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New prodrugs derived from 6-aminodopamine and 4-aminophenol as candidates for melanocyte-directed enzyme prodrug therapy (MDEPT)†

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Two novel tyrosinase mediated drug delivery pathways have been investigated for the selective delivery of cytotoxic units to melanocytes from urea and thiourea prodrugs. The synthesis of these prodrugs is reported, as well as oximetry data that illustrate that the targets are substrates for tyrosinase. The stability of each of the prodrugs in (i) phosphate buffer and (ii) bovine serum is discussed, and the urea prodrugs are identified as lead candidates for further studies. Finally, HPLC studies and preliminary cytotoxicity studies in a melanotic and an amelanotic cell line, that illustrate the feasibility of the approach, are presented.

Introduction

Melanocyte-Directed Enzyme Prodrug Therapy (MDEPT) is an acronym coined for a prodrug strategy directed towards the treatment of melanoma.[1] MDEPT differs from some other enzyme prodrug therapies such as Antibody-Directed Enzyme Prodrug Therapy (ADEPT)[2] in that the activating enzyme, tyrosinase, necessary for drug release is naturally expressed within the host and does not need to be artificially introduced. This therefore overcomes a major drawback of some current prodrug delivery systems that require the delivery of sufficient non-mammalian enzyme to the tumour site, as well as prodrug activation in a selective manner, for effective therapy. It has been reported that when melanocytes become malignant, the genes expressing tyrosinase become up-regulated, resulting in a marked increase in the tyrosinase levels within the cancerous cells.[3] Thus, since tyrosinase is naturally present in the tumour and virtually absent from other cells it provides an in-built drug delivery mechanism that will be selective for melanoma tumours over both healthy cells and normal, healthy melanocytes. A number of tyrosinase dependent prodrug strategies have been investigated for the treatment of melanoma.[3,4] For example, non-toxic phenol and catechol prodrugs have been oxidised by tyrosinase to afford toxic quinones within the vicinity of melanoma tumours.[4] Initial studies within the MDEPT strategy have also illustrated that tyrosinase can be utilised to mediate the release of a cytotoxic agent from carbamate and urea prodrugs *via* a cyclisation–drug release mechanism.[1]

† This paper is dedicated to Professor S. V. Ley on the occasion of his 60th birthday.

In this paper we wish to describe our recent work in this area that has involved the synthesis of further classes of urea and thiourea prodrugs that rely on complementary tyrosinase mediated pathways for drug release. An analysis of the ability of the prodrugs to act as tyrosinase substrates and release cytotoxic units upon exposure to tyrosinase, *in vitro*, is also presented. A nitrogen mustard (aniline *N*-mustard) has been selected as the cytotoxic agent for selective delivery since *N*-mustards have been approved for the clinical treatment of melanoma,[5] and have also been incorporated within ADEPT strategies.[6] Moreover, it has been demonstrated that alkylating agents can be administered repeatedly with less induced resistance than other classes of anticancer drugs.[7] The tyrosinase substrates and the cytotoxic unit are connected within the prodrugs *via* urea or thiourea linkages, to probe the stabilities of the different linkages *in vitro*.

The general structure of the first class of prodrugs is illustrated in Fig. 1.

The trigger units, 4-aminophenol or 4-amino-2-hydroxyphenol, were found to be substrates for tyrosinase by oximetry, oxidising at 70% of the rate of the oxidation of L-tyrosine, the natural substrate for tyrosinase. It was hypothesised that the prodrugs could be activated *via* the mechanism described below in Scheme 1. Since the onset of drug release will be dependent upon tyrosinase activation, this approach should allow drug release solely within the melanoma tumour.

