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Antitumour Benzothiazoles. Part 20:† 3′-Cyano and 3′-Alkynyl-Substituted 2-(4′-Aminophenyl)benzothiazoles as New Potent and Selective Analogues

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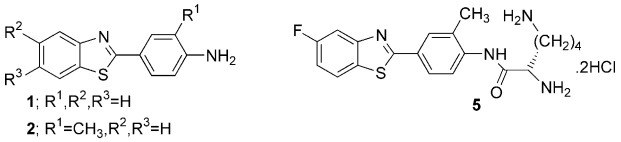
Abstract—The synthesis of a new series of antitumour 2-(4-aminophenyl)benzothiazole analogues, substituted in the 3′-position by cyano or alkynyl groups, is described. Several of the analogues, notably the 5-fluorinated compounds **7c** and **9c**, were found to possess potent in vitro activity against MCF-7 and MDA 468 human cancer cell lines. More comprehensive in vitro analysis (NCI 60-cell line) established compound **7c** as a particularly potent and selective 2-(4-aminophenyl)benzothiazole analogue.

The structural simplicity and synthetic accessibility of the 2-(4-aminophenyl)benzothiazole series belie remarkable antitumour properties. The original (unsubstituted) member of this series, 2-(4-aminophenyl)benzothiazole, **1**, was found to exhibit potent and selective activity against certain breast carcinoma cell lines in vitro (e.g., MCF-7, MDA 468, IC_{50} <1 nM) irrespective of oestrogen receptor status and with an unusual biphasic dose–response relationship.[2] Compound **1** was subsequently superceded as a lead compound with the discovery that certain 3′-substituents (Me, Cl, Br, I) conferred low nanomolar growth inhibitory activity in an extended set of human tumour cell lines, from which 2-(4-amino-3-methylphenyl)benzothiazole **2** was selected as lead candidate for further development on the basis of superior in vivo activity.[3–5]

Studies towards elucidation of the mechanism of action of this unique series of antitumour agents uncovered the crucial role of the cytochrome P450 isoform CYP1A1 as a bioactivation event preceding cell death.[6] In addition to its essential role in mediating antitumour activity, interaction of **2** and CYP1A1 additionally produced an inactive exportable metabolite, 2-(4-amino-3-methylphenyl)-6-hydroxybenzothiazole (**3**),[6,7] that was found to be antagonistic with respect to CYP1A1 activity and growth inhibition. Moreover, in oestrogen receptor positive (ER+) cells, concentrations of **3** between 300 nM and 30 μM were found to be mitogenic (observations which underlie the biphasic dose–response relationship observed in active compounds in this series). Circumvention of this deactivating hydroxylation was achieved by the synthesis and evaluation of a series of fluorinated analogues[8,9] from which 2-(4-amino-3-methylphenyl)-5-fluoro-benzothiazole **4** emerged as the lead candidate, retaining the exquisite potency and selectivity profile of **2** but bereft of inactive exportable metabolites. Compound **4**, as a lysyl-amide water-soluble prodrug **5**[1,10] is scheduled to enter Phase 1 clinical evaluation, under the auspices of Cancer Research UK, in early 2003 (Fig. 1).



1; R^1,R^2,R^3=H
2; R^1=CH_3,R^2,R^3=H
3; R^1=CH_3,R^2=H,R^3=OH
4; R^1=CH_3,R^2=F,R^3=H

Figure 1. Structures of antitumour 2-(4-aminophenyl)benzothiazoles **1**–**5**.

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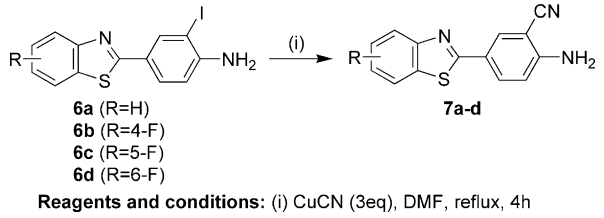
In this paper, we report the synthesis and evaluation as antitumour agents of a new generation of 2-(4-aminophenyl)benzothiazoles substituted in the 3-position of the phenyl ring by cyano- or alkynyl substituents, and fluorinated analogues thereof. Since previous structure–activity relationship studies had demonstrated that potent activity is retained in 2-(4′-aminophenyl)-benzothiazoles containing small 3′-substitutents (both electron-donating and electron-withdrawing), we were interested here in the effect of the small electron-withdrawing cyano-substituent and the related isosteric ethynyl-substituent on antitumour activity.

