The Synthesis of a Potential Anti-Cancer Agent Containing the Caffeine and 1,2,4-Benzotriazine Moieties

John Parrick* and Lina K. Mehta

Department of Chemistry, Brunel University, Uxbridge, Middlesex, UB8 3PH, U.K.

Richard J. Hodgkiss

Gray Laboratory of the Cancer Research Campaign, P.O. Box 100, Mount Vernon Hospital, Northwood, Middlesex, HA6 2JR, U.K.

Received September 17, 1992

The potential anti-cancer agent **6** has been synthesised from 4-(4-chlorobutoxy)-2-nitroaniline, **15b** by the conversion of the nitroaniline to the benzotriazine *N*-oxide **16**. Theophylline has been reacted with **16** to give the *N*-oxide **9** and this has been oxidised to the required *N,N'*-dioxide, **6**. The compound **6** has been found to be ineffective as a radiosensitizer.

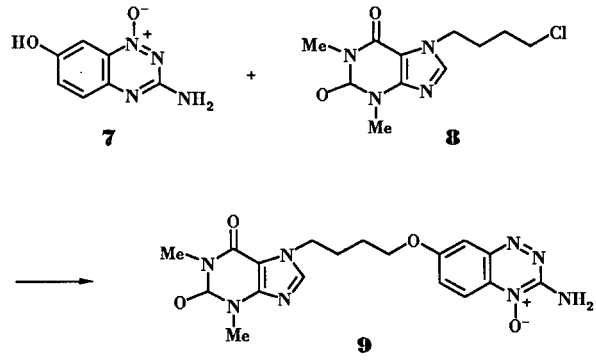
J. Heterocyclic Chem., **30**, 323 (1993).

The presence of hypoxic cells in a tumor arise as a result of rapid growth outstripping the available supply of oxygen. It is known [1] that these cells are about three times more resistant to radiation than the oxic cells. Several methods of overcoming this problem have been used clinically. An approach to overcoming this problem in radiotherapy is to develop chemicals called radiosensitizers, which mimic the sensitizing effect of oxygen and selectively increase the radiation sensitivity of hypoxic cells [2].

Included in the group of oxygen mimetic compounds are the electron-affinic sensitizers. Chemical structures associated with high electron affinity led to the discovery of metronidazole **1**, misonidazole **2** and SR-2508 **3** as very potent radiosensitizers for mammalian cells in culture [3-6]. Clinical evaluations [7-11] are encouraging but the neurotoxic properties of these compounds substantially limit their clinical usefulness.

Recently, a derivative of 2-nitroimidazole, 7-[4-(2-nitroimidazol-1-yl)butyl]theophylline (NITP) **4** has been found [12] to give an additional component of radiosensitization when present *after* irradiation, which is probably due to its ability to selectively inhibit the repair of radiation damage. However, no such effect has been obtained with **2** because this and other radiosensitizers must be present at the time of irradiation in order to exert their effect. A series of non-nitro compounds, benzotriazine dioxides described by Brown's group [13,14] have shown significant selective toxicity for hypoxic mammalian cells both *in vitro* and *in vivo*. The most active compound of the series was found to be 3-aminobenzo-1,2,4-triazine 1,4-dioxide (SR 4233) **5**.

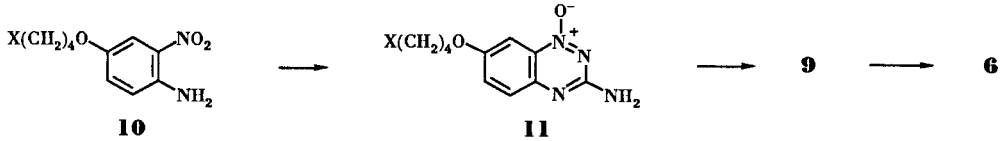
Scheme 1



Our interest in the development of novel cytotoxic agents for hypoxic cells and in radiosensitizers encouraged us to synthesise 3-amino-7-(4-[theophyllin-7-yl]butoxy)benzo-1,2,4-triazine 1,4-dioxide (L6) **6** an analogue of **4** but with a different electron affinic moiety. The ether group was incorporated in the structure for convenience in the synthesis.