Preliminary results suggesting the feasibility of this approach have recently been reported from our laboratory where we have illustrated that amines can be liberated from ureas derived from 4-aminophenol, upon exposure to tyrosinase—this has culminated in the development of novel enzyme labile protecting group methodology.[8]

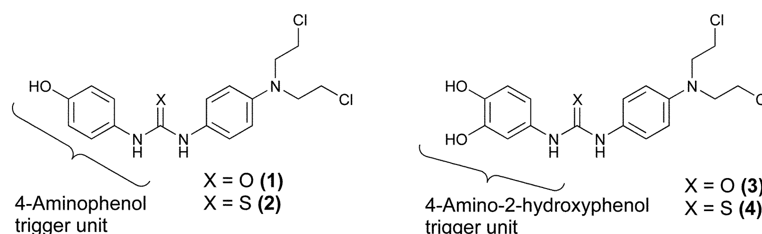

Fig. 1 Structures of novel prodrugs for use within MDEPT: Series 1.

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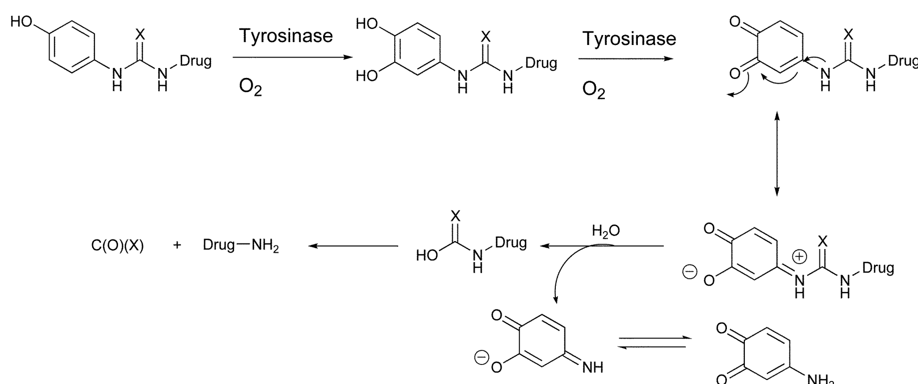


Scheme 1 Proposed drug release pathway.

The general structure of the second group of prodrugs is displayed in Fig. 2.

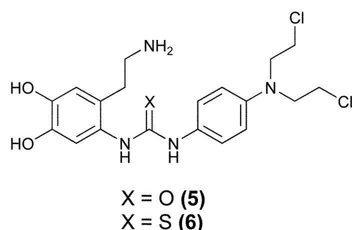


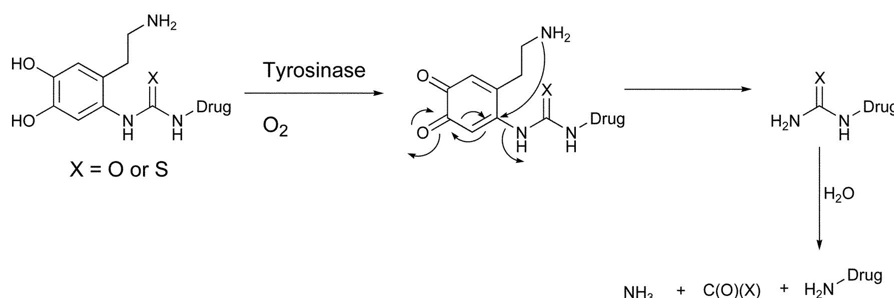
Fig. 2 Structures of novel prodrugs for use within MDEPT: Series 2.

The release mechanism for this series of prodrugs elaborates a report that 6-aminodopamine is a good substrate for tyrosinase, with oxidation occurring to afford the corresponding orthoquinone.[9] This orthoquinone then undergoes a rapid intramolecular cyclization to initiate excision of the 6-substituent. This study therefore sought to investigate whether the tyrosinase mediated manipulation could be expanded to allow delivery of a cytotoxic moiety to malignant melanocytes as illustrated in Scheme 2.

