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Spectral Assignments and Reference Data

A ^{15}N NMR investigation of a series of benzotriazinones and related antitumour heterocycles

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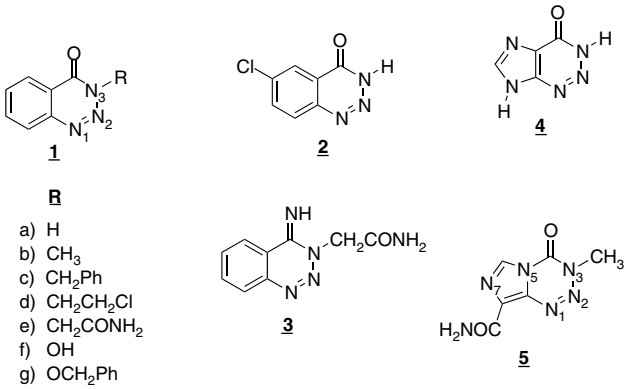
A series of 3-substituted 1,2,3-benzotriazin-4-ones, 1 and 2, were synthesized by standard methods and the ^{15}N NMR spectra were recorded. All spectra were obtained using the natural abundance of the nitrogen-15 isotope. The chemical shifts appear in the normal range for N-1, N-2 and N-3 of the triazine ring, and also correlate with the chemical shifts in the spectra of the imidazolotriazinone, 4, and the imidazolotetrazinone, 5. Significantly, the spectra of 1a, 2 and 4, recorded with full NOE, show inversion of the singlet assigned to N-3, demonstrating that these compounds exist in the tautomeric form shown. The structure of the 4-iminobenzotriazinone (3) was confirmed by this ^{15}N NMR analysis. The spectrum shows a signal for the NH-bearing imino-nitrogen atom, which is an inverted singlet in the NOE spectrum, whereas the signal from the N-3 atom of 3 is not inverted in the NOE spectrum. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{15}N NMR; benzotriazinones; antitumour drugs

INTRODUCTION

The antitumour drug temozolomide (**5**) (Scheme 1) is of considerable current interest and is now used widely for the treatment of malignant glioma.[1] A previous study[2] reported a multinuclear NMR study of temozolomide and the related drug mitozolomide. The synthesis of ^{15}N isotopically labelled temozolomide allowed the assignment of all the ^{15}N resonances of the compound. In the present work, we recorded the ^{15}N NMR spectra of a series of 1,2,3-benzotriazinones (**1a**–**g** and **2**), the imino-1,2,3-benzotriazine (**3**) and 2-azahypoxanthine (**4**), and assigned the chemical shifts to the respective nitrogen atoms by comparison with the chemical shifts observed for temozolomide (**5**).



Scheme 1

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EXPERIMENTAL

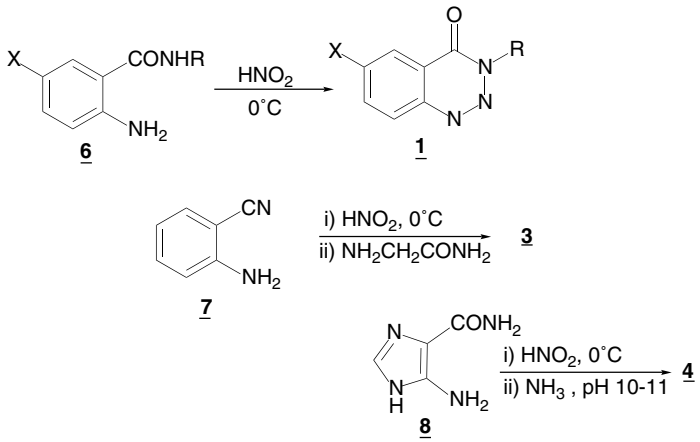
Sample preparation

*1,2,3,-Benzotriazin-4(3H)-one (**1a**)*
This was prepared by diazotization of anthranilamide (**6** X = R = H) according to the method of Heller[3] (Scheme 2).

*6-Chloro-1,2,3-benzotriazin-4(3H)-one (**2**)*
6-Chloroanthranilamide (**6**, X = Cl, R = H) (Aldrich Chemical) was diazotized in 2 M hydrochloric acid with sodium nitrite and neutralized to afford 6-chloro-1,2,3-benzotriazin-4(3*H*)-one (**2**).

*3-Methyl-1,2,3-benzotriazin-4-one (**1b**) and related compounds*
Methyl anthranilate (**9**) was diazotized and the resulting diazonium salt coupled with methylamine according to the method of LeBlanc and Vaughan,[4] to afford the 1-aryl-3-methyltriazene (**10** R = Me) (Scheme 3), which was cyclized by heating in ethanolic solution to afford the triazinone (**1b**).

