Antitumour Imidazotetrazines. Part 12.[1] Reactions of Mitozolomide and its 3-Alkyl Congeners with Oxygen, Nitrogen, Halogen, and Carbon Nucleophiles

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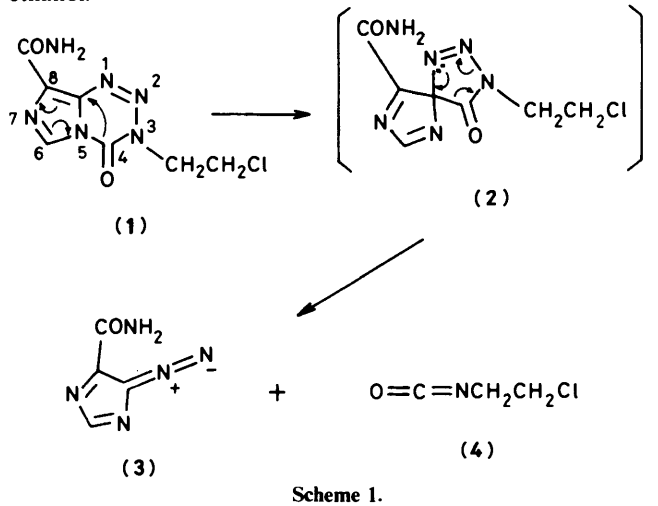
Mitozolomide (**1**) and its 3-alkyl congeners ring-open in aqueous sodium carbonate to form 5-(3-alkyltriazen-1-yl)imidazole-4-carboxamides. The 3-methyl and 3-ethyl analogues of mitozolomide decompose in alcohols to form 2-azahypoxanthine and 5-amino-1-alkoxycarbonylimidazole-4-carboxamides. In hydrazine hydrate mitozolomide yields, principally, 5-azidoimidazole-4-carboxamide, whereas the 3-alkyl-derivatives form 5-amino-4-carbamoylimidazole-1-carbohydrazide.

5-Diazoimidazole-4-carboxamide, generated by thermolysis of mitozolomide in acetic acid or pyridine, can be trapped by reactive methylenic ketones, nitriles or esters to afford imidazo-[1,2,4]triazines.

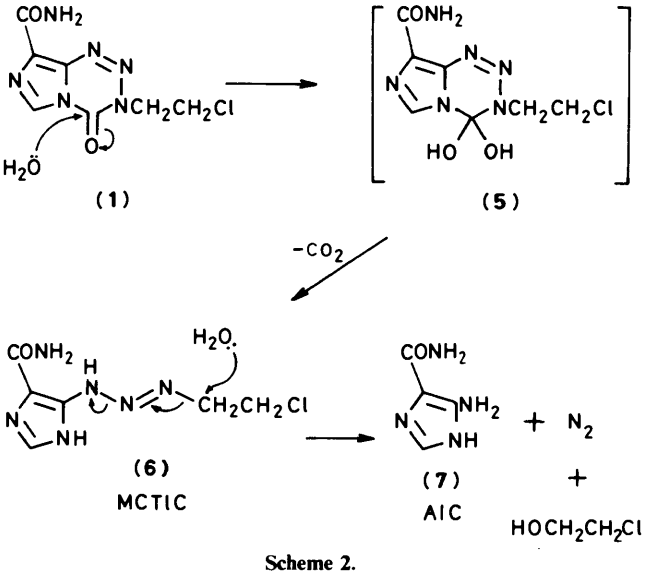
The antitumour agent mitozolomide (**1**) decomposes by two main pathways. In hot non-nucleophilic solvents, *e.g.* acetonitrile, the compound slowly fragments to regenerate the two moieties from which it is synthesized—5-diazoimidazole-4-carboxamide (**3**) and 2-chloroethyl isocyanate (**4**).[2] The mechanism of the thermal breakdown may involve a [1,5] sigmatropic shift *via* an unstable spirobicycle (**2**) (Scheme 1); alternatively, an ionic mechanism which is a reversion of the proposed synthetic pathway [2] may operate. In contrast, decomposition at pH 7.4 in aqueous systems proceeds by nucleophilic attack at C-4 to form a gem-diol (**5**) which ring-opens with loss of carbon dioxide to afford an unstable chloroethyltriazene (MCTIC) (**6**) which subsequently decomposes to form 5-amino-imidazole-4-carboxamide (AIC) (**7**), nitrogen and 2-chloroethanol.[2]



Scheme 1.

Evidence has been adduced that MCTIC can cross-link DNA[3–5] and the antitumour[6] and biological properties[7] of mitozolomide correlate well with its being considered a pro-drug form of MCTIC. However, the 3-methylimidazotetrazinone (**8**) also has pronounced antitumour properties, but with a different spectrum of activity,[8] and it is implausible that this derivative could lead to a species capable of cross-linking with DNA.

We now report a comparative study of the reactions of (**1**) and (**8**) with a range of nucleophiles in the expectation that the results might provide an insight into a chemical explanation for the different antitumour activities of the two structurally related



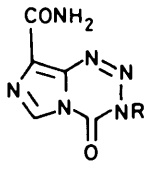
Scheme 2.

