A new route to the antitumour drug temozolomide, but not thiotemozolomide

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Interaction of 5-aminoimidazole-4-carboxamide with alkyl isocyanates yields *N*-substituted 1-carbamoylimidazoles which can be cyclised to imidazo[5,1-*d*][1,2,3,5]tetrazin-4(3*H*)-ones, including temozolomide 3a, on nitrosation; a similar reaction with methyl isothiocyanate, followed by nitrosation, affords the nitrosomethylamino derivative 11 of a new ring-system, imidazo[1,5-*b*][1,2,4]thiadiazole.

The antitumour drug temozolomide **3a** has shown useful clinical activity against brain tumours[1] and metastatic melanoma,[2] two malignancies hitherto considered poorly treatable.

The original synthesis of temozolomide[3] started with 5-diazoimidazole-4-carboxamide **1** which was reacted with methyl isocyanate following a route to azolotetrazinones originally discovered by Ege.[4] The (presumed) dipolar intermediate **2a** cyclised spontaneously to temozolomide in high yield in a Me_2SO–ethyl acetate mixed solvent system at 25 °C (Scheme 1). Analogues of temozolomide **3b**–**d** can be prepared similarly. The diazoimidazole **1** does not react with methyl isothiocyanate in Me_2SO at 40 °C nor in the same solvent containing the Lewis acids zinc iodide and boron trifluoride–diethyl ether or the base triethylamine, so 4-thiotemozolomide (**3a**; O = S) is not available by this route.

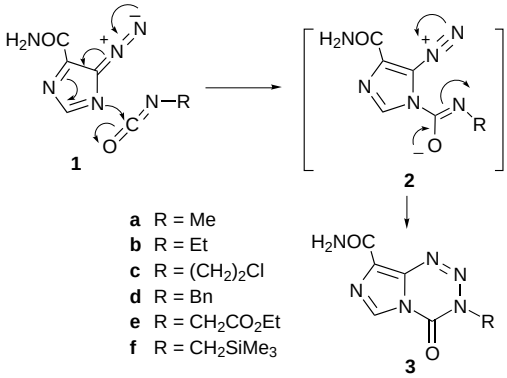
Our earlier work to develop alternative syntheses of temozolomide had exploited imidazotetrazinones *e.g.* **3e**,**f** prepared from less volatile 'masked' methyl isocyanates, which can be deprotected under conditions which retain the integrity of the base-fragile ring-system.[5] Here we report a new synthesis of temozolomide starting with 5-aminoimidazole-4-carboxamide hydrochloride **4**, which adapts another route developed by Ege[6] for the synthesis of pyrazolotetrazinones, so obviating the use of the potentially unstable diazoimidazole **1**. A parallel synthesis designed to furnish 4-thiotemozolomide surprisingly took an entirely different path and led to the construction of a new ring-system, imidazo[1,5-*b*][1,2,4]thiadiazole.

Interaction of the free base of **4** with methyl, ethyl, 2-chloroethyl and benzyl isocyanates in Me_2SO or acetonitrile afforded the ureas **5a**–**d**†‡ regioselectively (75–95%) despite the availability of competing sites for carbamoylation. Nitrosative cyclisation of **5a** in 2 m hydrochloric acid in the temperature range 5–25 °C gave a red solution from which temozolomide **3a** precipitated in 30–35% yield. A by-product, 2-azahypoxanthine (15%), is probably formed by competing nitrosative cyclisation to the primary carboxamide group followed by loss of methyl isocyanate. Compared with the ease with which *N*-alkylanthranilamides cyclise to 3-alkyl-1,2,3-benzotriazin-4(3*H*)-ones[7] the yield of temozolomide is disappointing, but accountable for by the weakly acidic nature of the urea proton in the diazonium species **6a** which suppresses generation of the dipolar intermediate **2a**, the presumed precursor to temozolomide. Higher yields of the imidazotetrazinones **3b**–**d** were isolated (50–70%) by cyclisation of the corresponding ureas **5b**–**d**: also we have shown previously[5] that, in the case of the urea **5e** formed from ethyl isocyanatoacetate, cyclisation gave the imidazotetrazine **3e** (93%) consistent with the hypothesis that an electron-withdrawing group R facilitates ionisation of the urea proton, and hence cyclisation (Scheme 2).

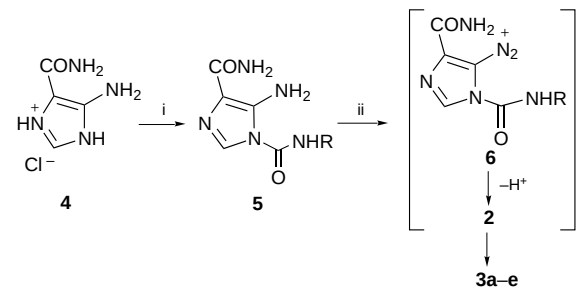
Interaction of the aminoimidazole carboxamide **4** with methyl isothiocyanate gave a thiourea (90%) which on nitrosation with excess nitrous acid at 25 °C gave a red product (85%), originally considered to be thiotemozolomide on the basis of its ^1H and ^{13}C NMR spectra which were comparable with that of temozolomide. However, elemental and mass spectral analysis confirmed a molecular formula $C_6H_6N_6O_2S$ (thiotemozolomide + 16 mass units). An X-ray structure determination (Fig. 1) revealed that the product was 2-nitrosomethylaminoimidazo[1,5-*b*][1,2,4]thiadiazole-4-carboxamide **11**.

