Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 2, pp. 185-188, 1996

0960-894X/96 $15.00 + 0.00

0960-894X(95)00586-2

SYNTHETIC STUDIES OF 8-CARBAMOYLIMIDAZO-[5,1-D]-1,2,3,5-TETRAZIN-4(3H)-ONE: A KEY DERIVATIVE OF ANTITUMOUR DRUG TEMOZOLOMIDE

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Abstract: 5-Diazoimidazole-4-carboxamide **4** reacted with trimethylsilyl isocyanate in acetonitrile to afford 8-carbamoylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one **1**, which was undergoing a methylation to give antitumour drug temozolomide **2**; while 1,5-dicarbamoyl aminoimidazole **6** failed in an azo-cyclization to give **1** but accomplished a carbon-cyclization to produce 8-carbamoylimidazo[1,5-a] s-triazin-4(3H)-one **7**.

In the progress of our research in the development of antitumour drugs from imidazotetrazinones[1], one of those compounds, 8-carbamoylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one **1**, has long remained a challenging target in order to expand knowledge of the chemistry of the imidazotetrazinones and the understanding of their bioactive mechanism. 8-Carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one **2**, temozolomide, is a robust antitumour drug of significant benefit to brain tumour patients, and currently is undergoing phase II clinical trials both in Europe and USA.[2] The difference in chemical structures between **2** and **1** is that the latter is not methylated at N-3; therefore it was named as nortemozolomide. It is widely believed that the methyl group in **2** gives rises to a nucleophilic methylation on an O^6-guanine residue in the major groove of guanine-rich sequences in DNA, resulting in antitumour activity.[3] The process of activating **2** was envisaged as follows: once the trigger in **2**, the carbonyl group in the tetrazinone, is attacked by an activated nucleophilic water molecule, a fugitive methyldiazonium fragment was formed first which then underwent the methylation on nucleophilic O^6 in guanine.[4] Since **1** is not a methyl donor, it would be very interesting to know whether or not it shows any antitumour activity. It is also of significance to reveal the chemical properties of **1** and to demonstrate if a methyl-like group could be incorporated onto **1** to generate an efficient synthetic route to **2**. Herein we report for the first time the synthesis of nortemozolomide **1** and the results of preliminary studies of its chemical properties.

In recent publications[5] we have described alternative synthetic routes to **2**. Those successful experiences and synthetic strategies facilitated the attempts to obtain **1**. The benzyl group is one of the most successful protecting groups used in syntheses of primary and secondary amines.[6] The first envisaged approach to **1** was to apply benzyl isocyanate to a reaction with diazoimidazocarbamide **4** to produce 3N-benzylimidazotetrazinone **5**, which then undergoes a catalytic-hydrogenation deprotection to give **1**. Compound **5**[7] was made accordingly[5] in a good yield of 90%, but the deprotection failed in a number of attempts under various conditions. To our surprise, **5** showed intrinsic stability to the catalytic hydrogenation since under most of the reaction conditions **5** was recovered without decomposition. This was contrary to the situation in which hydride reducing reagents, such as sodium borohydride, were employed, where the tetrazinone ring of **5** cleaved to give **3** (Scheme 1).

We have demonstrated that **3** underwent a selective carbamoylation with alkyl and aryl isocyanates to give the urea type compounds which then underwent an azo-cyclization into imidazotetrazinones by treatment with nitrous acid. 1,5-Dicarbamoyl aminoimidazole **6**[8] was synthesized by reaction of **3** with trimethylsilyl isocyanate in dry DMSO followed by a precipitation with iced water in 75% yield. The diazotization-driven