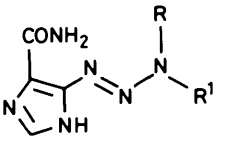
compounds. In addition, we have examined some reactions of three other alkyltetrazinones (**9**)—(**11**) which have proved to be inactive in some antitumour tests in which mitozolomide (**1**) and its 3-methyl derivative (**8**) are active.[9]

Reactions under Aqueous Conditions.—When mitozolomide (**1**) and its 3-alkyl homologues (**8**) and (**9**) are boiled in water the main product is AIC (**7**). In the case of mitozolomide AIC is contaminated by a coloured impurity. The rate of decomposition of mitozolomide is profoundly influenced by pH, in the critical range spanning physiological pH the decomposition $t_{\frac{1}{2}}$ varying from 2.08 h (pH 7.0) to 0.9 h (pH 7.5).[10] This pH sensitivity probably has clinical and toxicological significance since the distribution and activation of the drug in body compartments will have been influenced by the pH of the cellular microenvironments.

Mitozolomide has been converted preparatively into MCTIC (**6**) in aqueous 5% sodium carbonate.[2] In the present work the methyl- (**8**) and ethyl-tetrazinone (**9**) were shown to ring-open smoothly to MTIC (**12**) and ETIC (**13**) under similar conditions. Recently, MTIC has been shown to display differential cytotoxicity to a human colon carcinoma cell line (BE) and a virally transformed human embryonic cell line (VA-13), both of which are deficient in their capacity to repair O^6-methyl

lesions (Mer^-), compared to their repair proficient (Mer^+) counterparts. ETIC shows no differential cytotoxicity and is less cytotoxic generally.[11] Surprisingly, mitozolomide and the 3-methyl derivative (**8**) were stable in warm (60—65 °C) concentrated sulphuric acid and were recovered unchanged upon dilution of the acid solutions; in addition aqueous solutions of both compounds were photostable. This stability contrasts markedly with the photosensitivity of the clinically used and structurally related imidazolyltriazene DTIC (**14**).[12]





(**8**) R = Me

(**9**) R = Et

(**10**) R = Pr

(**11**) R = CH_2CH_2OMe

(**12**) R = H, R^1 = Me MTIC

(**13**) R = H, R^1 = Et ETIC

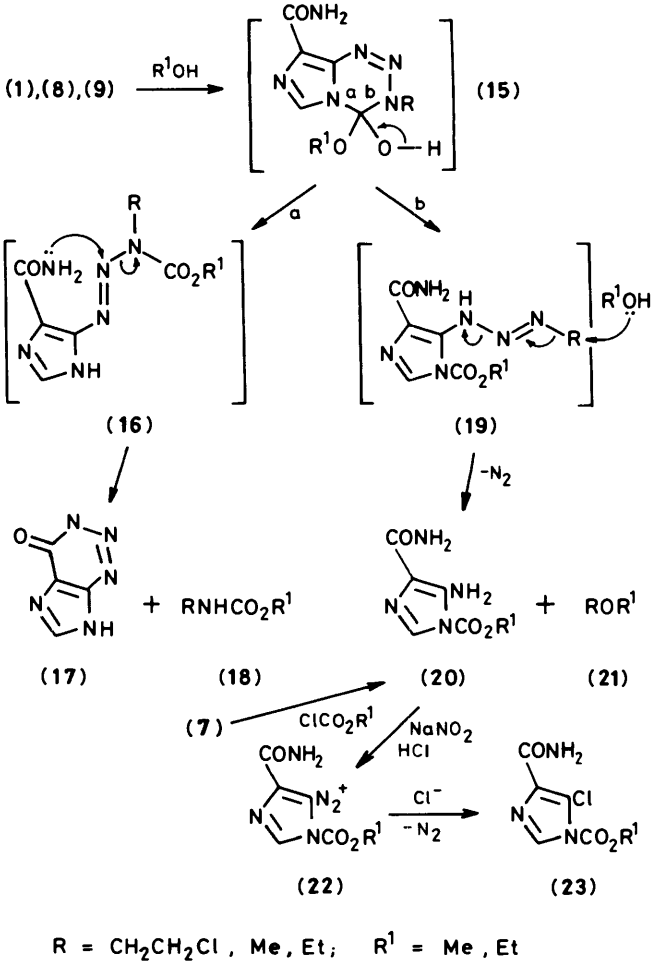
(**14**) R = R^1 = Me DTIC

Reactions with Alcohols.—Mitozolomide (**1**) is unstable in hot methanol or ethanol and affords 2-azahypoxanthine (**17**) and *N*-(2-chloroethyl) carbamates (**18**; R = CH_2CH_2Cl; R^1 = Me or Et).[2] The mechanism is believed to involve initial attack by the nucleophiles at C-4 to generate hemiacetals (**15**; R = CH_2CH_2Cl, R^1 = Me or Et) which ring-open by cleavage of the 4,5-bond to give unstable triazenes (**16**) and thence undergo intramolecular cyclisation to 2-azahypoxanthine with loss of the isocyanate moiety (Scheme 3a).

Both the 3-methyl- (**8**) and 3-ethylimidazotetrazinones (**9**) were more stable in boiling methanol than the 3-(2-chloroethyl) derivative. After 10 days the products from both compounds were 2-azahypoxanthine (**17**) (80%) and a colourless solid (20%) the analysis for which corresponded with the molecular formula $C_6H_8N_4O_3$; in agreement the mass spectrum showed a molecular ion at *m/z* 184. The i.r. spectrum indicated the presence of ester and amide carbonyl groups ($\nu_{C=O}$ 1 765 and 1 665 cm^{-1}) together with NH absorptions at 3 450 and 3 390 cm^{-1}. The ^1H n.m.r. spectrum confirmed the presence of a methyl ester resonance at δ 3.92. Structure (**20**; R^1 = Me) was assigned to this product since it was identical with a sample of 5-amino-1-methoxycarbonylimidazole-4-carboxamide prepared by treating 5-aminoimidazole-4-carboxamide (AIC; **7**) with methyl chloroformate under alkaline conditions and recently shown by Russian workers[13] to have structure (**20**; R^1 = Me). Reactions between (**8**) or (**9**) and boiling ethanol followed a similar course to give azahypoxanthine (**17**) and a product $C_7H_{10}N_4O_3$ (*m/z* 198) with ethyl ester absorptions in its i.r. and ^1H n.m.r. spectrum. The latter product was identical to the ester (**20**; R^1 = Et) formed by reacting AIC (**7**) with ethyl chloroformate in tetrahydrofuran–aqueous sodium hydroxide. Shaw[14] erroneously claimed that this product was 5-ethoxycarbonylaminoimidazole-4-carboxamide.