The same method was used to prepare 3-(2-chloroethyl-)1,2,3-benzotriazin-4-one (**1d**), 3-benzyl-1,2,3-benzotriazin-4-one (**1c**)[4] and 3-carbamoylmethyl-1,2,3-benzotriazin-4-one (**1e**). Full details of the characterization of **1e** are reported elsewhere.[5]



Scheme 2

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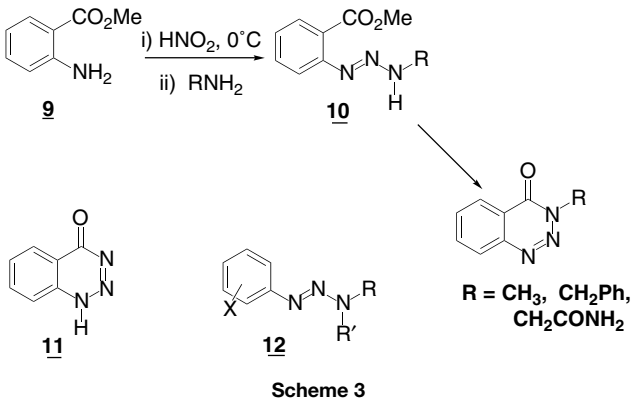

Scheme 3

*3-Hydroxy-1,2,3-benzotriazin-4-one (***1f***) and the 3-benzoyloxy compound (***1g***)*
Methyl anthranilate was treated with hydroxylamine in the presence of sodium hydroxide to afford the *o*-aminobenzhydroxamic acid (**6** X = H, R = OH), which was diazotized in hydrochloric acid with sodium nitrite according to the method of Ahern *et al*.[6] to afford 3-hydroxy-1,2,3-benzotriazin-4-one (**1f**).

Reaction of **1f** with benzoyl chloride in pyridine afforded 3-benzoyloxy-1,2,3-benzotriazin-4-one (**1g**).[6]

*3-(Carbamoylmethyl)-4-imino-1,2,3-benzotriazine (***3***) (Scheme 2)*
Anthranilonitrile (**7**) was diazotized in hydrochloric acid with sodium nitrite to afford the diazonium salt, which was coupled with glycinamide. The resulting intermediate triazene cyclized spontaneously to give the 4-iminotriazine (**3**). Full details of the characterization of **3** are described elsewhere.[5]

*2-Azahypoxanthine (***4***) (Scheme 2)*
5-Aminoimidazole-4-carboxamide (**8**) (0.50 g) was dissolved in 1 M hydrochloric acid (6.0 ml), cooled to 0 °C and then added slowly to a well-stirred ice-cold solution of sodium nitrite (2.0 g) in water (15 ml). The mixture was stirred for 10 min and then treated with concentrated ammonia to pH 10–11. The resulting clear brown solution was stirred for a few minutes until precipitation was complete. The product was filtered and dried to afford 2-azahypoxanthine (**4**) (0.31 g, 57%), m.p.>250 °C.

*Temozolomide (***5***)*
This was obtained from Aston Molecules (Birmingham, UK).

Spectroscopy techniques
^{15}N NMR spectra were recorded in DMSO solution on a Bruker AC250 spectrometer (at the Institute of Cancer Research, Sutton) at 25.36 MHz using a multinuclear 10 mm probe. Solutions were prepared by dissolving 1 mmol of each compound in dimethyl sulphoxide (2.4 ml) containing 30% (w/w) DMSO-d_6 with 30 mg of chromium acetylacetonate. A concentric capillary tube containing nitromethane was used as external reference. Spectra were recorded at 305 K. All spectra were obtained with the natural abundance of the nitrogen-15 isotope; no isotope enrichment was necessary to allow unequivocal assignment of all signals.

The microprogram INVGATE.AU (standard Bruker software) was used to acquire NOE-suppressed proton-decoupled ^{15}N spectra by inverse-gated heteronuclear decoupling. Confirmation of proton attachment was obtained by heteronuclear gated decoupling with the GATEDEC.AU microprogram which provided ^{1}H coupled spectra with full NOE. The parameters used were pulse width 14 μs (45°), decoupler power 18 H and 90° pulse for composite pulse decoupling 163 μs. The spectral width was 20 kHz and with 16 K data points gave an acquisition time of 0.41 s and a digital resolution of 2.44 Hz per point. Typically, a total of 32 000 scans were acquired for unlabelled compounds; the FID was exponentially multiplied (line broadening 5 Hz) before Fourier transformation.