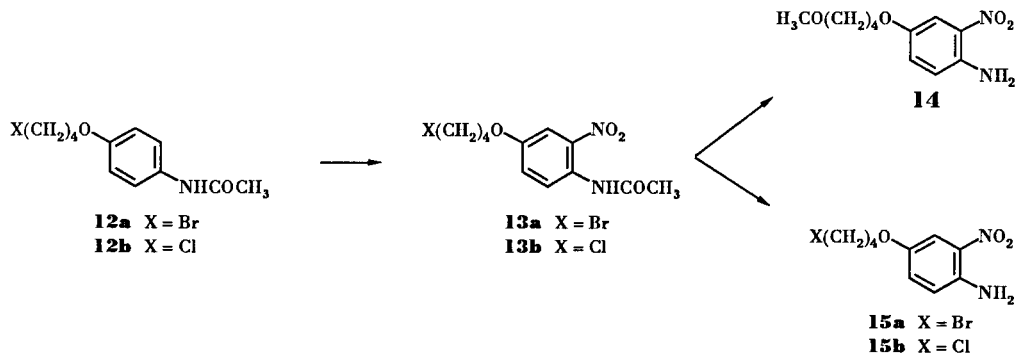
The most direct approach to the synthesis of **6** was to oxidise the product **9** (Scheme 1) expected to be obtained by the reaction of 3-amino-7-hydroxybenzo-1,2,4-triazine 1-oxide **7** [15] with 7-(4-chlorobutyl)theophylline **8** [16]. However, all out attempts to obtain **9** by this approach were unsuccessful.

Scheme 2



X = Halogen

Scheme 3



The majority of 3-aminobenzo-1,2,4-triazine 1-dioxides have been prepared by cyclization of 2-nitroanilines with cyanamide [17]. Thus, an alternative route to the desired **9** appeared to be the preparation of 4-(4-halogenobutoxy)-2-nitroaniline **10** and its treatment with cyanamide to obtain **11** (Scheme 2). The first problem was then the synthesis of **10**.

4-Bromobutoxyacetanilide **12a** was obtained by *O*-bromoalkylation [18] of *p*-hydroxyacetanilide and this on nitration [19] gave **13a**. Removal of the protecting group with cold alcoholic potassium hydroxide solution [20] gave the methyl ether **14** and the required **15a** in yields of 22 and 4%, respectively (Scheme 3).

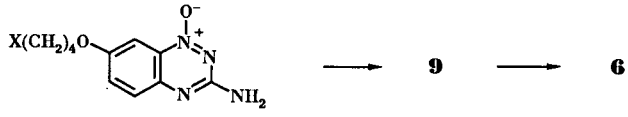
The hydrolysis of **13a** under acidic conditions was studied. Attempted deacetylation of **13a** under acidic conditions described by Lothrop [19] gave a mixture of three components. When less concentrated alcoholic hydrochloric acid was used, a product (45%) mainly composed of **15a** but containing **15b** was obtained as indicated by elemental analysis, but we were unable to separate the mixture. Hydrolysis of **13a** with hydrobromic acid in ethanol gave **15a** in 45% yield.

Later we found that the chloro compound **13b** was readily obtained from 4-(4-chlorobutoxy)acetanilide **12b** and was readily hydrolysed in good yield (75%) to **15b** with alcoholic hydrochloric acid. The chloro compound **15b** was used in the majority of subsequent work.

The conversion of the chloro compound **15b** to the benzotriazine 1-oxide **16** by the standard cyclization procedure [17] was inefficient giving **16** in only 13% yield. This result was in contrast to a cyclization of **14** which gave **17** in 63% yield, thus indicating the adverse effect of the presence of the alkyl halide function on the cyclization reaction.

N-Alkylation of the sodium salt of theophylline with **16** in the presence of 15-crown-5 [16] readily gave the derivative **9** (Scheme 4). The conversion of **9** into the dioxide **6** proved to be troublesome because **9** was insoluble in acetic acid, the usual medium for the *N*-oxidation of benzotriazine 1-oxides [17]. However, some success was achieved by the use of trifluoroacetic acid but an optimum yield of only 30% of **6** was achieved.

Scheme 4



16 X = Cl
17 X = OMe

Radiobiology.

Hypoxic toxicity data for L6 and SR 4233 are shown in Figure 1. Hall *et al.* [21] [22] and later others [23] [24] plotted the logarithm of the drug concentrations (C) against the logarithm of the incubation time with cells required to reduce the surviving fraction 0.1. From the slope of a straight line fit ($C = 1/k^*T^m$) to the data the time-dependence of toxicity (m) can be determined, suggesting a $CT^{0.65}$, $CT^{1.56}$, CT^2 and $CT^{1.1}$ relationship for L6 (**6**), SR 4233 (**5**), misonidazole (**2**) and NIPT (**4**), respectively. These values