The minor products (**20**) of these degradation must arise from the hemiacetals (**15**) by cleavage of the 3,4-bond to yield unstable monoalkyltriazenes (**19**; R = Me or Et, R^1 = Me or Et) which then suffer further breakdown by nucleophilic attack at the electrophilic alkyl group (Scheme 3b). The dialkyl ethers (**21**) proposed as by-products have not been isolated but the transformation (**19**) ⟶ (**20**) + (**21**) is fully consistent with the known chemistry of monoalkyltriazenes.[15] Thus breakdown of the hemiacetals (**15**) involves fission of the 4,5-bond exclusively when group R = 2-chloroethyl, whereas cleavage of the 3,4-bond occurs when group R = Me or Et and 1,4,5-trisubstituted imidazoles (**20**) are formed to a minor extent. It is interesting to note that a recent study has revealed that the C-4 position in a series of imidazotetrazinones is the most electron-deficient nucleus in the ring-system and presumably most vulnerable to nucleophilic attack.[16] Furthermore, in the ring structure of mitozolomide, the 4,5-bond is the longest and weakest bond.[17] Definitive bond lengths in the hemiacetals (**15**) are not available since these unstable intermediates cannot be isolated for crystallographic analysis. The propensity for heterolysis of the 3,4- or 4,5-bonds is presumably controlled by the steric and electronic effects of substituents R and R^1 and the competitive leaving group affinities of the imidazolyl and triazenyl fragments.

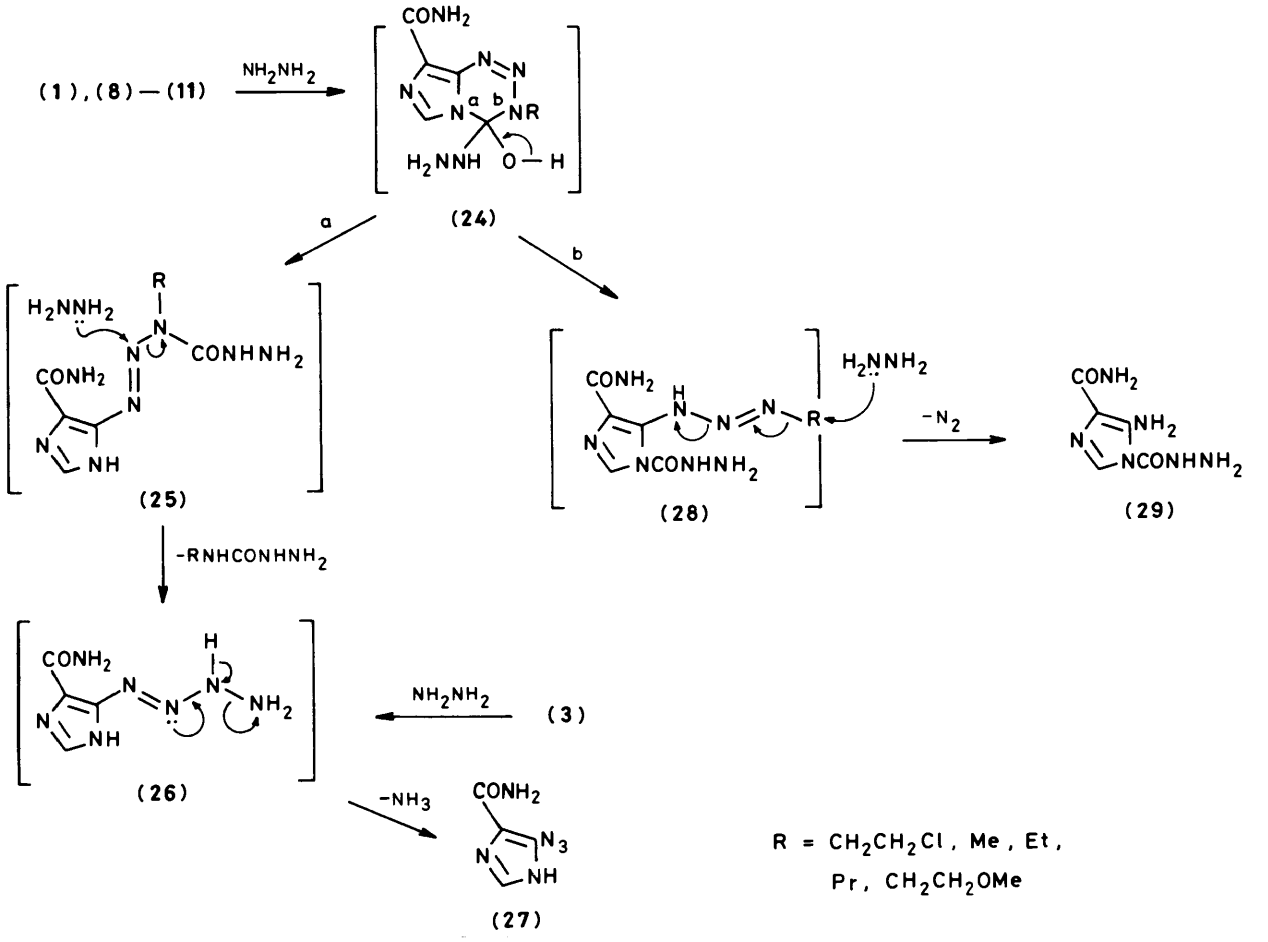
The possibility of forming an internally compensated diazo derivative [*cf.* 5-diazoimidazole-4-carboxamide (**3**)] is not open to the coloured diazonium species (**22**) formed on nitrosation of the 1-alkoxycarbonylimidazoles (**20**; R^1 = Me or Et) in dilute hydrochloric acid. Instead, the products are the 5-chloroimidazoles (**23**; R^1 = Me or Et) formed by Sandmeyer-type displacements.