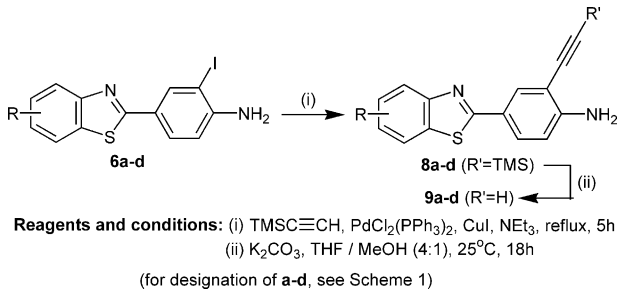
Chemistry

The synthesis of the 3′-cyano analogues **7a–d**[11] was accomplished in one step from the corresponding 2-(4-amino-3-iodophenyl)benzothiazoles **6a–d**,[2,8] using copper(I) cyanide in refluxing DMF (Scheme 1). Compounds **7a–d** all displayed a characteristic C≡N intrared stretching vibration at around 2220 cm^{-1}.

The synthesis of the 3′-alkynyl derivatives was accomplished in two steps from the corresponding 2-(4-amino-3-iodophenyl)benzothiazoles **6a–d** (Scheme 2). Palladium(0)-catalysed coupling (Sonogashira conditions) of the aryl iodide with (trimethylsilyl)acetylene gave the intermediate TMS-protected 3′-alkynyl derivatives **8a–d** which were readily deprotected using potassium carbonate in THF/methanol (4:1) to give the required 3′-alkynyl derivatives **9a–d**[12] in good overall yields. All terminal alkynes synthesised displayed a characteristic but weak infrared triple-bond stretching vibration at around 2100 cm^{-1}.



Scheme 1. Synthesis of 3′-cyano analogues.



Scheme 2. Synthesis of 3′-alkynyl analogues.

Biological Results

Compounds **7a–d**, **8a–d** and **9a–d** have been evaluated for in vitro antitumour activity in the human breast cancer cell lines MCF-7 (oestrogen receptor positive) and MDA 468 (oestrogen receptor negative). GI_{50} values for each cell line were determined using the three day MTT assay previously used for studies on the antitumour 2-(4-aminophenyl)benzothiazole series.[8] The results of these studies are shown in Table 1.

A number of interesting conclusions can be drawn from the results shown in Table 1.

Within the group of 3-cyano substituted compounds **7a–d**, the 5-fluoro analogue **7c** stands out as by far the most potent analogue. Analysis of in vitro activity in the NCI Developmental Therapeutics Program panel of sixty human cancer cell lines (2-day assay) confirms the exceedingly potent antitumour activity of **7c** in sensitive cell lines such as MCF-7 and MDA 468. In addition, this analogue retains the characteristic cell line selectivity profile unique to the 2-(4-aminophenyl)benzothiazole series. In terms of mean GI_{50} values across the cell panel **7c** is equipotent (4.47 μM vs 4.37 μM) and equally selective as 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole **4**. The NCI mean GI_{50} graph for **7c** is shown in Figure 2.

