.60 (1 H, br s, NH), 8.30 (1 H, s, H-6), 7.93 (1 H, s, H-2), 7.13 (2 H, br s, NH_2); δ_C[$(CD_3)_2SO$] 163.4, 150.5, 145.7 (CH), 138.5, 127.62 (CH), 125.17 (Found: C, 39.2; H, 2.9; N, 37.9. $C_6H_5N_5O_2$. 0.33 H_2O requires C, 38.9; H, 3.1; N, 37.8%).

8-Carbamoyl-3-methyl-2-phenylimidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-one 16g. From **7a** and triethyl orthobenzoate in DMSO (80–100 °C), the imidazotriazinone (40%) had mp 295–297 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3417, 3308, 3120, 1723 (C=O), 1672 (C=O), 1586, 1475, 1423; δ_H[$(CD_3)_2SO$] 8.45 (1 H, s, H-6), 7.60 (5 H, m, Ph), 7.35 (2 H, br s, NH_2), 3.33 (3 H, s, CH_3); δ_C[$(CD_3)_2SO$] 163.4, 155.6, 145.6 (CH), 136.8, 134.5, 131.4, 129.5 (CH), 129.3 (CH), 127.7 (CH), 125.1, 35.3 (CH_3) (Found: C, 57.7; H, 4.4. $C_{13}H_{11}N_5O_2$ requires C, 58.0; H, 4.1%).

8-Carbamoyl-3-(2-chloroethyl)-2-phenylimidazo[1,5-*a*][1,3,5]-triazin-4(3*H*)-one 16h. From **7b** and triethyl orthobenzoate in DMSO (80–100 °C), the imidazotriazinone (45%) had mp 229–231 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3425, 3127, 1735 (C=O), 1672 (C=O), 1586, 1384, 1277, 1121; δ_H[$(CD_3)_2SO$] 8.34 (1 H, s, H-6), 7.71 (5 H, m, Ph), 7.35 (2 H, br s, NH_2), 4.39 (2 H, t, CH_2), 3.85 (2 H, t, CH_2) (Found: C, 52.8; H, 4.0. $C_{14}H_{12}ClN_5O_2$ requires C, 53.0; H, 3.8%).

3-Benzyl-8-carbamoyl-2-phenylimidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-one 16i. From **7e** and triethyl orthobenzoate in DMSO (80–100 °C), the imidazotriazinone (45%) had mp 204–206 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3431, 3321, 3137, 1718 (C=O), 1674 (C=O), 1584, 1385, 708; δ_H[$(CD_3)_2SO$] 8.50 (1 H, s, H-6), 7.50 (5 H, m, Ph), 7.44 (2 H, br s, NH_2), 7.35 (5 H, m, Ph), 5.08 (2 H, s, CH_2) (Found: C, 65.9; H, 4.8. $C_{19}H_{15}N_5O_2$ requires C, 66.1; H, 4.4%).

NMR studies

(i) Decomposition of 8-carbamoyl-3-ethylimidazo[5,1-*d*]-[1,2,3,5]tetrazin-4(3*H*)-one 3c. Phosphate buffer (0.5 cm^3, 0.2 M, pD 7.7), $CDCl_3$ and imidazotetrazinone **3c** [0.2 cm^3 of a solution of 0.021 g in $(CD_3)_2SO$ (0.5 cm^3)] were incubated in a sealed tube at 37 °C for 4 d with occasional shaking. The tube was cooled in ice before opening and the layers were separated. The organic layer was dried (magnesium sulfate); the aqueous layer was treated with 2 drops of concentrated DCl. NMR analysis was conducted on both layers.

Organic layer: δ_H(with presaturation of $CHCl_3$) 5.32 (s, ethene); 3.58 (q, *J* 7.0 Hz) and 1.14 (t, *J* 7.0 Hz, ethanol). The organic solution was treated with 2 drops of a 5% solution of bromine in $CDCl_3$ and after 30 min the ^{1}H NMR spectrum showed conversion of ethene to 1,2-dibromoethane (δ 3.57, s).

Aqueous layer: δ_H(with presaturation of HOD) 7.20 (s, AIC **1**); 3.66 (quint, *J* 7.0 Hz) and 1.08 (t, *J* 7.0 Hz, ethyl phosphate); 3.52 (q, *J* 7.0 Hz) and 1.05 (t, *J* 7.0 Hz, ethanol). δ_P 5.9 (s, phosphate buffer); 7.1 (t, *J* 6 Hz, ethyl phosphate). Peak assignments were corroborated by spiking with authentic samples of AIC, ethanol, ethyl phosphate and 1,2-dibromoethane.

(ii) Decomposition of 8-carbamoyl-3-(2,2,2-trifluoroethyl)-imidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-one 3i. A solution of **3i** in $(CD_3)_2SO$ (*ca.* 0.02 g cm^{-3}) was left to stand at 25 °C (24 h). The spectrum showed disappearance of the residual water peak (δ 3.4) and the appearance of new signals: δ_H 14.92 (1 H, br), 14.1 (1 H, br), 8.51 (1 H, s), 6.82 (1 H, t, *J* 6.4, exch. D_2O), 3.86 (2 H, dq, *J* 6.6, 9.9); δ_F −72.3. Spiking of the sample with azahypoxanthine **11** confirmed the identity of the aromatic product.

The reaction of a $(CD_3)_2SO$ solution of **3i** (0.1 cm^3) with phosphate buffer (0.5 cm^3, 0.2 M, pD 7.7) at 37 °C was monitored by ^{1}H and ^{19}F NMR spectroscopy. After 48 h no further spectral changes were observed. The ^{1}H NMR spectrum showed imidazole resonances at δ 7.60 (major) and 8.09 (minor, azahypoxanthine **11**) and four trace products, which included AIC free base **1**. In the region δ 4.5–3.0 there were at least six quartets, the largest of which was $CF_3CH_2NH_2$ (by spiking); other important peaks were attributable to $CF_3CH_2NHCO_2H$ and CF_3CH_2OH.

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