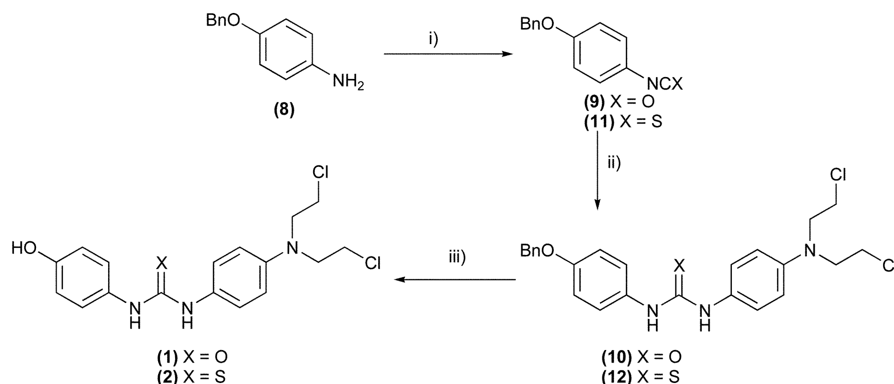
Results and discussion

Synthesis of the prodrugs

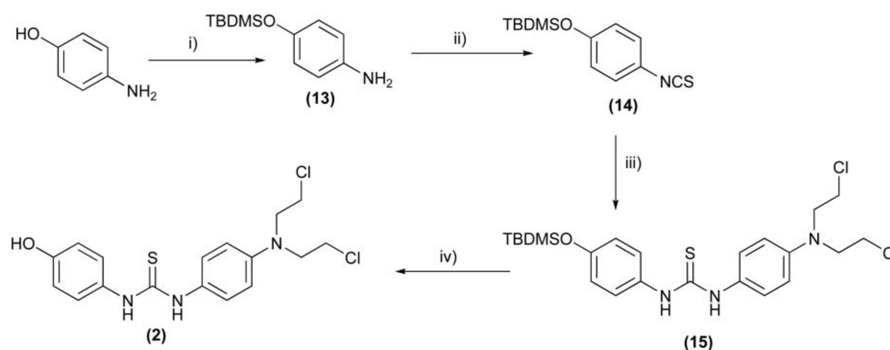
It was envisaged that syntheses of the urea and thiourea prodrugs from both Series 1 and Series 2 could be achieved *via* reaction of aniline *N*-mustard with isocyanates and isothiocyanates derived from the trigger units (Scheme 3). The *N*-mustard (**7**) was prepared according to multi-step literature procedures.[10] Entry to the urea prodrug (**1**) was achieved from isocyanate (**9**), which was itself prepared in 96% yield by reaction of benzyl protected phenol amine (**8**) with four equivalents of triphosgene for 2 h. A sharp peak at 2267 cm^{-1} in the IR spectrum, characteristic of the isocyanate stretch was indicative of the formation of (**9**). With isocyanate (**9**) available, coupling to the *N*-mustard (**7**) was achieved by reaction in DCM under $N_{2\ (g)}$ to yield (**10**), Scheme 3, in 50% yield. This was converted to prodrug (**1**) in 81% yield by deprotection of the benzyl group using 10% Pd/C and $H_{2\ (g)}$. In a similar fashion, amine (**8**) was converted to the isothiocyanate derivative (**11**) using thiophosgene in an excellent yield of 90%. Subsequent coupling to the *N*-mustard (**7**) afforded thiourea (**12**) in 48% yield. However, removal of the benzyl ether protecting group from (**12**) using



Scheme 2 Proposed drug release pathway.



Scheme 3 Synthesis of prodrug (**1**) and attempted synthesis of prodrug (**2**) *via* benzyl protected 4-aminophenol. (i) X = O, triphosgene, EtOAc, reflux, 2 h, 96%; X = S, thiophosgene, EtOAc, rt, 2 h, 90%; (ii) X = O, *N*-mustard (**7**), DCM, rt, 12 h, 50%; X = S, *N*-mustard (**7**), DCM, rt, 24 h, 48%; (iii) Pd/C, H_2, EtOAc, rt, 2 h, 81%.



Scheme 4 Synthesis of prodrug (**2**) *via* silyl protected 4-aminophenol. (i) TBDMSCl, imidazole, THF, rt, 90%; (ii) thiophosgene, DCM, reflux, 87%; (iii) *N*-mustard (**7**), DCM, rt, 55%; (iv) TBAF, rt, 62%.

DDQ[11] was unsuccessful and the starting material was instead recovered.