Compounds **8a–d** (containing a (trimethylsilyl)ethynyl substituent) are consistently less potent than their deprotected counterparts **9a–d**. This difference in activity is likely to be due to the poor affinity for the cytochrome P450 1A1 binding site in the more sterically encumbered trimethylsilyl derivatives compared to ethynyl derivatives **9a–d**. The superior potency of the 5-fluorinated analogues, notably compound **9c**, in these mini-series is again noteworthy. The remarkable effect on potency of the 5-fluoro substituent (in compounds **7–9c**) has previously been described in related antitumour benzothiazoles.[8]

Table 1. 50% growth inhibitory dose (GI_{50}) values for compounds **7a–d**, **8a–d** and **9a–d** in breast cancer cell lines MCF-7 and MDA 468 following 72 h treatment

Compd	MCF-7 GI_{50}, μM[a]	MDA 468 GI_{50}, μM[a]
7a	0.58	0.34
7b	0.18	0.080
7c	0.0057	0.0078
7d	0.82	0.32
8a	0.71	0.56
8b	0.52	0.23
8c	0.16	0.21
8d	0.68	0.40
9a	0.049	0.015
9b	0.11	0.030
9c	0.011	0.0068
9d	0.11	0.023

[a]Values are means of at least three experiments.

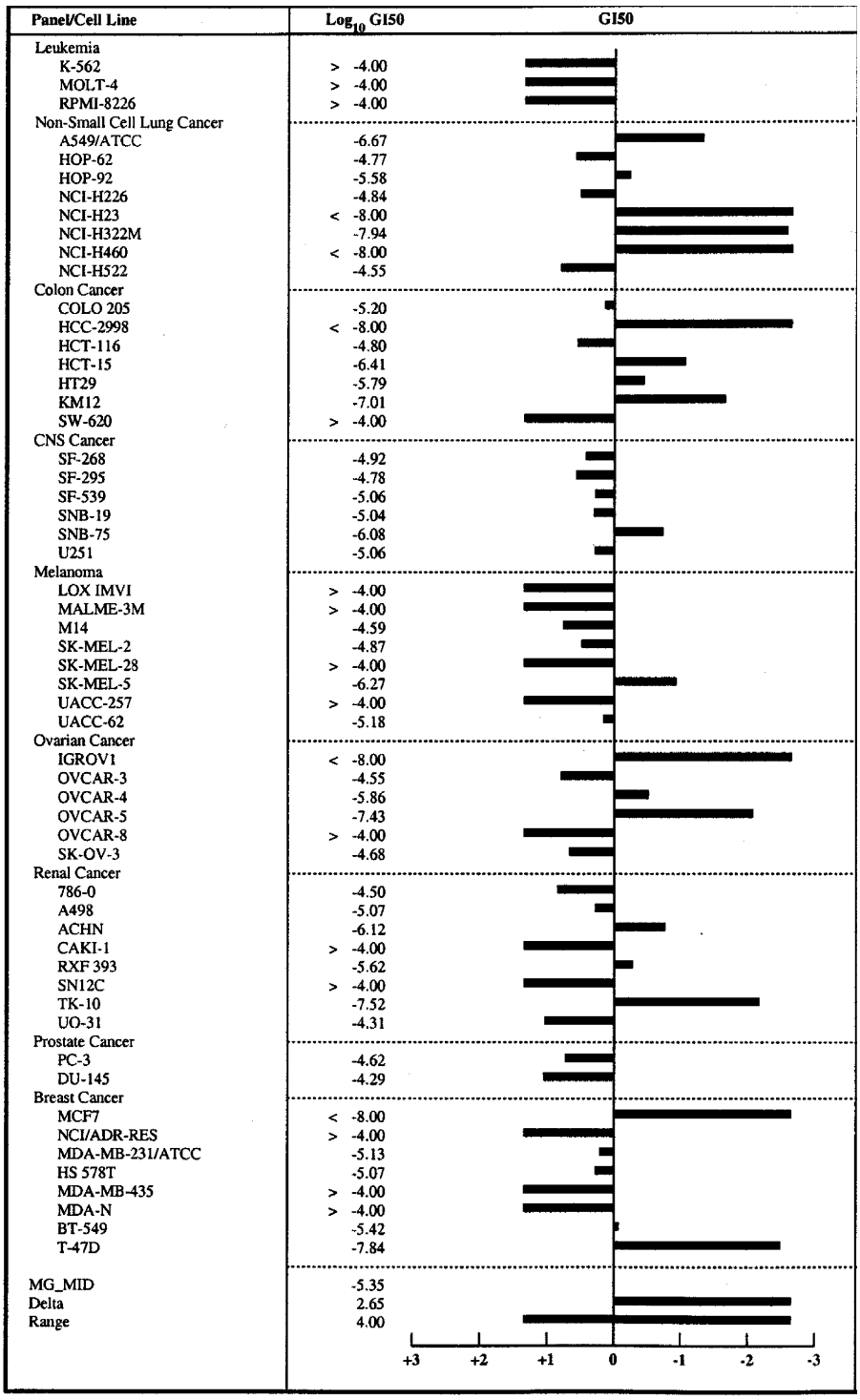


Figure 2. NCI mean GI_{50} graph for 5-fluoro-2-(4-amino-3-cyanophenyl)benzothiazole **7c**. Cell lines more sensitive than the mean across the 60 cell lines are represented by bars to the right, whilst cell lines less sensitive than the mean are shown as bars to the left (logarithmic scale).

Conclusions

A new series of fluorinated 3′-cyano and 3′-alkynyl-substituted 2-(4-aminophenyl)benzothiazoles related to previously described antitumour benzothiazoles has been synthesised. In vitro antitumour screening in MCF-7 and MDA 468 human cancer cell lines revealed that in general compounds **7a–d** and **9a–d** were potent agents in these antitumour benzothiazole-sensitive cell lines. The effect of the fluorination pattern in these series was dramatic with the 5-fluorinated derivatives being especially potent. NCI in vitro 60-cell line analysis has shown compound **7c** to be one of the most potent and selective antitumour analogues in this enigmatic series.