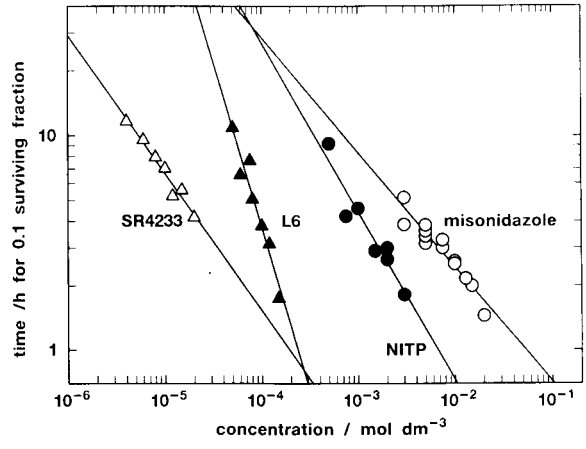


Figure 1. Concentration-time relationships required for a constant surviving fraction of 0.1. (△) SR4233; (▲) L6; (●) NITP; (○) misonidazole. The function fitted is $C=1/(k^*T^m)$, yielding slopes m = 0.64, 1.54, 0.92 and 0.50 for SR4233, L6, NITP and misonidazole respectively. Each point is derived from a full survival curve.

of the parameter m can be used to predict the likely effect on toxicity of a drug at constant concentration if the exposure time is altered, as may happen because of different plasma half-lives in different species. The solubilities of L6 and NITP were too low for similar assessments of their aerobic toxicity.

The novel compound had little effect on the radiosensitivity of V79 379A Chinese hamster cells when present at the time of hypoxic irradiation and for 24 hours afterwards under aerobic conditions (Figure 2). This result is in marked contrast to the effects observed [24] when the protocol was used with **4** where large radiosensitizing effects were seen in the absence of any toxicity.

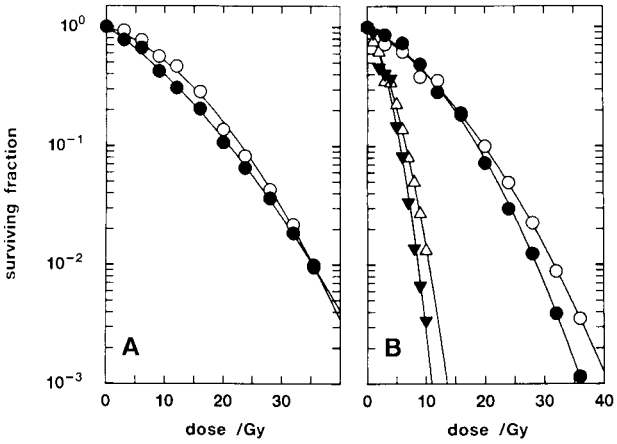
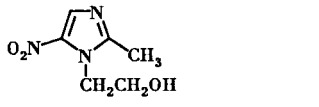
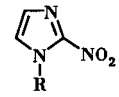


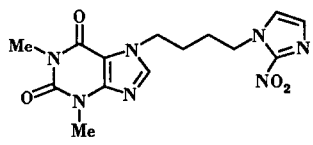
Figure 2. Radiation survival curves for Chinese hamster cells. A) (○) irradiated in hypoxia; (●) irradiated in hypoxia with 0.1 mmol dm^{-3} L6 with 24 h postirradiation aerobic contact with the drug. B) (△) aerobic irradiation; (○) hypoxic irradiation. Radiation survival curves for cells pretreated with L6 (0.1 mmol dm^{-3}, 2 h, S.F. = 0.5) (▲) aerobic irradiation; (●) hypoxic irradiation.



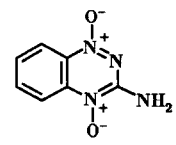
1



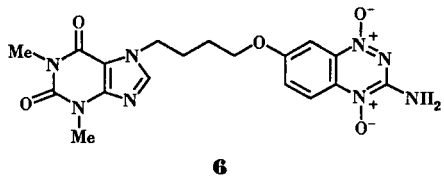
2 R = $CH_2CH(OH)CH_2OCH_3$
3 R = $CH_2CONHCH_2CH_2OH$



4



5



6

A toxic pretreatment of hypoxia with **6** made both aerobic and hypoxic cells slightly more radiosensitive than untreated cells. It has been reported that a toxic treatment of cells with **5** reduces the size of the shoulder of the aerobic radiation survival curve [25]. In our hands, the radiosensitizing action of a toxic treatment with **5** was small and mainly directed against hypoxic cells.