An alternative protecting group for 4-aminophenol was therefore employed—thus, 4-aminophenol was converted to amine (**13**) by treatment with *tert*-butyldimethylsilylchloride in the presence of a weak base, imidazole, in 90% yield, Scheme 4. Amine (**13**) was next converted to the thioisocyanate (**14**) by reaction with thiophosgene, in 87% yield. Subsequent reaction of (**14**) with the *N*-mustard (**7**) then afforded the protected prodrug (**15**), in 55% yield. The deprotection of the silyl group was achieved by using TBAF as a source of fluoride ions and in this way the target prodrug (**2**) was obtained in 62% yield, Scheme 4.

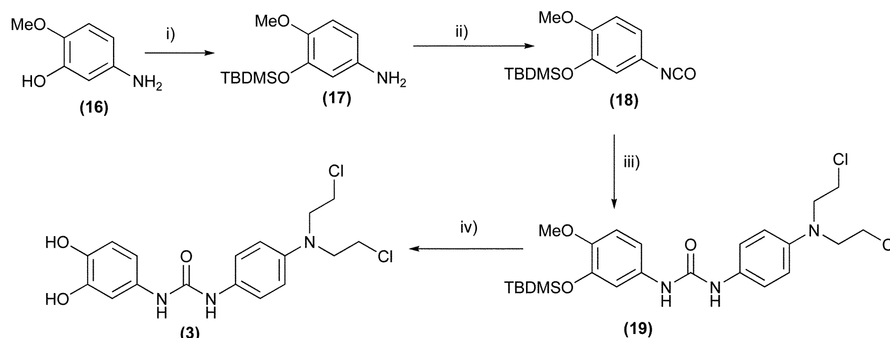
For comparative purposes, prodrug (**1**) was also synthesised using the silyl protected 4-aminophenol starting material (**13**), in an overall yield of 49%. This compared favourably with the overall yield of 34% obtained when benzyl protected 4-aminophenol (**8**) was utilised as the starting material.

Synthesis of the catechol analogue of (**1**), namely prodrug (**3**), was achieved from commercially available amine (**16**). Thus alcohol (**16**) was converted to silyl ether (**17**) in 65% yield using TBDMSCl and a catalytic amount of DMAP. Silyl ether (**17**) was then converted to the isocyanate (**18**), by treatment with triphosgene, in 93% yield, and this was then coupled to the *N*-mustard (**7**) to afford urea (**19**) in 71% yield. It was hoped that (**19**) could then be deprotected using conc. HCl to afford prodrug (**3**). These conditions, however, failed to deprotect the methyl ether group even after prolonged reaction times, thus affording the partially deprotected target. Removal of the methyl ether protecting group to afford the target prodrug (**3**) was accomplished in 91% yield using BBr_3 at low temperature, Scheme 5.

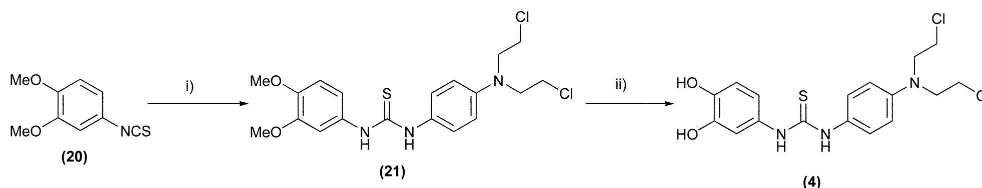
Synthesis of prodrug (**4**) required reaction of the commercially available isothiocyanate (**20**) with *N*-mustard (**7**) to afford thiourea (**21**) in 93% yield. Subsequent deprotection of the methyl ether protecting groups, again with BBr_3, afforded prodrug (**4**) in 92% yield, Scheme 6.

Preparation of prodrugs (**5**) and (**6**) from Series 2 again involved the coupling of protected isocyanate and isothiocyanate derivatives (**27**) and (**28**) with aniline *N*-mustard (**7**) as illustrated in Scheme 7. The isocyanate (**27**) and isothiocyanate (**28**) were prepared by reaction of protected amine (**26**) with phosgene and thiophosgene respectively.