Acknowledgements

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11. General experimental method for compounds **7a**–**d**. A mixture of the 2-(4-amino-3-iodophenyl)benzothiazole (**6a**–**d**)[8] and copper (I) cyanide (3 mol equiv) in DMF was heated under reflux for 4 h. After cooling, DMF was removed in vacuo and the residue extracted using excess hot ethyl acetate followed by filtration. The filtrate was concentrated in vacuo and the crude product recrystallised from ethanol to give the required 3′-cyano substituted product. **7a**: Yield 77%, ^{1}H NMR ($CDCl_3$) 8.11 (1H, d, J = 2.2 Hz, H-2′), 8.04 (1H, dd, J = 8.8, 2.2 Hz, H-6′), 7.98 (1H, dd, J = 8.0, 1.3 Hz, H-7), 7.85 (1H, dd, J = 7.8, 1.3 Hz, H-4), 7.46 (1H, dt, J = 7.8, 1.3 Hz, H-5), 7.34 (1H, dd, J 8.0, 1.3 Hz, H-6), 6.81 (1H, d, J = 8.8 Hz, H-5′), 4.73 (2H, s, NH_2); **7b**: Yield 68%, ^{1}H NMR (DMSO-d_6) 8.12 (1H, d, J = 2.3 Hz, H-2′), 8.05 (1H, dd, J = 8.9, 2.3 Hz, H-6′), 7.93 (1H, d, J = 7.5, 1.5 Hz, H-7), 7.39 (2H, m, H-5, H-6), 6.94 (1H, d, J = 8.9 Hz, H-5′), 6.91 (2H, s, NH_2); **7c**: Yield 65%, ^{1}H NMR (DMSO-d_6) 8.13 (1H, dd, J = 9.0, 5.8 Hz, H-7), 8.10 (1H, d, J = 2.3 Hz, H-2′), 8.00 (1H, dd, J = 8.8, 2.3 Hz, H-6′), 7.80 (1H, dd, J = 9.0, 2.5 Hz, H-4), 7.31 (1H, dt, J = 9.0, 2.5 Hz, H-6), 6.94 (1H, d, J = 8.8 Hz, H-5′), 6.88 (2H, s, NH_2); **7d**: Yield 58%, ^{1}H NMR (DMSO-d_6) 8.06 (1H, dd, J = 10.8, 2.5 Hz, H-7), 8.04 (1H, dd, J = 8.8, 2.5 Hz, H6′), 8.00 (1H, d, J = 2.5 Hz, H-2′), 7.99 (1H, dd, J = 9.0, 5.0 Hz, H-4), 7.39 (1H, dt, J = 9.0, 2.5 Hz, H-5), 6.94 (1H, d, J = 8.8 Hz, H-5′), 6.86 (2H, s, NH_2).
12. General experimental method for compounds **9a**–**d**. (Trimethylsilyl)acetylene (1.5 mol equiv) was added to a mixture of the 2-(4-amino-3-iodophenyl)benzothiazole,[2,8] dichlorobis (triphenylphosphine)palladium(II) (0.05 mol equiv) and copper(I) iodide (0.1 mol equiv) in triethylamine at ambient temperature with stirring. The mixture was heated at reflux for 16 h then allowed to cool and the triethylamine