R = CH_2CH_2Cl, Me, Et; R^1 = Me, Et

Scheme 3.

Reactions with Hydrazine Hydrate.—There was vigorous effervescence of nitrogen when a cold ethanolic solution of mitozolomide (**1**) was treated with hydrazine hydrate. The products were 5-azidoimidazole-4-carboxamide (**27**) and traces (t.l.c.) of the carbohydrazide (**29**). Formation of the azide can be rationalised by the mechanism outlined (Scheme 4a). Following attack by hydrazine at C-4 the intermediate covalent adduct (**24**) must fragment by cleavage of the 4,5-bond to generate the unstable triazene (**25**; R = CH_2CH_2Cl). Whereas in the presence of weaker nucleophiles (*e.g.* alcohols) a triazene of

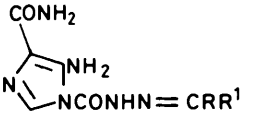


Scheme 4.

this type undergoes intramolecular cyclisation to 2-azahypoxanthine (see Scheme 3a), the strongly nucleophilic hydrazine intercedes to divert the reaction to the tetrazene (**26**). Subsequent decomposition of the tetrazene to azide (**27**) is feasible since the azide can be prepared in high yield by reaction of 5-diazoimidazole-4-carboxamide (**3**) and hydrazine hydrate.[18]

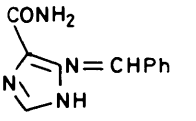
The reactions of 3-alkylimidazotetrazinones (**8**)—(**11**) when treated with hydrazine hydrate under comparable conditions were much slower and the only solid product isolated was the carbohydrazide (**29**). The mechanism proposed for this transformation (**24**) ⟶ (**28**) ⟶ (**29**) (Scheme 4b) exactly parallels that for the corresponding ring-opening in alcohols (see Scheme 3b). The structure of the carbohydrazide (**29**) was confirmed by spectroscopic analysis and its conversion into a hydrazone (**30**) on treatment with acetone. Surprisingly, attempted derivatisation of (**29**) with benzaldehyde did not afford the related hydrazone (**31**); instead the product was the benzylidene derivative (**32**). The structure of the latter product was confirmed by an independent synthesis from AIC (**7**) and benzaldehyde. The same benzylidene derivative was formed in poor yield when mitozolomide or its 3-methyl analogue (**8**) were heated in aqeuous benzaldehyde: in these cases the AIC required as reactant is formed as a hydrolytic product of the imidazotetrazinones (see earlier).

Reactions with Halide Ions and Reactive Methylenic Substrates.—Although we do not believe that 5-diazoimidazole-4-carboxamide (**3**) is a discrete intermediate in any of the aforementioned reactions between imidazotetrazinones and nucleophiles, there is no doubt that it is generated in thermal



(**30**) R = R^1 = Me

(**31**) R = H, R^1 = Ph

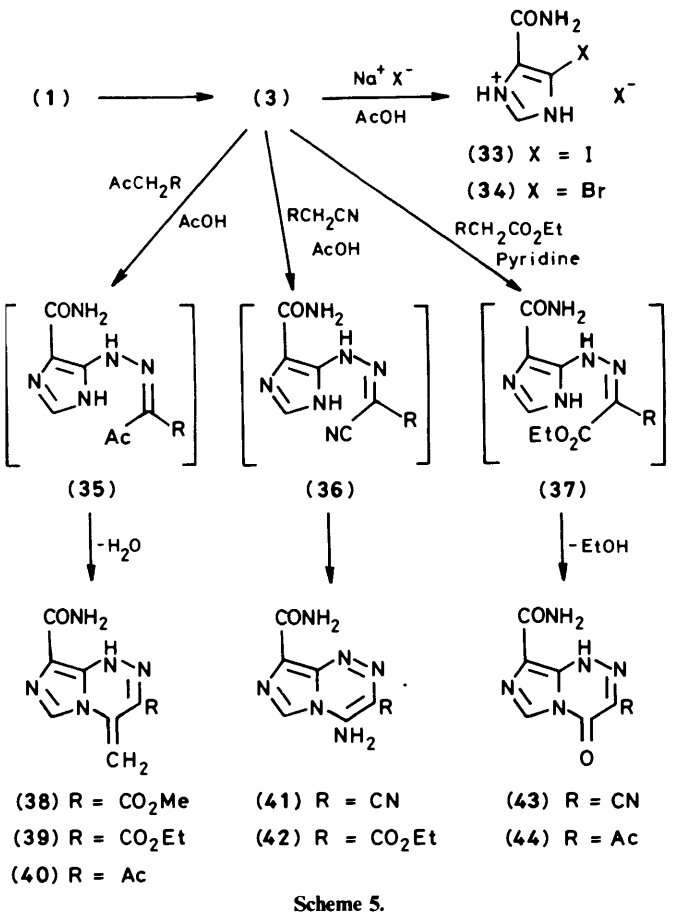


(**32**)