EXPERIMENTAL

Melting points were obtained in open capillary tubes using an electrothermal digital melting point apparatus and are uncorrected. The ir spectra were recorded for potassium bromide discs on a Perkin-Elmer 1420 Ratio recording infrared spectrophotometer. The ^1H nmr spectra were obtained on a Varian CFT-20 (80 Hz) and Jeol FX-200 (200 Hz) instrument using tetramethylsilane as internal standard. Low resolution electron impact mass spectra were obtained at 70 eV using a modified MS 902 spectrometer. Tlc was carried out on 0.25 nm silica gel precoated plastic plates obtained from CamLab. Flash column chromatography was performed using silica gel MPD 60 Å (40-60 microns). Elemental analyses were performed by Medac Limited, Brunel University. Petroleum ethers refers to the fraction of boiling range 40-60°.

General Procedure for the *O*-(4-Halogenoalkylation) of *p*-Hydroxyacetanilide to give **12a** and **12b**.

A mixture of *p*-hydroxyacetanilide (1.51 g, 10 mmoles), potassium carbonate (1.38 g, 10 mmoles) and 1,4-dihalogenobutane (15 mmoles) in ethanol (50 ml) was heated at reflux. When the reaction was complete (tlc about 8 hours), the mixture was poured into crushed ice and the precipitate filtered off and crystallised from carbon tetrachloride.

4-(4-Bromobutoxy)acetanilide (**12a**).

This compound was obtained as a colourless solid, yield 52%, mp 101-102° (lit [26] 102.5°).

4-(4-Chlorobutoxy)acetanilide (**12b**).

The compound was obtained in 67% yield, mp 103-104°; ir: ν NH 3320, CO 1650 cm^{-1}; ^1H nmr (deuteriochloroform): δ 7.31 (d, 2H, 2- and 6-H, J = 10 Hz), 7.01 (broad, 1H, NH, deuterium oxide exchangeable), 6.77 (dd, 2H, 3- and 5-H, J = 3, 6 Hz), 4.01-3.87 (m, 2H, OCH_2), 3.65-3.50 (m, 2H, CH_2Cl), 2.01 (s, 3H, $COCH_3$), 2.06-1.85 (m, 4H, CH_2CH_2); ms: m/z 243 (M^+ for ^{37}Cl, 8), 241 (M^+ for ^{35}Cl, 25), 151 (12), 109 (100).

Anal. Calcd. for $C_{12}H_{16}NO_2Cl$: C, 59.63; H, 6.67; N, 5.79. Found: C, 59.90; H, 6.80; N, 5.72.

General Procedure for the Nitration of 4-(4-Halogenobutoxy)-acetanilides to give **13a** and **13b**.

A slurry of the halogenobutoxyacetanilide **12a** or **12b** (1.7 mmoles) in glacial acetic acid (0.5 ml) was mixed with nitric acid (1.2 ml, 18%) and allowed to stand at room temperature for 24 hours. The solid was filtered off and washed with water. The washings and filtrate were poured into ice-water and the precipitated nitro compound collected. A further quantity was obtained by extraction of the initial solid with boiling ethanol, filtration and evaporation of the filtrate. The combined solids were crystallised from aqueous ethanol.

4-(4-Bromobutoxy)-2-nitroacetanilide (**13a**).

The nitro compound **13a** was obtained (0.4 g), yield 69%, mp 78-79°; ir: ν NH 3360, CO 1690, NO_2 1520 and 1340 cm^{-1}; ^1H nmr (deuterioacetone): δ 8.93 (broad, 1H, NH, deuterium oxide exchangeable), 8.19 (d, 1H, 6-H, J = 9 Hz), 7.56 (d, 1H, 3-H, J = 3 Hz), 7.27 (dd, 1H, 5-H, J = 3, 9 Hz), 4.23-4.07 (m, 2H, OCH_2), 3.66-3.50 (m, 2H, CH_2Br), 3.29 (s, 3H, $COCH_3$), 2.17-1.88 (m, 4H, CH_2CH_2); ms: m/z 332 (M^+ for ^{81}Br, 8), 330 (M^+ for ^{79}Br, 8), 290 (11), 288 (11), 154 (50), 135 (100).

Anal. Calcd. for $C_{12}H_{15}N_2O_4Br$: C, 43.52; H, 4.57; N, 8.46. Found: C, 43.28; H, 4.50; N, 8.74.

4-(4-Chlorobutoxy)-2-nitroacetanilide (**13b**).