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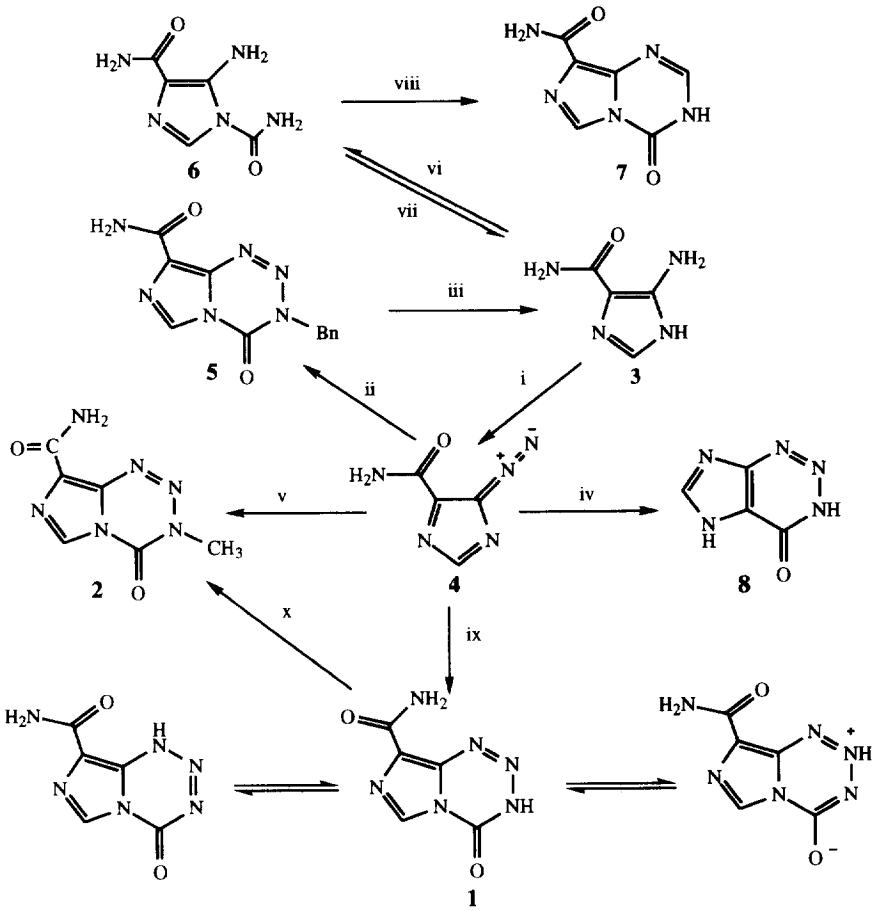
cyclization of **6** failed to afford the imidazotetrazinone product but **3** was recovered. In contrast, the cyclization of **6** with triethyl orthoformate in DMSO at 45 °C overnight gave a pure single product, 8-carbamoylimidazo[1,5-a] s-triazin-4(3H)-one **7**[9], in 55% yield (Scheme1).



Scheme 1. *Reagents and conditions*: (i) $NaNO_2$ (excess), 2N-HCl, 0 °C; (ii) benzyl isocyanate, DMSO, 25 °C; (iii) $NaBH_4$, EtOH, 0 °C; (iv) NH_3, H_2O, 25 °C; (v) methyl isocyanate, DMSO, 25 °C; (vi) a, trimethylsilyl isocyanate, DMSO, 5—25 °C; b, ice, H_2O; (vii) $NaNO_2$, 2N-HCl, 0 °C; (viii) triethyl orthoformate, DMSO, 40 °C; (ix) trimethylsilyl isocyanate, CH_3CN, 25 °C; (x) dimethyl sulfate, DMSO, triethylamine, 25 °C.

The original synthesis of **2**, performed in dichloromethane, suffered an unbearably slow completion.[10] Later by changing the solvent to DMSO, the reaction rate was increased more than 100 fold. However when this approach was applied to the synthesis of **1** by the reaction **4** with trimethylsilyl isocyanate, though the starting material disappeared rapidly, a mixture of products, mainly 2-azohypoxanthine **8**[11], was obtained (Scheme 1). The tendency for an intra-molecular cyclization into **8** was well known from the time when **4** was first synthesized.[12] As the cyclization of methyl isocyanate with **4** in dichloromethane was taking a month to complete, but 3-4 h. in DMSO, it was conclued that solvents play a crucial role in this reaction. Bearing this constraint in mind we started to try the reaction of **4** with TMS-isocyanate in different media. No imidazotetrazinone product was found in the reaction mixture except a small amount of **8** when

dichloromethane, hexane, ethyl acetate, chloroform or toluene were used as a solvent in a period of a month. When the reaction was performed in acetonitrile at room temperature overnight, one single product was obtained in a good yield of 70%. ^{1}H, ^{13}C and ^{15}N NMR spectroscopy and microanalysis established that this product was nortemozolomide **1**.[13] We experienced strong solvent effects on the outcome of the reaction, and also observed slow completion and competition of cyclizing into **8** when the reaction was performed on a gram scale in pure acetonitrile.

