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Antitumour Benzothiazoles. Part 15:[1] The Synthesis and Physico-Chemical Properties of 2-(4-Aminophenyl)benzothiazole Sulfamate Salt Derivatives

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Abstract—A series of sulfamate salt derivatives of the potent and selective 2-(4-aminophenyl)benzothiazole antitumour agents has been prepared and their evaluation as potential prodrugs for parenteral administration carried out. The salts were sparingly soluble under aqueous conditions (pH 4–9), and degradation to the active free amine was shown to occur under strongly acidic conditions. The salts were found to be markedly less active than their parent amines against sensitive human tumour cell lines in vitro.

Introduction

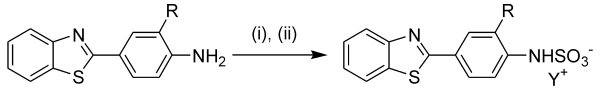
The structurally simple and synthetically accessible 2-(4-aminophenyl)benzothiazoles **1** possess potent and selective activity in certain human tumour cell lines in vitro and in vivo.[2] As a series, their biological profile is unlike any known investigational anticancer agents, and for these reasons they are currently the focus of extensive preclinical study.[3]

The lipophilicity of these agents, however, presents limitations on drug formulation and bioavailability, posing a pharmaceutical challenge as an aqueous iv formulation is desired to minimise the possibility of first pass deactivating metabolism[4] and improve drug bioavailability. Compounds of this class are known to be weak bases, for example, compound **1a** (R = H) has a pK_a of 3.0 in cyclohexane,[5] and the salts undergo dissociation in the pH range suitable for parenteral administration. In previous studies in this area the simple mono- and di-hydrochloride salts of series **1** have been synthesised, together with salts with methane- and ethane-sulfonic acids, and analysis of the sites of protonation carried out through observation of changes in NMR chemical shifts on titration with TFA.[6,7] As part of our attempts to develop water-soluble prodrugs which generate the active free base under physiological conditions, we report here on the synthesis and physicochemical properties of one such class of agent, the 2-(4-aminophenyl)benzothiazole sulfamate salts.

It is known that *N*-arylsulfamic acids are normally too unstable to isolate and only a few are known in the free state; the corresponding salts however are fairly stable in neutral solution.[8] In order to investigate the previously unexplored potential for sulfamate salts to act as prodrugs suitable for parenteral administration, sodium and ammonium salts of the most potent members of the 2-(4-aminophenyl)benzothiazole series were prepared.

Chemistry

The *N*-arylsulfamic acid salts **2a–d** were prepared in good yields according to the method of Sureau and Obellianne[9] from 2-(4-aminophenyl)benzothiazoles **1a–d** as shown in Scheme 1.[10,11]



1a (R=H)
1b (R=Me)
1c (R=Cl)
1d (R=I)
1e (R=Br)

2a (R=H, Y=Na or NH_4)
2b (R=Me, Y=Na)
2c (R=Cl, Y=Na)
2d (R=I, Y=Na)

Reagents and conditions: (i) $ClSO_3H$, 2-picoline; (ii) aq. Na_2CO_3 or NH_3

Scheme 1. The synthesis of sulfamic acid salt prodrugs.

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Physico-Chemical Properties

In order to evaluate the potential of sulfamate salt prodrugs for potential iv formulation, we conducted a number of studies on the physico-chemical properties of both the sulfamate salts and the parent free amines. In this respect log P determinations, solubility in solvents suitable for parenteral administration, and the effect of pH on the aqueous solubility and stability profiles were determined. Studies were concentrated on compounds where R was either a methyl or halogen group (Cl or I) since these compounds have previously been shown to be more potent and have an extended spectrum of activity in certain human tumour cell lines as compared to the unsubstituted (R = H) compound.[3]

Log P determination

The partitioning of benzothiazole free amines and the sulfamate salts **2b** and **2c** was determined at ambient temperature for systems of octanol:pH 7.4 phosphate buffer and cyclohexane:pH 7.4 phosphate buffer with the following results (Table 1).

Effect of pH on aqueous solubility and stability

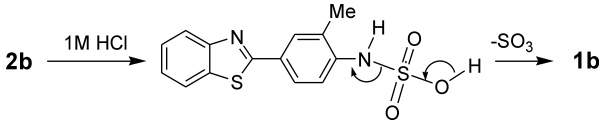
Preliminary preparative chemical studies indicated that sulfamate salt **2b** degraded to free base **1b** rapidly in 1 M HCl (aq) (59% conversion over 8 h at room temperature) (Scheme 2) but was essentially stable in water.

The solubility of the sulfamate salt **2b** was also determined at pH 3 and pH 7.4 using a citrate–citric acid and a phosphate buffer, respectively. In each case, **2b** was shaken vigorously with the buffer for 1 h prior to filtering the suspension through a 0.2 μm polysulfone filter unit. The filtrate was collected for HPLC analysis,[12] and solubility quantified by comparison of compound peak areas in test samples with those containing known concentrations of **2b**. The solubilities at pH 3 and pH 7.4 were found to be 0.01 and 2.1 mg/mL, respectively, lower than was anticipated.