A logical route to this unexpected compound is outlined in Scheme 3. Methylsulfanylcarbamoylation of the free base of amine **4** evidently affords the thiourea **7**†‡ substituted at the exocyclic amino group. Nitrosation of the thiol tautomer **7′** would generate an *S*-nitroso intermediate **8** which might cyclise to the imidazothiadiazole **10** directly (−HNO) or, probably, undergo further oxidation in the excess nitrous acid to the *S*-nitro intermediate **9** which would then cyclise ($-HNO_2$) to afford the bicycle **10**. Further nitrosation of the *sec*-amine group of **10** to afford the nitrosamine **11**‡§ is to be expected, although the exact timing of this step is not clear.

Of the five other imidazothiadiazole ring-systems reported in the literature only one, imidazo[1,2-*b*][1,2,4]thiadiazole,[8] contains an imidazole bridgehead nitrogen atom attached to sulfur.



Scheme 1



Scheme 2 *Reagents and conditions*: i, RNCO, Me_2SO or MeCN, 0–25 °C, NEt_3; ii, $NaNO_2$ in 2 m HCl, 0–25 °C

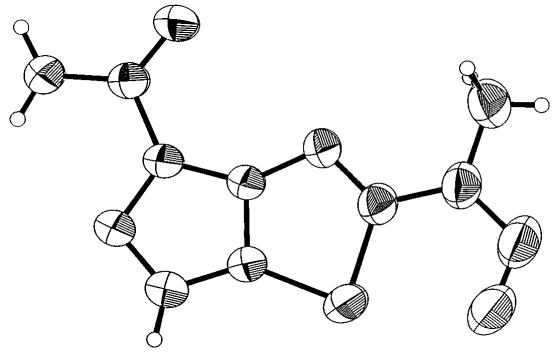
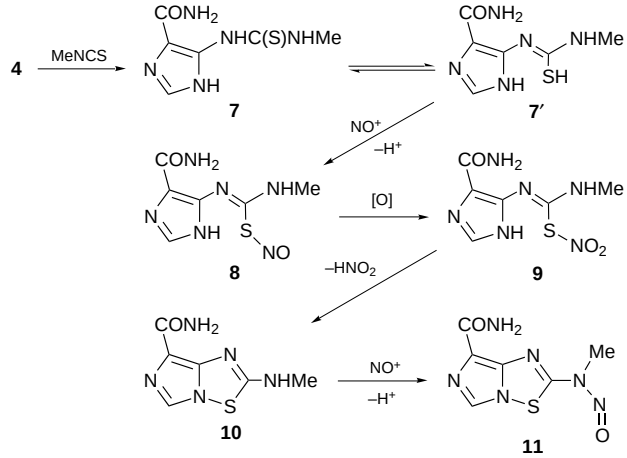


Fig. 1 ORTEP view of the structure of 2-nitrosomethylaminoimidazo-[1,5-*b*][1,2,4]thiadiazole-4-carboxamide **11**. Displacement elipsoids are shown at the 50% probability level.



Scheme 3

This new synthesis of **11** in two steps from the aminoimidazole **4** is notable for the high yield and mild conditions and may be adapted for the synthesis of other examples of this intriguing new bicyclic system.

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Footnotes

† *General experimental method* for ureas **5** and thiourea **7**. A solution or suspension of 5-aminoimidazole-4-carboxamide hydrochloride (0.5 g) and dry triethylamine (1 ml) in dry Me_2SO or acetonitrile (10 ml) was treated dropwise (1 h) with the isocyanate or isothiocyanate (1.2 equiv.) at 10 °C (−10 °C in the case of acetonitrile). The mixture was stirred overnight at 25 °C, quenched with water (25 ml), and products collected and washed successively with water and ethyl acetate. Yields of ureas were **5a** (85%), **5b** (75%), **5c** (70%), **5d** (95%) and the thiourea **7** (85%). Satisfactory microanalytical data were obtained for new compounds.

