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Antitumour properties of fluorinated benzothiazole-substituted hydroxycyclohexa-2,5-dienones ('quinols')☆

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Abstract—The synthesis and in vitro antitumour evaluation of a new series of fluorinated benzothiazole-substituted 4-hydroxycyclohexa-2,5-dienones ('quinols') is described. The new compounds were found to be of comparable activity compared to the non-fluorinated precursor PMX 464, in terms of antiproliferative activity in sensitive human cancer cell lines (nanomolar GI_{50} values) and inhibitory activity against the thioredoxin signalling system.

The selective introduction of fluorine into small molecules can have a dramatic effect on therapeutic efficacy.[2] The reasons for the ability of fluorine to influence the properties of drug candidates are diverse; however circumvention of P450-induced metabolism, and the increasing use of the fluorine isotope ^{18}F as a tracer atom in positron emission tomography (PET)[3] are particularly noteworthy. Perhaps the most well-known example of a class of agents where fluorine is essential for therapeutic efficacy is the fluoroquinolone class of antibiotics.[4]

Our own previous experience in the synthesis and characterisation of the antitumour properties of fluorinated 2-phenylbenzothiazole derivatives has been well documented;[5] the structures of the relevant compounds described below are presented in Figure 1. The initial lead antitumour agent 2-(4-amino-3-methylphenyl)benzothiazole (DF 203)[6] was found to possess potent and selective activity against a range of human cancer cell lines. However, DF 203 produced the inactive and antagonistic 6-hydroxylated benzothiazole metabolite (6OH 203) through binding to the arylhydrocarbon receptor[7,8] and induction of cytochrome P450 1A1,[9] resulting in a biphasic growth inhibitory dose–response curve.[10] Synthesis and testing of fluorinated DF 203 derivatives led to the identification of the corresponding 5-fluorobenzothiazole analogue (5F 203) as a new lead compound able to circumvent deactivating metabolism whilst preserving CYP1A1-induced bioactivation.[11] The lysyl amide prodrug of 5F 203 (Phortress)[5,12–15] is currently undergoing Phase 1 clinical evaluation by Cancer Research UK. Other more recent work has led to the discovery of 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW 610), a compound possessing potent and selective in vitro antitumour activity (sub-nanomolar GI_{50} in certain colon, lung and breast cancer cell lines).[16] Remarkably the corresponding non-fluorinated derivative of GW 610 is completely devoid of antitumour activity.

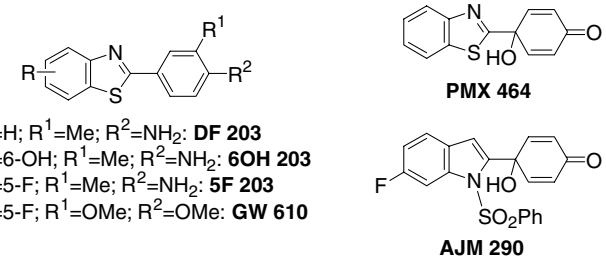

Figure 1. Structures of bioactive benzothiazoles and 4-substituted-4-hydroxycyclohexa-2,5-dienones.

Keywords: Antitumour agents; Fluorinated benzothiazoles; Thioredoxin.

☆ This is Part 6 of the series 'Quinols as Novel Therapeutic Agents'. For Part 5 of the series see Ref. 1.

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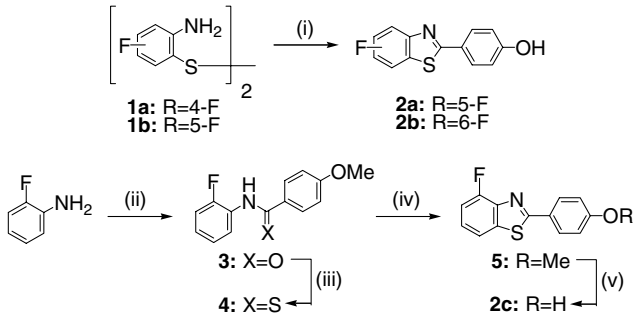
We have recently described the synthesis and antitumour evaluation of a novel series of (hetero)aromatic 4-hydroxycyclohexa-2,5-dienones ('quinols'), exemplified by the lead compounds PMX 464[17,18] (formerly AW 464) and AJM 290.[19] These phenolic oxidation products[20] exhibited potent and selective antitumour activity concentrated in certain colon, renal and breast cancer cell lines, in part through inhibition of the cellular redox protein thioredoxin.[21,22] Inspired by our previous success in the identification of promising fluorinated benzothiazole-based antitumour agents, we here report the synthesis and antitumour evaluation of the 4-, 5- and 6-fluoro analogues of PMX 464.