Table 1. Log P determinations for selected 2-(4-aminophenyl)benzothiazoles and their sulfamate salts

Compound	Log P[a]	Log P[b]	ΔLog P
1b	3.96	2.51	1.45
1c	>3.3	>3.2	Ind[c]
1d	>3.3	>2.7	Ind[c]
1e	>3.3	>2.8	Ind[c]
2b	−0.60	<−3.00	>2.40
2c	−0.02	<−2.19	>2.17

[a]Octanol:buffer (pH 7.4).
[b]Cyclohexane:buffer (pH 7.4).
[c]Ind, indeterminate.



Scheme 2. Degradation of sulfamate salt **2b** under acidic conditions.

The solubility of sulfamate salt prodrug **2c** was determined over a pH range of 4–9 using a citrate–citric acid buffer at pH 4, a phosphate buffer over the pH range of 5–7.5 and a glycine–sodium hydroxide buffer at pH 9, using the method described for salt **2b**. The solutions prepared for the assessment of solubility were divided and aliquots stored at ambient temperature and at 50 °C. After a period of 2 days samples were subjected to HPLC analysis for assessment of levels of degradation to free base **1c** (Table 2).

The results below indicate that **2c** has a rather limited aqueous solubility over the pH range investigated. In acidic media, there was significant degradation of **2c** to **1c** at 50 °C with 21% being converted to **1c** at pH 4.2 over 2 days. However, at neutral and alkaline pH this degradation was not significant, nor was loss of sulfur trioxide evident at ambient temperature over the pH range studied.

Examination of the stability of compounds **2b** and **2c** in the presence of freshly prepared human plasma at 37 °C over a 4 h period by the HPLC method revealed that the salts were stable under these conditions.

In view of the limited aqueous solubility of **2c**, its solubility was also evaluated in a series of potential co-solvents, resulting in greatly enhanced solubility, most notably when using DMSO as co-solvent (Table 3).

Biological Results

The sulfamate salts **2a–d** have been tested for in vitro antitumour activity against the human breast tumour cell lines MCF-7 (oestrogen receptor, ER + ve) and MDA 468 (ER–ve) and compared to the results

Table 2. Aqueous solubility and stability of salt **2c** over the pH range 4–9

Solution pH	Solubility of **2c** (mg/mL)	**1c** (% total peak area)	
		50 °C	Ambient temperature
4.2	0.5	21.3	0.4
5.4	0.3	3.3	0.3
6.0	0.4	0.7	0.1
7.1	0.3	0.2	0.1
7.5	0.3	<0.1	<0.05
9.0	0.7	0.2	0.1

Table 3. The effect of co-solvents on the solubility of **2c**

Co-solvent	Estimated solubility (mg/mL)
Ethanol	≥10
DMSO	≥140
Propylene glycol	≥5
5% Glucose	≥10
0.9% w/v Sodium chloride	<2
10% DMSO in 0.9% saline	3[a]

[a]Cannot be diluted further with aqueous media without precipitation.

Table 4. IC_{50} values in sensitive breast cell lines MCF-7 (ER^+) and MDA 468 (ER^-)

Sulfamate salt	IC_{50} (μM) (MCF-7)[a]	IC_{50} (μM) (MDA 468)[a]
2a (Y = Na)	3.4	7.5
2b	0.49	0.72
2c	0.42	9.0
2d	1.8	1.9

[a]Results are the mean of at least three determinations.

obtained on the parent free bases according to methods (MTT assay) previously described.[2] The sulfamate salts were found to be generally several orders of magnitude less active (typical IC_{50}s in the low micromolar range) in comparison to their parent amines (IC_{50}s in the range <0.0001–$0.001\ \mu M$)[2] in the MCF-7 and MDA 468 cell lines (Table 4).

Conclusions

A series of sulfamate salt derivatives of the antitumour 2-(4-aminophenyl)benzothiazole series has been synthesised in good yields. Studies focused on sulfamate salts **2b** and **2c** revealed that neither was particularly soluble in water over the pH range 4–9 and, furthermore, decomposition of salt **2c** to the active free base **1c** was evident only at acidic pH at 50 °C. Since no degradation of **2b** or **2c** was found in biological matrices conducted as part of this study, further development of sulfamate salts would depend on the salts demonstrating potency in their own right. However, tests carried out on human tumour cell lines in vitro, which were previously found to be exquisitely sensitive to the parent amines, revealed that the corresponding sulfamate salts were much less active (Table 4). The sulfamate prodrug approach in this instance, whilst a novel strategy in prodrug design, was not found to yield the expected physico-chemical characteristics for further drug development. The results concerning other related prodrug strategies in the 2-(4-aminophenyl)benzothiazole area will be reported in due course.