‡ *Selected physical data* for ureas **5**, thiourea **7** and imidazothiadiazole **11**. 5-Amino-1-(*N*-methylcarbamoyl)imidazole-4-carboxamide **5a**: mp 170 °C (decomp.); ν_{max} (KBr) 3409, 1718, 1661, 1535, 1453, 1311, 1241, 947 cm^{-1}; δ_H [$(CD_3)_2SO$] 8.46 (q, 1 H, NH), 7.62 (s, 1 H, H-2), 6.93 (br s, 1 H, NH), 6.83 (br s, 1 H, NH), 6.39 (br s, 2 H, NH_2), 2.78 (d, 3 H, CH_3); δ_C [$(CD_3)_2SO$] 167.2, 151.6, 144.3, 127.0, 112.1, 27.5; *m/z* 184 (M^+ + 1); 5-Amino-1-(*N*-ethylcarbamoyl)imidazole-4-carboxamide **5b**: mp 150–152 °C (decomp.); ν_{max} (KBr) 3476, 3360, 3276, 1718, 1656, 1532, 1294, 847 cm^{-1}; δ_H [$(CD_3)_2SO$] 8.51 (m, 1 H, NH), 7.64 (s, 1 H, H-2), 6.92 (br s, 1 H, NH), 6.81 (br s, 1 H, NH), 6.37 (br s, 2 H, NH_2), 3.22 (m, 2 H, CH_2), 1.11 (t, 3 H, CH_3); δ_C [$(CD_3)_2SO$] 167.2, 150.2, 150.9, 144.4, 127.1, 112.1, 35.8, 15.3; *m/z* 198 (M^+ + 1); 5-Amino-1-[*N*-(2-chloroethyl)carbamoyl]imidazole-4-carboxamide **5c**: mp 102–105 °C (decomp.); ν_{max} (KBr) 3432, 3364, 3257, 1720, 1651, 1550, 1502, 1325 cm^{-1}; δ_H [$(CD_3)_2SO$] 8.79 (m, 1 H, NH), 7.68 (s, 1 H, H-2), 6.92 (br s, 1 H, NH), 6.84 (br s, 1 H, NH), 6.40 (br s, 2 H, NH_2), 3.78 (t, 2 H, CH_2CH_2Cl), 3.59 (q, 2 H, CH_2CH_2Cl);δ_C [$(CD_3)_2SO$] 170.3, 154.4, 147.6, 130.1, 115.3, 46.9, 45.9; *m/z* 231/233 (M^+); 5-Amino-1-(*N*-benzylcarbamoyl)imidazole-4-carboxamide **5d**: mp 163–168 °C (decomp.); ν_{max} (KBr) 3323, 3202, 1735, 1637, 1532, 1492, 1316, 1250 cm^{-1}; δ_H [$(CD_3)_2SO$] 9.09 (t, 1 H, NH), 7.74 (s, 1 H, H-2), 7.34 (m, 5 H, Ph), 6.92 (br s, 1 H, NH), 6.84 (br s, 1 H, NH), 6.42 (br s, 2 H, NH_2), 4.45 (d, 2 H, CH_2); δ_C [$(CD_3)_2SO$] 168.2, 152.3, 145.5, 140.2, 130.3, 129.2, 129.0, 128.0, 113.1, 45.2; *N*-(4-Carbamoylimidazol-5-yl)-*N*′-methylthiourea **7**; mp 200–205 °C (decomp.); ν_{max} (KBr) 3374, 3205, 2914, 1663, 1574, 1528, 1481, 1327 cm^{-1}; δ_H [$(CD_3)_2SO$] 12.63 (br s, 1 H, NH), 10.26 (br s, 1 H, NH), 9.89 (br s, 1 H, NH), 7.82 (s, 1 H, H-2), 7.43 (br s, 2 H, NH_2), 3.08 (d, 3 H, CH_3); δ_C [$(CD_3)_2SO$] 183.8, 167.6, 149.5, 139.1, 110.9, 37.2; *m/z* 199 (M^+); 2-Nitrosomethylaminoimidazo[1,5-*b*][1,2,4]thiadiazole-4-carboxamide **11**; mp 145–150 °C (decomp.); ν_{max} (KBr) 3481, 3423, 3365, 3139, 3126, 3039, 1675, 1625 1507, 1125 cm^{-1}; δ_H [$(CD_3)_2SO$] 8.08 (s, 1 H, H-6), 7.30 (br s, 1 H, NH), 7.16 (br s, 1 H, NH), 4.50 (s, 3 H, CH_3); δ_C [$(CD_3)_2SO$] 163.9, 154.8, 145.1, 126.4, 119.9, 31.6; *m/z* 227 (M^+ + 1).

§ *Crystal data* for **11**: $C_6H_6N_6O_2S$, M = 226.23, monoclinic, space group $P2_1/n$, a = 7.522(7), b = 9.121(2), c = 13.383(7) Å, b = 97.73(6)°, U = 910(1) Å^3, Z = 4, D_c = 1.65 g cm^{-3}, $F(000)$ = 464, (Cu-Kα) = 1.54180 Å, μ = 3.148 mm^{-1}, A lath 0.65 × 0.40 × 0.24 mm grown from Me_2SO–Et_2O was mounted on an Enraf-Nonius CAD 4 diffractometer. 1734 unique reflections were collected by $\bar{\omega} - 2\theta$ for $2° \leqslant \theta \leqslant 75°$ and phased by direct methods.[8] Full-matrix least-squares refinement[10] on F^2 with anisotropic thermal parameters for non hydrogen atoms and all hydrogen atom positions determined from difference Fourier synthesis. At convergence, R = 0.076, R_w = 0.22 and GOF = 1.105 for 1479 observed reflections [$I > 2\,\delta I$]. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited in the Cambridge Crystallographic Data Centre (CDDC). See information for Authors, Issue No. 1. Any requests to the CDDC for this material should quote the full literature citation and the reference number 182/301.

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