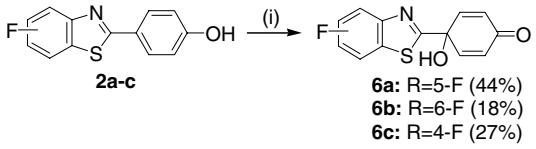
The synthesis of 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dienone (PMX 464) via deprotonation of benzothiazole followed by addition to 4,4-dimethoxycyclohexa-2,5-dien-1-one and ketal deprotection has previously been described.[17] Since the corresponding 4-, 5- and 6-fluorobenzothiazoles are not commercially available nor readily accessible, a different strategy was adopted for the synthesis of fluorinated derivatives of PMX 464 (**6a**–**c**). The starting point for the synthesis of the 5- and 6-fluorobenzothiazoles was the fluorinated *ortho*-aminothiophenol disulfides **1a**,**b**, available via hydrolytic cleavage of fluorinated 2-aminobenzothiazoles, and previously described as intermediates in the synthesis of 2-(4-aminophenyl)benzothiazoles.[11] One-pot reduction of disulfide (PPh_3) and acid-catalysed reaction with 4-hydroxybenzaldehyde gave access to 5- and 6–fluoro-2-(4-hydroxyphenyl)benzothiazoles **2a**,**b** following in situ oxidation of the initial dihydrobenzothiazole products (28% and 25% overall yields, respectively).[16] The synthesis of 4-fluoro-2-(4-hydroxyphenyl) benzothiazole **2c** using an equivalent route from 4-fluoro-2-aminobenzothiazole was not successful, and a different route to **2c** was employed. Reaction of 2-fluoroaniline with 4-methoxybenzoyl chloride was followed by conversion of the resulting amide **3** to thioamide **4** using P_4S_{10} in hexamethyldisiloxane (HMDO). Potassium ferricyanide (Jacobsen) cyclisation led to 4-fluoro-2-(4-methoxyphenyl)benzothiazole **5**. Demethylation using pyridinium hydrochloride afforded the desired 4-fluoro-2-(4-hydroxyphenyl)benzothiazole **2c** (20% overall yield) (Scheme 1).

Phenolic oxidation of fluorinated 2-(4-hydroxyphenyl)benzothiazoles **2a**–**c** using the hypervalent iodine oxidant di(trifluoroacetoxy)iodobenzene and the stable radical oxidant TEMPO in a mixture of acetonitrile and water[17] gave the desired fluorinated benzothiazole-substituted 'quinol' products **6a**–**c**[23] in moderate yields, as shown in Scheme 2.

Compounds **6a**–**c** have been evaluated for in vitro antitumour activity in the human breast cancer cell lines MCF-7 (oestrogen receptor positive) and MDA MB 468 (oestrogen receptor negative), and the human colon carcinoma cell lines HCT-116 and HT 29. The cell lines were chosen as some of those already known to be sensitive to the lead compound PMX 464, and the results (GI_{50} values, MTT assay)[11] are shown in Table 1.



Scheme 1. Synthesis of fluorinated 2-(4-hydroxyphenyl)benzothiazoles **2a**–**c**. Reagents and conditions: (i) 4-hydroxybenzaldehyde, PPh_3, *p*-TsOH, toluene, reflux; (ii) 4-methoxybenzoyl chloride, NEt_3, CH_2Cl_2, 0 °C; (iii) P_4S_{10}, HMDO, reflux; (iv) $K_3Fe(CN)_6$, NaOH(aq), EtOH, 90 °C; and (v) pyridium hydrocholoride, 210 °C.



Scheme 2. Synthesis of fluorinated 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dienones. Reagents and conditions: (i) $PhI(OCOCF_3)_2$, TEMPO, $MeCN/H_2O$.

Inspection of Table 1 reveals that the new fluorinated quinol analogues **6a**–**c** compare favourably with PMX 464 in terms of GI_{50} potency. For the 5-fluoro analogue (**6a**), more potent antiproliferative activity was observed in three out of four cell lines compared to PMX 464. In general GI_{50} values are in the sub-micromolar range, indicative of potent in vitro activity. Amongst the cell lines examined the colon cancer cell line HCT-116 was found to be the most sensitive, as was observed previously for PMX 464 and related quinols. New compounds **6a**–**c** were also screened for in vitro antitumour activity through the National Cancer Institute Developmental Therapeutics Program sixty human cancer cell line screen (two day assay). Mean GI_{50} values across the sixty cell lines for **6a**–**c** (0.43, 0.34, and 0.35 μM, respectively) were found to be similar to PMX 464 (0.23 μM) after repeat screening. Colon and renal cell sub-panels were found to have enhanced sensitivity to **6a**–**c**, as was the case for PMX 464 (data not shown).