removed in vacuo. The residue was partitioned between ethyl acetate and water then the layers separated. The aqueous layer was extracted using further ethyl acetate (×2), then dried ($MgSO_4$), filtered and concentrated to yield the crude product. Purification by flash column chromatography (1% methanol in chloroform as eluant) gave the pure (trimethyl)silylethynyl-substituted benzothiazole (**8a**–**d**) in good yields. The benzothiazole **8** was then dissolved in a mixture of THF and methanol (4:1) and potassium carbonate (1.1 mol equiv) added to the solution, which was then stirred at ambient temperature for 18 h. THF and methanol were removed in vacuo, and the residue dissolved in dichloromethane. After washing with water and brine the organic solution was concentrated to give the pure product (**9a**–**d**) in good yield. **9a**: Yield 58% (two steps), ^{1}H NMR (DMSO-d_6) 8.07 (1H, dd, J = 7.8, 1.4 Hz, H-7), 7.95 (1H, dd, J = 8.0, 1.0 Hz, H-4), 7.88 (1H, d, J = 2.3 Hz, H-2′), 7.83 (1H, dd, J = 8.6, 2.3 Hz, H-6′), 7.50 (1H, dt, J = 8.0, 1.0 Hz, H-5), 7.39 (1H, dt, J = 7.8, 1.1 Hz, H-6), 6.86 (1H, d, J = 8.6 Hz, H-5′), 6.18 (2H, s, NH_2), 4.51 (1H, s, C≡CH); **9b**: Yield 56% (two steps), ^{1}H NMR ($CDCl_3$) 8.09 (1H, d, J = 2.1 Hz, H-2′), 7.94 (1H, dd, J = 8.6, 2.1 Hz, H-6′), 7.62 (1H, dd, J = 8.0, 1.1 Hz, H-7), 7.23 (2H, m, H-5, H-6), 6.77 (1H, d, J = 8.6 Hz, H-5′), 4.65 (2H, s, NH_2), 3.46 (1H, s, C≡CH); **9c**: Yield 62% (two steps), ^{1}H NMR ($CDCl_3$) 8.05 (1H, d, J = 2.0 Hz, H-2′), 7.89 (1H, dd, J = 8.6, 2.1 Hz, H-6′), 7.77 (1H, dd, 6.3, 5.0 Hz, H-7), 7.67 (1H, dd, J = 9.8, 2.5 Hz, H-4), 7.11 (1H, dt, J = 8.8, 2.5 Hz, H-6), 6.78 (1H, d, J = 8.6 Hz, H-5′), 4.64 (2H, s, NH_2), 3.46 (1H, s, C≡CH); **9d**: Yield 68% (two steps) ^{1}H NMR ($CDCl_3$) 8.02 (1H, d, J = 2.2 Hz, H-2′), 7.92 (1H, dd, J = 8.9, 5.0 Hz, H-4), 7.85 (1H, dd, J = 8.5, 2.2 Hz, H-6′), 7.53 (1H, dd, J = 8.1, 2.6 Hz, H-7), 7.18 (1H, dt, J = 8.9, 2.6 Hz, H-5), 6.77 (1H, d, J = 8.5 Hz, H-5′), 4.63 (2H, s, NH_2), 3.46 (1H, s, C≡CH).