This compound was obtained as a yellow crystalline solid, yield 60%, mp 76-77°; ir: ν NH 3360, CO 1680, NO_2 1560 and 1330 cm^{-1}; ^1H nmr (deuteriochloroform): δ 10.05 (broad s, 1H, NH, deuterium oxide exchangeable), 8.62 (d, 1H, 6-H, J = 9 Hz), 7.65 (d, 1H, 3-H, J = 3 Hz), 7.22 (dd, 1H, 5-H, J = 3, 10 Hz), 4.07-3.99 (m, 2H, OCH_2), 3.65-3.47 (m, 2H, CH_2Cl), 2.26 (s, 3H, $COCH_3$), 2.04-1.93 (m, 4H, CH_2CH_2); ms: m/z 288 (M^+ for ^{37}Cl, 7), 286 (M^+ for ^{35}Cl, 18), 247 (15), 244 (45), 154 (100).

Anal. Calcd. for $C_{12}H_{15}N_2O_4Cl$: C, 50.27; H, 5.27; N, 9.72. Found: C, 50.74; H, 5.29; N, 9.64.

4-(4-Methoxybutoxy)-2-nitroaniline (**14**).

A mixture of **13a** (0.5 g, 1.5 mmoles) and aqueous alcoholic potassium hydroxide solution (1 ml) (made by dissolving potassium hydroxide (8.8 g) in water (6 ml) and dilution to 25 ml with methanol) was stirred and warmed on a steam-bath for 15 minutes, when the mixture had become a red paste. Water (0.5 ml) was added and the heating continued for 15 minutes. The mixture was extracted with chloroform and the chloroform extract evaporated to yield an oil which was separated by flash chromatography (silica gel, ethyl acetate in petroleum ether, 1:4) to give **14** (22%) and **15a** (4%).

4-(4-Methoxybutoxy)-2-nitroaniline **14** was obtained as a viscous red oil, bp 147-148° at 0.5 mm of mercury; ir: ν NH_2 3470, 3340, NO_2 1570, 1335 cm^{-1}; ^1H nmr (deuteriochloroform): δ 7.53 (d, 1H, 3-H, J = 3 Hz), 7.04 (dd, 1H, 5-H, J = 3, 9 Hz), 6.72 (d, 1H, 6-H, J = 9 Hz), 5.82 (s, 2H, NH_2, deuterium oxide exchangeable), 4.02-3.87 (m, 2H, OCH_2), 3.50-3.42 (m, 2H, CH_2), 3.33 (s, 3H, OCH_3), 1.89-1.52 (m, 4H, CH_2CH_2); ms: m/z 240 (M^+, 13), 108 (15), 87 (100).

Anal. Calcd. for $C_{11}H_{16}N_2O_4$: C, 54.99; H, 6.71; N, 11.66. Found: C, 55.34; H, 6.88; N, 11.31.

4-(4-Bromobutoxy)-2-nitroaniline (**15a**).

A mixture of **13a** (1 g, 3 mmoles) and hydrobromic acid (0.7 ml, 49%) was refluxed in ethanol (20 ml) until the reaction was complete (tlc 36 hours). The mixture was made neutral by the addition of aqueous ammonia and the solvent evaporated. Water was added and the mixture extracted with chloroform. Evaporation of the extract gave a residue which was crystallised from aqueous ethanol to give red **15a** (0.4 g), yield 45%, mp 83-85°; ir: ν NH_2 3470, 3350, NO_2 1570, 1335 cm^{-1}; ^1H nmr (deuteriochloroform): δ 7.46 (d, 1H, 3-H, J = 2 Hz), 7.01 (dd, 1H, 5-H, J = 9, 3 Hz), 6.69 (d, 1H, 6-H, J = 8 Hz), 5.82 (broad s, 2H, NH_2, deuterium oxide exchangeable), 3.99-3.85 (m, 2H, OCH_2), 3.52-3.37 (m, 2H, CH_2Br), 2.13-1.82 (m, 4H, CH_2CH_2); ms: m/z 290 (M^+ for ^{81}Br, 19), 288 (M^+ for ^{79}Br, 20), 154 (56), 137 (98), 135 (100).

Anal. Calcd. for $C_{10}H_{13}N_2O_3Br$: C, 41.54; H, 4.53; N, 9.69. Found: C, 41.81; H, 4.56; N, 9.69.

4-(4-Chlorobutoxy)-2-nitroaniline (**15b**).