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References and Notes

1. Part 14 of the series: Hutchinson, I.; Chua, M.-S.; Browne, H. L.; Trapani, V.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **2001**, in press.
2. Shi, D.-F.; Bradshaw, T. D.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M. F. G. *J. Med. Chem.* **1996**, *39*, 3375.
3. Bradshaw, T. D.; Wrigley, S.; Shi, D.-F.; Schultz, R. J.; Paull, K. D.; Stevens, M. F. G. *Br. J. Cancer* **1998**, *77*, 745.
4. Kashiyama, E.; Hutchinson, I.; Chua, M.-S.; Stinson, S. F.; Phillips, L. R.; Kaur, G.; Sausville, E. A.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **1999**, *42*, 4172.
5. Dey, J. K.; Dogra, S. K. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 3142.
6. Wheelhouse, R. T.; Shi, D.-F.; Wilman, D. E. V.; Stevens, M. F. G. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1271.
7. Shi, D.-F. PhD Thesis, University of Nottingham, 1995.
8. Gilbert, E. E. *Chem. Rev.* **1962**, *62*, 549.
9. Sureau, R. F. M.; Obellianne, P. M. J. US Patent 2,789,132; *Chem. Abstr.* **1957**, *51*, 15571.
10. In the general method, anhydrous 2-picoline (five equivalents) was slowly added to chlorosulfonic acid at 0 °C. Addition of the amine was followed by heating at 50 °C for 1 h. After cooling to room temperature an aqueous solution of either 10% sodium carbonate or 35% ammonia was added. Upon concentration in vacuo a white precipitate appeared which was collected by filtration, washed with water and recrystallised from chloroform to give the desired sulfamate salt. All new compounds were characterised by ^1H and ^{13}C NMR, IR spectroscopy, and mass spectrometry.
11. Yields and ^1H NMR data for salts **2a–d**: **2a**: (Y = Na) Yield 86%, ^1H NMR (DMSO-d_6) 8.55 (1H, s, NH), 8.06 (1H, d, J = 7.7 Hz, H-4), 7.97 (1H, d, J = 7.8 Hz, H-7), 7.84 (2H, d, J = 8.7 Hz, H-2′, H-6′), 7.49 (1H, dt, J = 1.3, 7.6 Hz, H-5), 7.38 (1H, dt, J = 1.2, 7.7 Hz, H-6), 7.17 (2H, d, J = 8.7 Hz, H-3′, H-5′); **2a** (Y = NH_4) Yield 69%, ^1H NMR (DMSO-d_6) 8.65 (1H, s, NH), 8.06 (1H, d, J = 7.8 Hz, H-4), 7.96 (1H, d, J = 8.0 Hz, H-7), 7.84 (2H, d, J = 8.6 Hz, H-2′, H-6′), 7.48 (1H, t, J = 7.4 Hz, H-5), 7.38 (1H, t, J = 7.5 Hz, H-6), 7.18–7.15 (6H, m, H-3′, H-5′, N^+H_4); **2b**: Yield 82%, ^1H NMR (DMSO-d_6) 8.07 (1H, d, J = 7.8 Hz, H-4), 7.97 (1H, d, J = 8.0 Hz, H-7), 7.78–7.75 (2H, m, H-2′, H-6′), 7.61 (1H, d, J = 8.3 Hz, H-5′), 7.50 (1H, t, J = 7.6 Hz, H-5), 7.39 (1H, t, J = 7.6 Hz, H-6), 3.37 (1H, br s, NH), 2.21 (3H, s, CH_3); **2c**: Yield 75%, ^1H NMR (DMSO-d_6) 8.08 (1H, d, J = 7.8 Hz, H-4), 7.96 (1H, d, J = 7.8 Hz, H-7), 7.91 (1H, d, J = 2.0 Hz, H-2′), 7.75 (1H, dd, J = 2.1, 8.7 Hz, H-6′), 7.70 (1H, d, J = 8.7 Hz, H-5′), 7.52 (1H, t, J = 7.7 Hz, H-5), 7.42 (1H, t, J = 7.7 Hz, H-6), 6.63 (1H, s, NH); **2d**: Yield 79%, ^1H NMR (DMSO-d_6) 8.38 (1H, d, J = 2.0 Hz, H-2′), 8.11 (1H, d, J = 7.5 Hz, H-4), 8.01 (1H, d, J = 7.8 Hz, H-7), 7.97 (1H, dd, J = 2.1, 8.7 Hz, H-6′), 7.62 (1H, d, J = 8.7 Hz, H-5′), 7.53 (1H, t, J = 7.6 Hz, H-5), 7.43 (1H, t, J = 7.6 Hz, H-6), 6.63 (1H, s, NH).
12. HPLC methodology: Primesphere column, 5 μm, C18 HC; mobile phase 45% acetonitrile in 0.04% v/v trifluoroacetic acid; flow rate 0.8 mL/min; UV detection at 220 nm; approximate retention times: **2c** = 3 min and **1c** = 30 min.