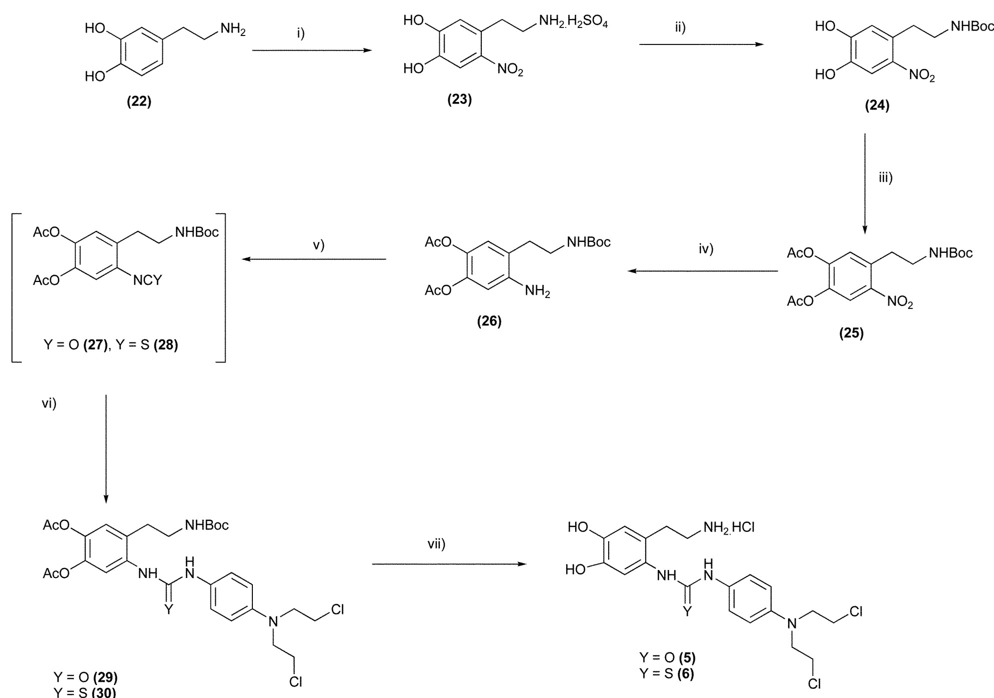
Synthesis of amine (**26**) commenced with nitration of dopamine (**22**) using sodium nitrite and a 20% solution of sulfuric acid in water at 0 °C, according to the procedure of Napolitano *et al.*[9] Precipitation of the hydrogen sulfate salt (**23**) occurred in the reaction vessel, and after filtration, washing and drying, it could be isolated as a mustard coloured solid in 93% yield. Introduction of a Boc protecting group to amine (**23**) using Boc_2O and NaOH in DMF proved low yielding (typically 22%). However, use of Boc–ON[12] and NEt_3 in a THF–H_2O mixture allowed access to the desired Boc-protected amine (**24**) in 59%. Subsequent acetyl protection of the catechol moiety using Ac_2O and pyridine proceeded well to afford nitro derivative (**25**) in quantitative yield. Nitro reduction, to afford



Scheme 5 Synthesis of prodrug (**3**). (i) TBDMSCl, DMAP, NEt_3, THF, rt, 65%; (ii) triphosgene, EtOAc, reflux, 93%; (iii) *N*-mustard (**7**), DCM, rt, 71%; (iv) conc. HCl, reflux, 77% then BBr_3, DCM, −78 °C, 91%.



Scheme 6 Synthesis of prodrug (**4**). (i) *N*-Mustard (**7**), DCM, rt, 93%; (ii) BBr_3, DCM, 92%.