Table 1. ^{15}N chemical shift data (δ, ppm) for a series of 3-alkyl-1,2,3-benzotriazinones (fully ^{1}H decoupled, without NOE)

Compound	N-1[a]	N-2	N-3	Other
1a	−16.5	29.3	−152.5[b]	—
1b	−16.0	32.8	−153.8	—
1c	−13.5	32.4	−143.8	—
1d	−14.0	31.8	−150.3	
1e	−14.7	33.1	−151.3	−274.6[c]
1f	−23.8	24.7	−113.4	
1g	−17.1	25.2	−104.1	
2	−18.8	30.0	−152.5[d]	
4	−35.3	22.4	−142.0	—[e]

[a]See Scheme 1 for atom notation.
[b]−152.6 ppm, inverted singlet when ^{1}H coupled with full NOE.
[c]−274.7 ppm, inverted triplet when ^{1}H coupled with full NOE.
[d]−152.5 ppm, inverted doublet when ^{1}H coupled with full NOE.
[e]N atoms of imidazole ring not observed.

DISCUSSION

Table 1 gives the ^{15}N chemical shifts of the benzotriazinones (**1a**–**g** and **2**) and of azahypoxanthine (**4**). The chemical shift of N-1 is in the range −13 to −19 ppm for all of the triazinones, except for **1f** at −23.8, compared with the chemical shift of ca −35 ppm in the imidazolo-fused compounds (**4** and **5**). The chemical shift of N-2 is in the range 20–33 ppm for all compounds and N-3 is in the range −142 to −153 ppm for most compounds of the series, with the exception of temozolomide, with an upfield shift to −180 ppm, and the N-oxy compounds (**1f** and **1g**), which show a downfield shift to −104 to −113 ppm. Compounds **1e**, **3** and **5** exhibit a signal a ca −275 ppm arising from the amide N-atom; this signal has been unambiguously assigned to the amide nitrogen by full ^{1}H NOE, which shows the signal as an inverted triplet. Compound **5** shows the expected signals for the imidazole nitrogens at −105.6 and −199.8 ppm. Surprisingly, the N-atoms of the imidazole ring in azahypoxanthine (**4**) are not observed, possibly owing to a combination of signal broadening and the effect of exchange of the hydrogen atom between both nitrogen sites of the imidazole ring.

When the spectra of **1a**, **2** and **4** are recorded with ^{1}H coupled NOE, the signal from N-3 is inverted in each case, showing that a hydrogen atom is bonded to N-3. This observation confirms that these molecules do indeed exist in the tautomeric form shown (e.g. **1a**), rather than the alternative tautomer with the hydrogen atom located at N-1 as in structure **11** (Scheme 3). The spectra of **2**, with and without NOE, are shown in Fig. 1 to illustrate these observations. The appearance of a doublet for the N-3 nitrogen in the spectrum of **2** with NOE [Fig. 1(b)] allows the measurement of the coupling constant ^1J(N,H), which is found to be 99 ± 5 Hz in this case. This value is very close to previously reported ^1J(N,H) coupling constants.[2]

The value of these ^{15}N chemical shift assignments is evident in the analysis of the ^{15}N NMR spectrum of 3-(carbamoylmethyl)-4-imino-1,2,3-benzotriazine (**3**). In an independent study,[5] a series of 1-aryl-3-carbamoylmethyltriazenes, ArN═N—NH—CH_2CONH_2, were prepared and characterized. As part of this study, the diazotization of anthranilonitrile (**7**) was carried out and the resulting diazonium salt was coupled with glycinamide, $NH_2CH_2CONH_2$, to afford an unstable triazene, which undergoes spontaneous cyclization. The cyclization product was assigned structure **3** on the basis of IR and ^{1}H and ^{13}C NMR spectroscopic evidence, but the structure was not unequivocal. The ^{15}N NMR spectrum of **3** removed the ambiguity from the structural assignment; the ^{15}N chemical shifts of **3** are shown in Table 2. Chemical shifts for N-1, N-2 and N-3 are all consistent with the previous observations, and the amide nitrogen at −275.05 ppm shows the appropriate inversion with NOE. The crucial evidence is the signal at −177 ppm, which

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Spectral Assignments and Reference Data