decompositions in boiling acetic acid since when pure mitozolomide (**1**) is recrystallised from hot acetic acid the recovered material contains an impurity with a diazo band (2 198 cm^{-1}) in its i.r. spectrum. When the reaction is performed in acetic acid containing an excess of sodium iodide the product is the iodoimidazole (**33**) isolated as a golden hydroiodide salt; similarly reaction in acetic acid–sodium bromide or acetic acid containing 45% hydrobromic acid yields the bromoimidazole hydrobromide (**34**). These Sandmeyer reactions recall the related degradation of 3-phenyl-1,2,3-benzotriazin-4(3*H*)-one to 2-halogenobenzanilides in boiling acetic acid containing sodium halides.[19]

The same intermediate diazoimidazole (**3**) can be trapped by reactive methylenic substrates in boiling acetic acid. Thus the intermediate hydrazones formed from ketones (**35**) and nitriles (**36**) cyclise *in situ* to 4-methyleneimidazo-1,2,4-triazines (**38**)—(**40**) and 4-aminoimidazo-1,2,4-triazines (**41**) and (**42**), respectively, whereas the esters (**37**) cyclise in hot pyridine to imidazo-1,2,4-triazinones (**43**) and (**44**) (Scheme 5). These pathways have been corroborated previously by independent synthesis of the series of hydrazones (**35**)—(**37**) from pure diazoimidazole (**3**) and subsequent cyclisation in acetic acid or pyridine.[20] The

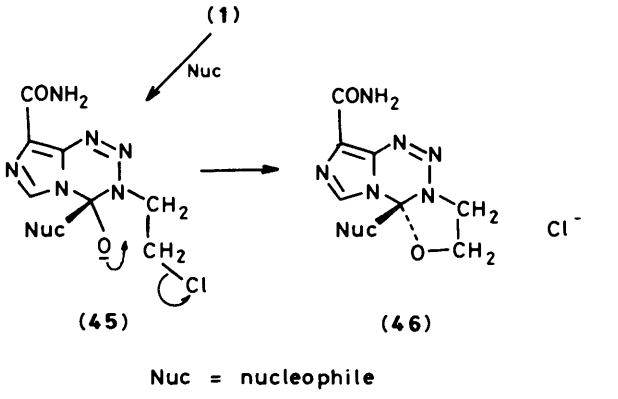
present examples represent a facile 'one-pot' conversion of an imidazo[5,1-*d*]-1,2,3,5-tetrazinone into a series of imidazo-[5,1-*c*][1,2,4]triazines. The 3-methyl- (**8**) and 3-ethyl-tetrazinone (**9**) are stable in hot acetic acid containing sodium halides or reactive methylenic compounds.



Scheme 5.

We cannot assess the significance of the minor differences observed in the chemistry of mitozolomide and its 3-alkyl derivatives. The former compound appears to cleave in the presence of oxygen and nitrogen nucleophiles exclusively at the C(4)–N(5) bond whereas the 3-methyl and 3-ethyl homologues also fragment at the N(3)–C(4) bond to a minor extent. It has been pointed out to us* that the tetrahedral adduct (**45**) formed by attack of nucleophiles at C(4) in mitozolomide might be aligned favourably for intramolecular cyclisation to form an oxazolidine intermediate (**46**). Such a species can only be formed from the chloroethyl substituted tetrazine (Scheme 6). Possibly this intermediate might cleave exclusively at the observed bond although it is difficult to see why this should occur selectively. We cannot exclude this possibility for the present work since we did not isolate and characterise the products from the chloroethyl fragment. However, in earlier studies,[2] we confirmed the presence of 2-chloroethanol, 2-chloroethyl carbamates and 2-chloroethylureas from the reactions of mitozolomide and water, alcohols and arylamines, respectively implying that the C–Cl bond remains intact in the presence of these nucleophiles.

Finally, the chemical studies described herein do not shed any light on the reasons for the antitumour activity ranking in the 3-alkylimidazotetrazinones: chloroethyl ≫ methyl > ethyl.[9] Other series of antitumour agents which display the same



Scheme 6.

activity feature include 1-aryl-3,3-dialkyltriazenes, alkylnitrosoureas, and alkylhydrazines[21–23] but not *N*-alkylformamides.[24] We are presently searching for a biochemical explanation for this phenomenon for it may have crucial biological significance.

Experimental

Mass spectra were recorded on a VG Micromass 12 instrument at 70 eV; source temperature 200—300 °C. I.r. spectra were measured on a Pye-Unicam SP 200 spectrometer as KBr discs. ^{1}H N.m.r. spectra were recorded on a Varian EM 360 A in $(CD_3)_2SO$. Ethanol refers to absolute ethanol.

5-(3-*Methyltriazen*-1-*yl*)*imidazole*-4-*carboxamide* (**12**).—(i) A mixture of 8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (**8**) (0.2 g)[2] and 5% aqueous sodium carbonate (10 ml) was stirred at 25 °C for 20 minutes. The buff triazene (0.09 g) was collected and, when dried *in vacuo*, had m.p. 178 °C (with explosion!) (lit.,[25] m.p. 175—180 °C); $\nu_{max.}$ 3 400 and 3 250 (NH), and 1 680 cm^{-1} (C=O); $\lambda_{max.}$(EtOH) 230 and 317 nm; δ 3.0 (3 H, s, CH_3), 7.6 (4 H, br s, NH and NH_2), and 7.50 (1 H, s, 2-H).