A mixture of **13b** (1 g, 3.5 mmoles) and hydrochloric acid (1 ml, 49%) was refluxed in ethanol (20 ml) until the reaction was complete (tlc 48 hours). The reaction mixture was worked up as described for **15a** to give red crystals of **15b** (0.64 g), yield 75%, mp 78-80°; ir: ν NH_2 3450, 3330, NO_2 1560, 1325 cm^{-1}; ^1H nmr (deuteriochloroform): δ 7.50 (d, 1H, 3-H, J = 3 Hz), 7.01 (dd, 1H, 5-H, J = 3, 9 Hz), 6.69 (d, 1H, 6-H, J = 9 Hz), 5.82 (s, 2H, NH_2, deuterium oxide exchangeable), 4.00-3.86 (m, 2H, OCH_2), 3.66-3.50 (m, 2H, CH_2Cl), 2.00-1.81 (m, 4H, CH_2CH_2); ms: m/z 246 (M^+ for ^{37}Cl, 13), 244 (M^+ for ^{35}Cl, 42), 154 (100), 108 (55).

Anal. Calcd. for $C_{10}H_{13}ClN_2O_3$: C, 49.09; H, 5.36; N, 11.45. Found: C, 49.38; H, 5.46; N, 11.27.

3-Amino-7-(4-chlorobutoxy)benzo-1,2,4-triazine 1-Oxide (**16**).

A mixture of **15b** (0.88 g, 3.6 mmoles) and cyanamide (1 g, 24 mmoles) was warmed until the mixture was molten. The cooled mixture was then treated with concentrated hydrochloric acid (1.5 ml) and warmed briefly until a vigorous reaction occurred. A solution of sodium hydroxide (1 g) in water (1.5 ml) was then added to the cooled mixture and the resultant solution heated on a steam-bath until the precipitation of a brilliant yellow solid was complete (about 15 minutes). The mixture was extracted with chloroform, the extract evaporated and the residue purified by flash chromatography (silica gel, ethyl acetate in petroleum ether, 1:1) to give **16** (0.13 g) in 13% yield which was crystallised from aqueous methanol, mp 192-194°; ir: ν NH_2 3350 cm^{-1}; ^1H nmr (DMSO-d_6): δ 7.48 (s, 3H, 5-, 6- and 8-H), 6.96 (s, 2H, NH_2, deuterium oxide exchangeable), 4.12-4.09 (m, 2H, OCH_2), 3.71-3.68 (m, 2H, CH_2Cl), 1.91-1.87 (m, 4H, CH_2CH_2); ms: m/z 270 (M^+ for ^{37}Cl, 10), 268 (M^+ for ^{35}Cl, 30), 178 (100), 161 (12).

Anal. Calcd. for $C_{11}H_{13}N_4O_2Cl$: C, 49.17; H, 4.88; N, 20.85. Found: C, 49.38; H, 4.90; N, 20.41.

3-Amino-7-(4-methoxybutoxy)benzo-1,2,4-triazine 1-Oxide (**17**).

A similar procedure to that used for the preparation of **16** but this time starting with **14** gave a yellow solid which was filtered off and crystallised from aqueous methanol to give **17** (0.12 g) in 65% yield, mp 186-188°; ir: ν NH_2 3370, 3320 cm^{-1}; ^1H nmr (deuteriochloroform): δ 7.5 (dd, 2H, 5- and 6-H, J = 2, 8 Hz), 7.43 (d, 1H, 8-H, J = 2 Hz), 5.04 (s, 2H, NH_2, deuterium oxide exchangeable), 4.11-4.01 (m, 2H, OCH_2), 3.45-3.37 (m, 2H, CH_2), 3.35 (s, 3H, OCH_3), 1.91-1.78 (m, 4H, CH_2CH_2); ms: m/z 264 (M^+, 8), 178 (13), 87 (100).

Anal. Calcd. for $C_{12}H_{16}N_4O_3$: C, 54.54; H, 6.10; N, 21.20. Found: C, 54.29; H, 6.12; N, 21.09.

3-Amino-7-(4-[theophyllin-7-yl]butoxy)benzo-1,2,4-triazine 1-Oxide (**9**).

A mixture of **16** (0.18 g, 67 mmoles), the sodium salt of theophylline (0.13 g, 67 mmoles) [16], 15-crown-5 (1 ml) and acetonitrile (70 ml) was refluxed for 5 days. The solid was filtered off from the hot mixture, washed with water and crystallised from aqueous dimethylsulfoxide to give **9** (0.45 g) in 80% yield, mp 240° dec; ir: ν NH_2 3420, 3310, CO 1700, 1650 cm^{-1}; ^1H nmr (DMSO-d_6 at 80°): δ 8.02 (s, 1H, 8-H of theophylline), 7.46-7.39 (m, 3H, 5-, 6- and 8-H), 6.76 (s, 2H, NH_2, deuterium oxide exchangeable), 4.37-4.30 (m, 2H, OCH_2), 4.10-4.04 (m, 2H, NCH_2), 3.40 (s, 3H, NCH_3), 3.22 (s, 3H, NCH_3), 2.04-1.93 (m, 2H, CH_2), 1.78-1.72 (m, 2H, CH_2); ms: m/z (M^+, 1), 396 (74), 235 (100), 221

(20), 193 (56), 178 (25).