Previous studies have established the potential of the thioredoxin/thioredoxin reductase system as an anticancer drug target,[24] and a small molecule inhibitor of this system (PX 12) is in clinical development.[25] Further biological studies focused on compounds **6a** and **6b**, the fluorinated quinols that exhibited the most interesting selectivity profile of antitumour activity across the NCI sixty human cancer cell line screen (data not shown). The abilities of fluorobenzothiazole quinols **6a** and **6b** to inhibit human thioredoxin signalling were compared with that of PMX 464, using a modified version of the insulin reduction assay previously described.[21] Insulin reduction was monitored over time and results presented in Table 2 tabulate % inhibition

Table 1. 50% Growth inhibitory dose (GI_{50}) values for PMX 464 and fluorinated analogues **6a–c** in human cancer cell lines MCF-7, MDA MB 468, HCT-116 and HT 29

Compound	MCF-7 GI_{50}, μM ±s.d.[a]	MDA 468 GI_{50}, μM ±s.d.[a]	HCT-116 GI_{50}, μM ±s.d.[a]	HT 29 GI_{50}, μM ±s.d.[a]
PMX 464	0.44[b]	0.41[b]	0.11[b]	0.59[b]
6a	0.37 ± 0.11	0.41 ± 0.03	0.08 ± 0.03	0.41 ± 0.05
6b	0.39 ± 0.11	0.48 ± 0.17	0.31 ± 0.10	0.49 ± 0.09
6c	0.43 ± 0.05	0.49 ± 0.20	0.18 ± 0.09	0.47 ± 0.07

[a] Values are means of at least three experiments.
[b] As reported in Ref. 17.

Table 2. Thioredoxin signalling inhibition by compounds PMX 464, **6a** and **6b** (30 min agent incubation time)

Compound concn (μM)	% Inhibition of insulin reduction[a]		
	PMX 464	**6a**	**6b**
10	13.2	10.1	8.6
25	35.1	15.8	31.8
50	94.8	94.6	93.3
100	95.8	97.4	95.8

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

of insulin reduction at given concentrations of PMX 464, **6a** and **6b**. The ability of the new fluorinated quinols **6a**,**b** to inhibit thioredoxin signalling at micromolar concentrations, as measured by inhibition of the thioredoxin substrate insulin, is apparent, and is within the same range as the lead thioredoxin-inhibitory quinol PMX 464.

We have described the synthesis and in vitro evaluation of new fluorinated 'quinols' based on the lead structure PMX 464. The new analogues are at least as potent (GI_{50}) in representative sensitive cancer cell lines, and additionally inhibit the thioredoxin redox signalling pathway, as is the case for PMX 464.

One potential advantage of compounds such as **6a–c** is that the ^{18}F radiolabelled analogues could be used in future non-invasive imaging of biodistribution using positron emission tomography (PET). Given the half-life of PET isotopes such as ^{18}F (110 min), this possibility would only be viable if the fluorine atom could be installed at a late (final or penultimate) stage of the synthesis. We have previously described the synthesis of [^{18}F]-5F 203 via treatment of a 5-(trimethylstannyl)benzothiazole precursor with [^{18}F-F],[26] however a synthetic method employing fluoride (rather than fluorine) to install the radiolabel would be greatly advantageous. Further developments on the therapeutic and diagnostic potential of these new compounds will be reported in due course.

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23. General experimental procedure for phenolic oxidation. 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO) (0.6 mmol) was added to a solution of fluorinated 2-(4-hydroxyphenyl)benzothiazole (6.0 mmol) in acetonitrile/water (9:1) (30 mL). Di(trifluoroacetoxy)iodobenzene (12.0 mmol) was then added, and the reaction mixture was stirred for 15 min. The acetonitrile was removed in vacuo, then further water added (30 mL) and the crude product extracted using diethyl ether (3× 30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography (ethyl acetate/hexane) gave the required fluorinated quinols. Compound **6a**: mp = 148 °C; ^{1}H NMR (DMSO-d_6) δ 8.23 (1H, dd, J = 8.8, 5.2 Hz, H-7), 7.89 (1H, dd, J = 9.8, 2.5 Hz, H-4), 7.59 (1H, s, OH), 7.43 (1H, dt, J = 8.8, 2.5 Hz, H-6), 7.12 (2H, d, J = 10.3 Hz, H-3′, H-5′), 6.33 (2H, d, J = 10.3 Hz, H-2′, H-6′); *m*/*z* (CI) 262 (M^++1). Compound **6b**: mp = 181 °C; ^{1}H NMR (DMSO-d_6) δ 8.00 (1H, dd, J = 9.0, 4.8 Hz, H-4), 7.58 (1H, dd, J = 7.9, 2.5 Hz, H-7), 7.29 (1H, dt, J = 9.0, 2.5 Hz, H-5), 7.26 (1H, s, OH), 7.01 (2H, d, J = 10.0 Hz, H-3′, H-5′), 6.38 (2H, d, J = 10.0 Hz, H-2′, H-6′); *m*/*z* (CI) 262 (M^++1). Compound **6c**: mp = 168 °C; ^{1}H NMR (DMSO-d_6) δ 8.02 (1H, d, J = 7.9 Hz, H-4), 7.64 (1H, s, OH), 7.53–7.49 (2H, m, ArH), 7.14 (2H, d, J = 10.1 Hz, H-3′, H-5′), 6.34 (2H, d, J = 10.1 Hz, H-2′, H-6′); *m*/*z* (CI) 262 (M^++1).
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