Scheme 7 Synthesis of prodrugs (**5**) and (**6**). (i) $NaNO_2$, H_2SO_4, H_2O, 93%; (ii) Boc–ON, NEt_3, THF, H_2O, 59%; (iii) Ac_2O, pyridine, 100%; (iv) H_2, Pd/C, 70%; (v) thiophosgene or triphosgene (Y = S and O respectively); (vi) pyridine, rt, 16 h, Y = O, 63%, Y = S, 71% (all yields quoted over 2 steps); (vii) 2 M HCl, acetone, room temperature, 2–4 h, Y = O, 100%, Y = S, 67%.

amine (**26**) was first attempted using Raney nickel and hydrazine but this resulted in a poor yield of formation of amine (**26**) (25%) with some *O*-acetyl deprotection also occurring under these reaction conditions. Hydrogenation of (**25**) over Pd/C proved more effective, affording amine (**26**) in 70% yield after purification by column chromatography. Amine (**26**) was next converted to either the isocyanate (**27**) or isothiocyanate (**28**) using triphosgene or thiophosgene respectively. The isocyanate (**27**) and isothiocyanate (**28**) were then reacted with the *N*-mustard (**7**) in pyridine at room temperature for 16 h to allow entry to the protected urea (**29**) and thiourea prodrugs (**30**) in synthetically useful yields. Removal of the acetate and Boc protecting groups from (**29**) and (**30**) was easily achieved in good to excellent yields for both targets upon treatment with 2 M HCl, in acetone, without any decomposition of the resulting urea (**5**) and thiourea (**6**) prodrugs.

Tyrosinase mediated processing of the prodrugs

Attention next focused on probing the ability of prodrugs (**1**)–(**6**) to be processed by tyrosinase, to effect drug release. Although human tyrosinase is not commercially available, mushroom-derived tyrosinase (E.C. 1.14.18.1) can be readily obtained, and so was utilised in this programme. Studies have shown that mushroom tyrosinase is a good model for human tyrosinase with the active sites of both mushroom and human tyrosinase containing a common binuclear copper centre.[13]

When molecules act as substrates for tyrosinase, oxygen is removed from the surrounding solution, and this can be measured *via* oximetry. The rate of oxygen depletion correlates with the tyrosinase-mediated oxidation of the substrates. Oxidation of each prodrug (**1**)–(**6**) was therefore monitored *via* oximetry and the rates of oxidation were compared with that for a natural substrate of tyrosinase, namely L-tyrosine (R_{max} = 17 nanomoles/min). Prodrugs (**1**)–(**4**) showed very similar oxidation profiles, and were oxidised at 70–78% the rate of oxidation of L-tyrosine. In contrast, prodrugs (**5**) and (**6**) demonstrated slower oxidation profiles, and were oxidised at 50% and 25% of the rate of oxidation of L-tyrosine, respectively. The relatively slow oxidation rates afforded by prodrugs (**5**) and (**6**) contrasted with that obtained for 6-hydroxydopamine which proved to be a better substrate for tyrosinase than L-tyrosine (R_{max} = 30 nanomole/min).

In addition to oximetry studies, HPLC studies were also performed to assess the viability of the tyrosinase mediated drug release protocol. Each prodrug was therefore solubilised in phosphate buffer, treated with tyrosinase and the solution analysed by HPLC for evidence of drug release. Table 1 displays the results obtained and illustrates that whilst urea linked prodrugs showed successful drug release, the thiourea linked prodrugs proved less effective. This is in contrast with the oximetry results that had suggested that both urea and thiourea prodrugs (**1**)–(**4**) were good substrates for tyrosinase. It is possible that drug release from prodrugs (**2**) and (**4**) was less effective than expected due to the generation of an inhibitor of tyrosinase—this hypothesis is supported by the observation that phenylthiourea is an inhibitor of tyrosinase.[14] It should be noted that for the urea linked prodrugs, the efficiency of drug release, as evidenced by the quantitative HPLC assays, reflected the ability of the prodrugs to act as substrates for tyrosinase. Thus drug release from the urea linked prodrugs (**1**) and (**3**) was more effective than from prodrug (**5**).