Anal. Calcd. for $C_{18}H_{20}N_8O_4 \cdot 1/4H_2O$: C, 51.86; H, 4.92; N, 26.89. Found: C, 51.81; H, 4.96; N, 26.40.

3-Amino-7-(4-[theophyllin-7-yl]butoxy)benzo-1,2,4-triazine 1,4-Dioxide (**6**).

Aqueous hydrogen peroxide (8 ml, 30%) was added to a solution of **9** (0.61 g, 1.5 mmoles) in trifluoroacetic acid (5.5 ml) and the mixture stirred at 50° for 1 hour. Sodium hydrogen carbonate was then added to neutralise the mixture and to cause the formation of a red precipitate. The solid was removed and purified by flash chromatography (methanol in chloroform, 1:99) to give **6** (0.19 g) in 30% yield. An analytical sample was crystallised from water, mp 223-225° dec; ir: ν NH_2 3300, CO 1700, 1670 cm^{-1}; ^1H nmr (DMSO-d_6): δ 8.06 (d, 1H, 5-H, J = 9 Hz), 7.7 (s, 1H, 8-H of theophylline), 7.54 (dd, 1H, 6-H, J = 2, 10 Hz), 7.44 (d, 1H, 8-H, J = 2 Hz), 6.83 (s, 2H, NH_2, deuterium oxide exchangeable), 4.37-4.30 (m, 2H, OCH_2), 4.14-4.07 (m, 2H, NCH_2), 3.40 (s, 3H, NCH_3), 3.21 (s, 3H, NCH_3), 2.03-1.93 (m, 2H, CH_2), 1.78-1.71 (m, 2H, CH_2); ms: (FAB) m/z 429 (M^+ + 1, 26), 391 (55), 149 (100).

Anal. Calcd. for $C_{18}H_{20}N_8O_5 \cdot 1\frac{1}{2}H_2O$: C, 47.47; H, 5.05; N, 24.61. Found: C, 47.37; H, 5.01; N, 24.24.

Radiobiology.

Dimethyl sulphoxide (DMSO) and other reagents were BDH AnalaR grade. Special gases (air + 5% CO_2, nitrogen + 5% CO_2), were obtained from British Oxygen Company. V79 379A Chinese hamster cells were maintained as exponentially growing suspension cultures in Eagle's Minimal Essential Medium with Earle's salts, modified for suspension culture, with 7.5% foetal calf serum. For toxicity measurements, cell suspensions (5 x 10^5 cells ml^{-1}) were stirred under air + 5% CO_2 or nitrogen + 5% CO_2 and incubated with drugs at 37° as previously described [27] [28]. Compounds **2**, **5** and **6** were initially dissolved at 10-50 mmol dm^{-3} in DMSO and **2** also at 50 mmol dm^{-3} in growth medium and small volumes of stock solutions added to cell suspensions in growth medium to give the appropriate drug concentration. DMSO alone was not toxic at the maximum final concentration used (1% or less). Following incubation, cells were separated from the drug by centrifugation and resuspended in fresh medium before dilution and plating on 5 cm petri dishes, for assessment of survival in a 7-day colony forming assay.

Cells were irradiated with 250 kVp X-rays (1.46 mm Cu half value layer) as monolayers on glass dishes at 20° in a special perspex jig [29]. Before irradiation, cells were equilibrated with air + 5% CO_2 or nitrogen + 5% CO_2 by passing the gas over the culture for 1 hour, in the presence or absence of drugs as appropriate. Following irradiation of cell monolayers, the medium was removed and the cells washed with PBS to remove residual drugs, before the addition of fresh medium. In some cases drugs were left in post-irradiation contact with cell monolayers at 37° and washed off 18 hours after the irradiation. Cells pre-treated with drugs before irradiation, were centrifuged to remove drug solutions and resuspended in fresh medium, before diluting and plating. Survival of cells was assessed in a 7-day colony-forming assay, with no drugs present in the culture medium.

Acknowledgement.

We are grateful to the Cancer Research Campaign for financial support and the Science and Engineering Research Council mass spectrometry service for the FAB mass spectra.