(ii) Methylamine hydrochloride (20 g) was dissolved in water (30 ml) and the solution covered with ethyl acetate (100 ml). Anhydrous potassium carbonate (60 g) was added and the mixture was stirred at 25 °C for 18 h. The ethyl acetate solution (50 ml) was decanted and stirred with 5-diazoimidazole-4-carboxamide (1.37 g) for 18 h in a foil-protected flask. The triazene (1.58 g) had m.p. 178 °C (explodes!) (Found: C, 35.5; H, 4.4; N, 49.6. Calc. for $C_5H_8N_6O$: C, 35.7; H, 4.8; N, 50.0%). The sample had i.r. and u.v. absorption characteristics identical with the foregoing sample.

5-(3-*Ethyltriazen*-1-*yl*)*imidazole*-4-*carboxamide* (**13**).—(i) Similarly prepared, by ring-opening of 8-carbamoyl-3-ethylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (**9**) (0.2 g)[26] and 5% aqueous sodium carbonate, this triazene (0.1 g) had m.p. 156 °C (explodes!) (lit.,[25] m.p. 154—158 °C); $\nu_{max.}$ 3 500, 3 300, and 3 100 (NH), 3 000—2 500 (br, NH), and 1 650 cm^{-1} (C=O); δ 1.23 (3 H, t, CH_3), 3.5 (2 H, q, CH_2), 7.5 (4 H, br, NH and NH_2), and 7.68 (1 H, s, 2-H).

(ii) A mixture of 70% aqueous ethylamine (40 ml) and ethyl acetate (100 ml) was stirred (2 days) with anhydrous potassium carbonate (50 g). The ethylamine-saturated ethyl acetate (25 ml) was stirred with 5-diazoimidazole-4-carboxamide (1.37 g) for 24 h. The off-white triazene (1.55 g) had spectroscopic characteristics identical with the foregoing sample (Found: C, 39.8; H, 5.4; N, 46.4. Calc. for $C_6H_{10}N_6O$: C, 39.6; H, 5.5; N, 46.15%).

5-*Amino*-1-*methoxycarbonylimidazole*-4-*carboxamide* (**20**; R^1 = Me).—(i) A solution of 8-carbamoyl-3-methylimidazo-

* We thank Professor C. W. Rees for this suggestion.

[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (**8**) (1.0 g) in methanol (100 ml) was boiled (10 days) and evaporated to dryness under reduced pressure. The crude pink solid was chromatographically fractionated on silica gel using ethanol–toluene (4:1) as eluant. The first fraction, on evaporation, yielded the aminomethoxycarbonylimidazolecarboxamide (0.2 g) (Found: C, 39.25; H, 4.3; N, 30.3%; M^+, 184. Calc. for $C_6H_8N_4O_3$: C, 39.2; H, 4.4; N, 30.4%; *M*, 184); $\lambda_{max.}$ 265 nm; $\nu_{max.}$ 3 450 and 3 390 (NH), 1 765 and 1 665 cm^{-1} (C=O); δ 3.92 (3 H, s, CH_3), 6.9 (2 H, br s, NH_2), and 7.50 (1 H, s, 2-H); m.p. 180—185 °C (from water). Evaporation of the second fraction from the original chromatographic separation afforded 2-azahypoxanthine (**17**) (0.58 g), identical with a sample prepared by cyclisation of 5-diazoimidazole-4-carboxamide (**3**) in aqueous ammonia.[12]

(ii) Similarly prepared, from the 8-ethylimidazotetrazinone (**9**) and boiling methanol (10 days) was a mixture of 5-amino-1-methoxycarbonylimidazole-4-carboxamide (20%) and 2-azahypoxanthine (80%).

(iii) A sample of 5-amino-1-methoxycarbonylimidazole-4-carboxamide prepared from 5-aminoimidazole-4-carboxamide hydrochloride and methyl chloroformate in chloroform containing triethylamine[13] was identical (i.r. and ^{1}H n.m.r.) with the two foregoing samples.

5-Amino-1-ethoxycarbonylimidazole-4-carboxamide (**20**; R^1 = Et).—(i) When the 3-methylimidazotetrazinone (**8**) (0.5 g) was boiled in ethanol (20 ml) (10 days) and the mixture chromatographically fractionated as before, the products were 2-azahypoxanthine (80%) and the aminoethoxycarbonylimidazolecarboxamide (20%) (Found: C, 42.35; H, 4.95; N, 28.2; M^+, 198. Calc. for $C_7H_{10}N_4O_3$: C, 42.4; H, 5.05; N, 28.3%; *M*, 198); $\lambda_{max.}$ 267 nm; $\nu_{max.}$ 3 440, 3 325, and 3 145 (NH), 1 750 and 1 665 cm^{-1} (C=O); δ 1.32 (3 H, t, CH_3), 4.42 (2 H, q, CH_2), 6.42 (2 H, brs, NH_2), and 7.60 (1 H, s, 2-H). The product recrystallised from water as white needles, m.p. 180—182 °C (resol. at 200 °C) and was identical with the imidazolecarboxamide prepared by the method of Shaw[14] (see below).