Table 1 Tyrosinase mediated drug release studies

Prodrug	$t_{1/2}$	Comment
(**1**)	85 min	Complete consumption of prodrug, and generation of drug, was observed
(**2**)	—	No evidence of drug release, even after 4 h. Formation of a metabolite was observed, RT = 2.0 min. $t_{1/2}$ = 110 min
(**3**)	58 min	Complete consumption of prodrug, and generation of drug, was observed
(**4**)	—	Some drug release observed, but the major components were two metabolites, RT = 1.8, RT = 4.0 min
(**5**)	100 min	Slow release of drug is evident, with complete drug release eventually being observed
(**6**)	—	No evidence for drug release, but instead formation of a single metabolite is evident

Table 2 Stability profiles for prodrugs (**1**)–(**6**)

Prodrug	Phosphate buffer[a]	Bovine serum[a]
(**1**)	100	65
(**2**)	98	45
(**3**)	99	98
(**4**)	0, $t_{1/2} = 20$ min	17, $t_{1/2} = 110$ min
(**5**)	98	80
(**6**)	96	0, $t_{1/2} = 10$ min

[a] % of the prodrug remaining after 5 h.

In order to ensure that drug release was truly dependent on tyrosinase, the stability of each prodrug in phosphate buffer, and in bovine serum, was examined. Thus the prodrugs were exposed to phosphate buffer or bovine serum, and the rate of undesirable drug release was determined by HPLC analysis. The results of these studies are illustrated in Table 2.

In all cases it was evident that the urea linked prodrugs (**1**), (**3**) and (**5**) were of greater stability in aqueous and biological media than the thiourea linked prodrugs (**2**), (**4**) and (**6**). This, together with the tyrosinase mediated drug release data, suggested that the urea prodrugs were better candidates for MDEPT than their thiourea counterparts

The final stage of the study involved an analysis of the cytotoxicity of the lead urea prodrugs (**1**), (**3**) and (**5**) in a tyrosinase rich (FF1, 0.55 nM/min/mg protein) and tyrosinase absent (A375) cell line.[15,16] Pleasingly greater cytotoxicity was evident in the tyrosinase rich cell line ($IC_{50} = 10.2$, 15.2 and 9.7 μM for (**1**), (**3**) and (**5**) respectively) than in the tyrosinase absent cell line ($IC_{50} = 20.2$, 25.8 and 30.2 μM for (**1**), (**3**) and (**5**) respectively). The *N*-mustard (**7**) exhibited an IC_{50} of 1 μM in each cell line. These results therefore support the hypothesis that tyrosinase can be utilised for the selective release of a cytotoxic drug from the two new series of prodrugs described herein. Whilst it is hypothesised that *ortho*quinones are generated as intermediates in the proposed drug release pathways, and *ortho*quinones are known to be cytotoxic, the biological effect for the protocol described herein is likely to be dominated by the DNA cross-linking properties of the *N*-mustard thus released. This is due to the rapid reaction of *ortho*quinones with cellular glutathione which, in the absence of ancillary treatment to depress glutathione synthesis, makes it difficult for the requisite quinone levels to be attained.[3c]

Conclusions

In conclusion, the synthesis and analysis of a range of prodrugs derived from 6-aminodopamine and 4-aminophenol, of potential use within MDEPT, has been described. Promising results have been obtained that indicate that the urea prodrugs (**1**), (**3**) and (**5**) are good leads for further studies. Future work will seek to further increase the differential cytotoxicity of the prodrugs within tyrosinase rich and tyrosinase absent cell lines to maximise the impact of this targeted approach.

Experimental

All NMR spectra were recorded on a Bruker WM250, Bruker AC250, Bruker Avance DPX 250, Bruker AMX400 or Jeol AX400 spectrometer, using $CHCl_3$ as an internal standard unless otherwise stated (7.26 ppm for ^1H NMR, 77.0 ppm for ^{13}C NMR). ^{13}C spectra were recorded using Distortionless Enhancement by Polarisation Transfer. Mass spectra were recorded on a Fisons VG Autospec. Infra red spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer. Melting points were determined using an Electrothermal digital melting point apparatus, and are uncorrected.