REFERENCES AND NOTES

[1] K. A. Kennedy, B. A. Teicher, S. Rockwell and A. C. Sartorelli, *Biochem. Pharmacol.*, **29**, 1 (1980).

[2] G. E. Adams, *Br. Med. Bull.*, **29**, 48 (1973).

[3] J. L. Foster and R. L. Willson, *Br. J. Radiol.*, **46**, 234 (1973).

[4] J. C. Asquith, M. E. Watts, K. Patel, C. E. Smithen and G. E. Adams, *Radiat. Res.*, **60**, 108 (1974).

[5] G. E. Adams, J. F. Fowler, S. Dische and R. H. Thomlinson, *Lancet*, 186 (1976).

[6] J. M. Brown, N. Y. Yu, D. M. Brown and W. W. Lee, *Int. J. Radiat. Oncol. Biol. Phys.*, **7**, 695 (1981).

[7] R. Utrasun, P. Band, J. D. Chapman, M. L. Feldstein, B. Mielke and C. Fryer, *N. Engl. J. Med.*, **294**, 1364 (1976).

[8] J. M. Brown and P. Workman, *Radiat. Res.*, **82**, 171 (1980).

[9] T. L. Phillips, T. H. Wasserman, R. J. Johnson, V. A. Levin and G. VanRaalte, *Cancer*, **48**, 1697 (1981).

[10] P. Workman, Advanced Topics on Radiosensitizers of Hypoxic cells, A. Breccia, C. Rimondi and G. E. Adams, eds, Plenum, New York, 1982, p 143.

[11] J. M. Brown, *Cancer*, **55**, 2222 (1985).

[12] R. J. Hodgkiss, G. Jones, A. Long, J. Parrick, K. A. Smith, M. R. L. Stratford and G. D. Wilson, *Br. J. Cancer*, **63**, 119 (1991).

[13] E. M. Zeman, J. M. Brown, M. J. Lemmon, V. K. Hirst and W. W. Lee, *Int. J. Radiat. Oncol. Biol. Phys.*, **12**, 1239 (1986).

[14] E. M. Zeman, M. A. Baker, M. J. Lemmon, C. J. Pearson, J. A. Adams, J. M. Brown, W. W. Lee and M. Tracy, *Int. J. Radiat. Oncol. Biol. Phys.*, **16**, 977 (1989).

[15] P. Workman, M. I. Walton, J. Johnson, J. F. Baker, G. R. Gordon, A. B. Kelson and M. Tracy, 7th International Conference on Chemical Modifiers of Cancer Treatment, Clearwater, Florida, D16, February 2-5, 1991.

[16] A. Long, J. Parrick and R. J. Hodgkiss, *Synthesis*, **21**, 709 (1991).

[17] J. C. Mason and G. Tennant, *J. Chem. Soc.*, B 91 (1970).

[18] W. Weigel and J. Prakt, *Chem.*, [4], **4**, 79 (1956).

[19] W. C. Lothrop, *J. Am. Chem. Soc.*, 1698 (1942).

[20] P. E. Fanta and D. S. Tarbell, Organic Syntheses, Vol **25**, W. E. Bachman, ed, John Wiley & Sons, Inc., London, 1945, p 78.

[21] E. J. Hall, R. Miller, M. Astor and F. Rini, *Br. J. Cancer*, **37**, Suppl. III, 120 (1978).

[22] E. J. Hall, M. Astor, R. Osmak, P. Shapiro and A. August, *Int. J. Radiat. Oncol. Biol. Phys.*, **5**, 1781 (1979).

[23] R. J. Hodgkiss and R. W. Middleton, *Biochem. Pharmacol.*, **34**, 2175 (1985).

[24] R. J. Hodgkiss, E. Kelleher and J. Parrick, *Int. J. Radiat. Biol.*, **62**, 797 (1992).

[25] E. M. Zeman and J. M. Brown, *Int. J. Radiat. Oncol. Biol. Phys.*, **16**, 967 (1989).

[26] W. Davis, J. J. Roberts and W. C. J. Ross, *J. Chem. Soc.*, 890 (1955).

[27] A. C. Begg, R. J. Hodgkiss, N. J. McNally, R. W. Middleton, M. R. L. Stratford and N. H. A. Terry, *Brit. J. Radiology*, **58**, 645 (1985).

[28] R. J. Hodgkiss, R. W. Middleton, M. R. L. Stratford and R. Del. Buono, *Biochem. Pharmacol.*, **36**, 1483 (1987).

[29] R. J. Hodgkiss and M. R. L. Stratford, *Int. J. Radiat. Biol.*, **54**, 601 (1988).