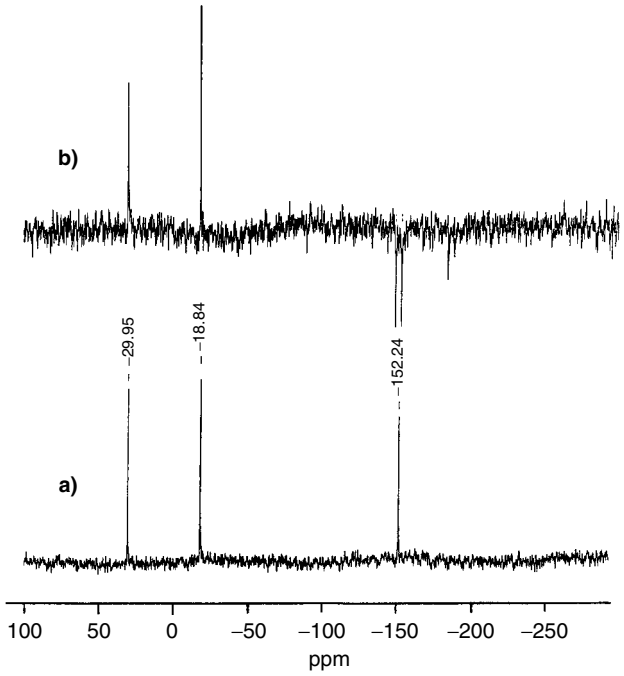

Figure 1. ^{15}N NMR spectra of 6-chloro-1,2,3-benzotriazin-4(3*H*)-one (**2**) in DMSO, (a) without NOE and (b) with NOE.

Table 2. ^{15}N chemical shift data (δ, ppm) for 3-carbamoylmethyl-4-imino- 1,2,3-benzotriazinone (**3**), with NOE

Atom	δ
N-1	−29.0
N-2	31.0
N-3	−168.4
Amide NH_2	−275.2 (inverted triplet)
Imino NH	−177.8 (inverted broad doublet)

is assigned to the imino-nitrogen atom at position 4 in the triazine ring. This nitrogen atom is shown to carry a hydrogen atom by the observed inversion with NOE.

The ^{15}N nucleus is relatively sensitive to changes in the atom or group immediately attached to the nitrogen, which can cause significant effects.[7] The ^{15}N chemical shift becomes more positive (i.e. shifts to lower field) with increasing electron withdrawal by attached groups. The chemical shift data reported in this paper are consistent with this general principle. For example, compare the chemical shift of N-3 in **1b** and **1f**; the electron-withdrawing effect of the oxygen atom in **1f**, compared with the methyl carbon in **1b**, causes a large downfield shift of ca 40 ppm. On the other hand, changing the substituent from a methyl group in **1b** to a chloroethyl group in **1d** causes only a marginal downfield shift of 3.5 ppm because the halogen atom is too far removed from the nitrogen to have a significant effect. The change from *N*-methyl in **1b** to *N*-benzyl in **1c** is more significant, causing a 10 ppm downfield shift. Introducing the chlorine substituent in the benzene ring as in **2** causes almost no change in any of the ^{15}N resonances when compared with those of **1a**; only N-1, the closest nitrogen to the halogen, is shifted marginally by 2.4 ppm.

Table 3. ^{15}N chemical shift data (δ, ppm) for 8-carbamoyl-3-methylimidazolo [5,1-*d*]-1,2,3,5-tetrazine-4-one (**5**)

Atom[a]	δ
N-1	−34.0
N-2	20.0
N-3	−180.4
N-5	−199.8
N-7	−105.6
Amide N	−274.9[b]

[a]See Scheme 1 for atom notation.
[b]Inverted triplet when ^{1}H coupled with full NOE.

The ^{15}N resonances assigned to N-1, N-2 and N-3 in the benzotriazines have chemical shifts with a similar order to the chemical shifts found in the open-chain analogues, the 1-aryl-3,3-dialkyltriazenes (**12**) (Scheme 3), for which typical values are N-1, −13 to −35 ppm, N-2, 68–75 ppm and N-3, −170 to −220 ppm.[8] The most important deviation in comparing the cyclic and open-chain variants is in the chemical shift of N-2, which differs by ca 40 ppm. The most likely explanation for this difference is in the differing geometry of the N-1═N-2 double bond; the cyclic benzotriazines are locked into the *cis* geometry, whereas the 1-aryl-3,3-dialkyltriazenes are known to prefer the *trans* geometry in the solid state.[9]

Acknowledgements

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REFERENCES

1. Friedman HS, Kerby T, Calvert H. *Clin. Cancer Res.* 2000; **6**: 2585.
2. Wheelhouse RT, Wilman DEV, Thompson W, Stevens MFG. *J. Chem. Soc.* 1995; 249.
3. Heller G. *J. Prakt. Chem.* 1925; **111**: 1.
4. LeBlanc RJ, Vaughan K. *Can. J. Chem.* 1972; **50**: 2544.
5. Hooper DL, Jollimore JV, Vaughan K. *J. Org. Chem.* 1996; **61**: 210.
6. Ahern TP, Navratil T, Vaughan K. *Can. J. Chem.* 1977; **55**: 630.
7. Levy GC, Lichter RL. *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*. John Wiley & Sons: New York, 1979; 28–107.
8. Wilman DEV. *Magn. Reson. Chem.* 1990; **28**: 729.
9. Neidle S, Wilman DEV. *Acta Crystallogr., Sect. B* 1992; **48**: 213.