(ii) Decomposition of the ethylimidazotetrazinone (**9**) in boiling ethanol as above similarly afforded a mixture of 2-azahypoxanthine (80%) and the aminoethoxycarbonylimidazolecarboxamide (20%).

(iii) 5-Aminoimidazole-4-carboxamide hydrochloride (0.82 g) in water (15 ml) containing potassium carbonate (0.6 g) was treated portionwise over 30 min with ethyl chloroformate (3 × 0.2 ml). The stirred mixture deposited crystals of the aminoethoxycarbonylimidazolecarboxamide (0.4 g) which was identical (i.r. and ^{1}H n.m.r.) with the foregoing samples. The structure of this compound was erroneously assigned by Shaw[14] as 5-ethoxycarbonylaminoimidazole-4-carboxamide.

1-Methoxycarbonyl-5-chloroimidazole-4-carboxamide (**23**; R^1 = Me).—The purple solution formed when 5-amino-1-methoxycarbonylimidazole-4-carboxamide (0.2 g) in 2M-hydrochloric acid (10 ml) was treated at 0 °C with sodium nitrite (0.05 g) in water (2 ml) was stirred for 4 h. The precipitated solid (0.13 g) crystallised from aqueous ethanol to yield the *chloroimidazole*, m.p. 150—152 °C [Found: C, 35.1; H, 2.8; N, 20.6%; M^+, 203(205). $C_6H_6ClN_3O_3$ requires C, 35.4; H, 2.9; N, 20.6%; *M*, 203(205)]; $\lambda_{max.}$ 267 nm; $\nu_{max.}$ 3 450, 3 350, and 3 150 (NH), and 1 778 and 1 660 cm^{-1} (C=O); δ 3.30 (3 H, s, CH_3), 6.62 (2 H, br s, NH_2), and 7.76 (1 H, s, 2-H).

1-Ethoxycarbonyl-5-chloroimidazole-4-carboxamide (**23**; R^1 = Et).—Similarly prepared (see above) by diazotisation of a 2M-hydrochloric acid solution of 5-amino-1-ethoxycarbonylimidazole-4-carboxamide, this *chloroimidazole* (60%) had m.p. 158—159 °C from aqueous ethanol [Found: C, 38.45; H, 3.6; N, 19.3%; M^+, 217(219). $C_7H_8ClN_3O_3$ requires C, 38.6; H, 3.7; N, 19.3%; *M*, 217(219)]; $\lambda_{max.}$ 267 nm; $\nu_{max.}$ 3 320 and 3 190 (NH), and 1 762 and 1 665 cm^{-1} (C=O); δ 1.30 (3 H, t, CH_3), 4.24 (2 H, q, CH_2), 6.7 (2 H, br s, NH_2), and 7.76 (1 H, s, 2-H).

5-Azidoimidazole-5-carboxamide (**27**).—A vigorous effervescence ensued and a solution was formed when 50% ethanolic hydrazine hydrate (2 ml) was added at 25 °C to a suspension of mitozolomide (**1**) (0.24 g) in ethanol (3 ml). After 0.5 h, the precipitated azide was collected (0.08 g) and had m.p. 145—147 °C (decomp.); $\nu_{max.}$ 3 450 and 3 195 (NH), 2 150 and 2 130 (N_3), 1 662 cm^{-1} (C=O); δ 7.64 (1 H, s, 2-H). The product was identical (m.p. and i.r. spectrum) with an authentic sample prepared by treating 5-diazoimidazole-4-carboxamide (**3**) with ethanolic hydrazine hydrate.[18]

5-Amino-4-carbamoylimidazole-1-carbohydrazide (**29**).—A suspension fo 3-methylimidazotetrazine (**8**) (0.25 g) in ethanol (3 ml) was treated with 50% ethanolic hydrazine hydrate (2 ml) for 0.5 h at 25 °C. The precipitated buff *carbohydrazide* (0.17 g) was washed with water and had m.p. 183—185 °C (Found: C, 32.6; H, 4.2; N, 46.0%; M^+, 184. $C_5H_8N_6O_2$ requires C, 32.6; H, 4.3; N, 45.65%; *M*, 184); $\lambda_{max.}$ 267 nm; $\nu_{max.}$ 3 500 and 3 390 (NH) and 1 715 and 1 660 cm^{-1} (C=O); δ 6.34 and 6.74 (7 H, br s, NH and NH_2) and 7.52 (1 H, s, 2-H).

(ii) The same carbohydrazide (60—70%) was formed when the 3-alkylimidazotetrazines (**9**)—(**11**) in ethanol were treated with ethanolic hydrazine hydrate at 25 °C.

Reactions of 5-Amino-4-carbamoylimidazole-1-carbohydrazide (**29**).—(i) A solution of carbohydrazide (**29**) (0.1 g) in acetone (20 ml) and ethanol (10 ml) was stirred at 25 °C for 5 days. Evaporation under reduced pressure left a brown residue which was redissolved in ethanol and left overnight. The pink *semicarbazone* (**30**) (0.09 g) had m.p. 220 °C (decomp.) (Found: C, 43.1; H, 5.4; N, 37.8%; M^+, 224. $C_8H_{12}N_6O_2$ requres C, 42.9; H, 5.4; N, 37.5%; *M*, 224); $\nu_{max.}$ 3 300 and 3 120 (NH) and 1 645 cm^{-1} (C=O); δ 1.82 (3 H, s, CH_3), 1.90 (3 H, s, CH_3), 6.69 (4 H, br s, NH), 7.10 (1 H, s, 2-H), and 9.14 (1 H, br s, NH).