Compound **1** has extremely poor solubility in most solvents including DMSO and DMF. It is stable in the solid state and showed one decomposition point at 190.95 °C in thermo-analysis. However, it is not stable in either acidic or basic medium. Its IR spectrum (KBr) showed typical tetrazinone peaks at 1776, 1679 and 1625cm^{-1}, and UV absorbancies at 273, 244 and 207nm. A test of its antitumour activities in comparison with temozolomide and a range of tetrazinones is being carried out on a variety of cancer cell lines and the results will be reported elsewhere.

Attempts to methylate **1** to produce temozolomide **2** have been made with variable success using a number of reagents. In most of cases, the reaction gave a mixture of products in which NMR studies showed the presence of temozolomide about 10—20% along with a few by-products of imidazotetrazinones and imidazoles. This result made a significant contribution to the imidazotetrazinone chemistry and established another potentially efficient route to **2**. Optimisation of the reaction conditions are still in progress: the main reasons ascribed to the outcome of the methylations of **1** could be the existence of three active hydrogens in **1**, each of which may promote methylation, and tautomerism of **1** is likely to create more sites for methylation (Scheme 1), as well as the sovent affecting the stability of **1**.

Acknowledgement: We thank the Cancer Research Campaign, UK for support of this work and Dr. Derry E. V. Wilman for the ^{15}N NMR spectrum.

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7. *Spectroscopic data* for compound **5**: mp 188 °C(decomp.); υ_{max}(KBr) cm^{-1} 3447, 3162, 3099, 1734, 1678, 1607, 1449 and 1366; δ_H[$(CD_3)_2SO$] 8,84(1H, s, H-6), 7.84(1H, brs, NH), 7.71(1H, brs, NH), 7.40(5H, m, phenyl), 5.52(2H, s, CH_2); δ_C[$(CD_3)_2SO$] 162.37, 140.03, 136.46 (2C), 135.29, 131.85, 129.87, 129.40, 128.81, 52.73.

8. *Spectroscopic data* for compound **6**: mp 168 °C(decomp.); υ_{max}(KBr) cm^{-1} 3444, 3363, 3187, 1750, 1673, 1592 and 1385; δ_H[$(CD_3)_2SO$] 7.95(2H, brs, NH_2) 7.65(1H, s, H-6), 6.93(1H, brs, NH), 6.82(1H, brs, NH), 6.43(2H, brs, NH_2); δ_C[$(CD_3)_2SO$] 171.87, 156.85, 149.23, 132.07, 116.65.

9. *Spectroscopic data* for compound **7**: mp 290 °C(decomp.); υ_{max}(KBr) cm^{-1} 3446, 3324, 3082, 2805, 2642, 1756, 1659, 1602 and 1487; δ_H[$(CD_3)_2SO$] 12.6(1H, brs, NH), 8.35(1H, s), 7.96(1H, s), 7.30(2H,

brs, NH_2); δ_C[$(CD_3)_2SO$] 163.37, 150.48, 145.70, 138.50, 127.62, 125.17; [Found: C, 39.2; H, 2.9; N, 37.9%; M^+, 179. $C_6H_5N_5O_2$ 1/3 H_2O requires C, 38.92; H, 3.06; N, 37.83%; M^+, 179].

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11. *Spectroscopic data* for compound **8**: mp 138-140 °C (decomp.); υ_{max}(KBr) cm^{-1} 3487, 3146, 2944, 2835, 2638, 2565, 1694, 1463 and 1211; δ_H[$(CD_3)_2SO$ + D_2O] 8.51(1H, s, H-6); δ_C[$(CD_3)_2SO$] 158.02, 157.74 148.83, 125.16.

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13. *Spectroscopic data* for compound **1**: mp 191 °C (decomp.); υ_{max}(KBr) cm^{-1} 3290, 3144, 2965, 1776, 1679, 1625, 1359, 1333 and 1172; δ_N[$(CD_3)_2SO$] 22.27, -35.21, -141.92, -151.55, -197.66, -245.64; δ_H[$(CD_3)_2SO$] 9.79(1H, brs, NH), 9.07(1H, s, H-6), 8.55(1H, brs, NH), 8.52(1H, NH); δ_C[$(CD_3)_2SO$] 154.01, 151.55, 145.14 (2C), 120.97; [Found: C, 33.2; H, 2.5; N, 44.8%; M + H, 181. $C_5H_4N_6O_2$ requires C, 33.3; H, 2.2; N, 44.7%, M + H, 181).

(*Received in Belgium* 12 *October* 1995; *accepted* 7 *December* 1995)