(ii) The carbohydrazide (**29**) (0.1 g) in ethanol (20 ml) was boiled with benzaldehyde (0.5 g) and the solution was left overnight. The buff crystalline *benzylidene derivative* (**32**) (0.1 g) was collected and had m.p. 242—245 °C (decomp.) (Found: C, 61.6; H, 4.6; N, 26.3%; M^+, 214. $C_{11}H_{10}N_4O$ requires C, 61.7; H, 4.7; N, 26.2%, *M*, 214); $\nu_{max.}$ 3 400 and 3 160 (NH) and 1 658 cm^{-1} (C=O); δ 7.48—7.82 (8 H, m, Ph and NH_2), 7.72 (1 H, s, 2-H), and 9.24 (1 H, s, C*H*Ph).

(iii) The same benzylidene derivative (95%) was obtained by heating 5-aminoimidazole-4-carboxamide base (0.5 g) and benzaldehyde (1.0 g) in refluxing ethanol (0.5 h) or 65 and 62%, respectively, when mitozolomide or the 3-methylimidazotetrazinone (**8**) were boiled for 1 h in water (5 ml) and benzaldehyde (0.5 g).

5-Iodoimidazole-4-carboxamide (**33**).[27]—The violet solid, deposited when mitozolomide (0.24 g) was boiled (2.5 h) in acetic acid (10 ml) containing sodium iodide (0.5 g) and then cooled, was rinsed with acetone to yield golden crystals of the *iodoimidazolecarboxamide hydroiodide* (0.05 g), m.p. 285—287 °C (Found: C, 12.7; H, 1.5; N, 12.0%; M^+, 237. $C_4H_4IN_3O$·HI requires C, 13.15; H, 1.4; N, 11.5%; *M*, 237 for the free base); $\nu_{max.}$ 3 420 and 3 190 (NH) and 1 675 cm^{-1} (C=O); δ 8.62 (1 H, s, 2-H).

5-Bromoimidazole-4-carboxamide (**34**).[27]—(i) The (cooled) solution formed when mitozolomide (0.25 g) was refluxed in acetic acid (10 ml) containing sodium bromide (0.5 g) for 3 h deposited brown crystals of the *bromoimidazolecarboxamide hydrobromide* (0.06 g), m.p. 210 °C [Found: C, 19.6; H, 1.9; N, 17.0%; M^+, 189(191). $C_4H_4BrN_3O$·HBr requires C, 19.2; H, 2.0;

N, 16.8%; *M*, 189(191)]; $\nu_{max.}$ 3 420 and 3 190 (NH) and 1 680 cm^{-1} (C=O); δ 8.60 (1 H, s 2-H).

(ii) The same bromoimidazolecarboxamide hydrobromide (60%) was formed when mitozolomide (0.24 g) was heated in a mixture of acetic acid (10 ml) and 45% hydrobromic acid in acetic acid (3 ml) for 1 h.

(iii) When 5-diazoimidazole-4-carboxamide (0.25 g) was stirred in 45% hydrobromic acid in acetic acid (10 ml) in the dark at 25 °C, the product was the same bromoimidazolecarboxamide hydrobromide.

Methyl 8-Carbamoyl-1,4-dihydro-4-methyleneimidazo[5,1-c]-[1,2,4]*triazine-3-carboxylate* (**38**).—A solution of mitozolomide (0.24 g), methyl acetoacetate (1.5 g), and acetic acid (6 ml) was boiled for 0.5 h. The cooled solution deposited golden crystals of the triazinecarboxylate as an acetic acid solvate (0.18 g), m.p. 250 °C. The product was identical (m.p., i.r. and ^{1}H n.m.r.) with an authentic sample prepared from 5-diazoimidazole-4-carboxamide (**3**) and methyl acetoacetate in acetic acid.[20]

Similarly prepared, from mitozolomide and a reactive methylenic compound, in boiling acetic acid, were the following: ethyl 8-carbamoyl-1,4-dihydro-4-methyleneimidazo[5,1-*c*]-[1,2,4]triazine-3-carboxylate (**39**) (85%), from ethyl acetoacetate; 3-acetyl-8-carbamoyl-1,4-dihydro-4-methyleneimidazo[5,1-*c*][1,2,4]triazine (**40**) (55%), from acetylacetone; 4-amino-8-carbamoyl-3-cyanoimidazo[5,1-*c*][1,2,4]triazine (**41**) (60%), from malononitrile; ethyl 4-amino-8-carbamoylimidazo[5,1-*c*][1,2,4]triazine (**42**) as an acetic acid solvate, from ethyl cyanoacetate.

All the aforementioned samples were identical (m.p. and i.r.) with authentic samples.[20]

8-Carbamoyl-3-cyanoimidazo[5,1-c](1,2,4]*triazin-4*(1*H*)*-one* (**43**).—Mitozolomide (0.24 g) and ethyl cyanoacetate (1.0 g) were boiled in pyridine (10 ml) and ethanol (15 ml) for 8 h. The precipitated triazinone (50%) had m.p. > 300 °C (decomp.) and was identical (m.p., i.r., and ^{1}H n.m.r.) with an authentic sample prepared from 5-diazoimidazole-4-carboxamide and ethyl cyanoacetate in pyridine–ethanol.[20]

Similarly prepared, from mitozolomide and methyl or ethyl acetoacetate in boiling pyridine–ethanol was 3-acetyl-8-carbamoylimidazo[5,1-*c*][1,2,4]triazin-4(1*H*)-one (**44**) in 48% (from methyl acetoacetate) or 35% yield (from ethyl acetoacetate).

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