HPLC was performed on a Perkin-Elmer 410 series LC pump fitted with a Gilson 231 auto-sampler injector, using a LUNA C18 reverse phase column (250 mm × 4.5 mm). Compounds were detected by UV at 254 nm. Method 1: 0 min H_2O (100%), 5 min H_2O (100%), 25 min MeCN (100%), 30 min MeCN (100%), 35 min H_2O (100%), flow rate 1 mL min^{-1}. Method 2: 0 min H_2O (100%), 5 min H_2O (100%), 25 min 1,4-dioxane (100%), 30 min 1,4-dioxane (100%), 35 min H_2O (100%), flow rate 1 mL min^{-1}. Method 3: H_2O–MeCN (3 : 7, v/v), flow rate 1 mL min^{-1}. Oximetry was performed using a YSI 5300 biological oxygen monitor, utilising a KCl electrolyte on a membrane bound oxygen probe. Readings were recorded on an ABB SE120 chart recorder operating at 1 cm min^{-1} chart speed and 10 mV sensitivity. Unless stated otherwise, all chemicals and materials were obtained from the Sigma-Aldrich Chemical Company, the B. D. H. Merck Chemical Company or Lancaster Chemicals and were used as received. Silica gel for column chromatography was obtained from Merck, with a pore diameter of 6 nm.

4-Benzyloxyphenyl isocyanate (9)

Et_3N (0.3 mL, 2.12 mmol) was added to a solution of 4-benzyloxyaniline hydrochloride salt (0.5 g, 2.12 mmol) in EtOAc (40 mL). The mixture was kept at 0–5 °C and triphosgene (2.5 g, 8.48 mmol, 4 eq.) was added. The reaction mixture was gradually brought to reflux at 77 °C for 2 h. The progress of the reaction was monitored by IR spectroscopy and TLC analysis. Excess solvent was removed *in vacuo* and the crude product was flashed through a short pad of SiO_2 to yield the pure product (**9**) as a pale pink crystalline solid (0.48 g, 96%). Mp 59–60 °C (lit.[17] 59–61.5 °C); R_f 0.54 (hexane–EtOAc, 7 : 3, v/v); ^1H NMR: δ 7.27–7.35 (5H, m, Ar*H*), 7.03 (2H, d, *J* 9.0 Ar*H* '*o*' to NCO), 6.92 (2H, d, *J* 9.0 Ar*H* '*m*' to NCO), 5.07 (2H, s, OCH_2); ^{13}C NMR: δ 155.6 (Ar*C*OBn), 137.4 (Ar*C*CH_2), 127.8–129.0 (8 × Ar*C*H), 121.1 (N*C*O), 115.7 (2 × Ar*C*H), 70.7 (*C*H_2); IR (thin film) ν cm^{-1} 2267 s (NCO), 1243 s; *m/z* (CI) 91 (100%), 225 (M + H, 30%); Found 225.0785. $[C_{14}H_{11}NO_2 + H]^+$ requires 225.0790.

1(4-Benzyloxy-3-{4-[bis-(2-chloroethyl)-amino]-phenyl} urea (10)

Benzylisocyanate (**9**) (0.4 g, 1.91 mmol) was dissolved in DCM (40 mL) and freshly prepared *N*-mustard (**7**) (0.4 g, 1.91 mmol) was added. The reaction mixture was left to stir for 12 h, concentrated *in vacuo* and purified by column chromatography (DCM–MeOH, 95 : 5, v/v) to yield the desired product (**10**) as a white solid (0.43 g, 50%). Mp 114–116 °C; R_f 0.38 (DCM–MeOH, 95 : 5, v/v); ^1H NMR: δ 7.36–7.39 (5H, m, Ar*H*Bn), 7.19–7.26 (4H, m, Ar*H*, '*o*' to urea), 6.95 (2H, d, *J* 9.0 Ar*H* '*o*' to OBn), 6.67 (2H, d, *J* 9.0 Ar*H*, '*o*' to NCH_2CH_2Cl), 6.39 (1H, br, s, N*H*), 6.31 (1H, br, s, N*H*), 5.02 (2H, s, CH_2), 3.73–3.78 (4H, m, 2 × CH_2CH$_2$Cl), 3.59–3.66 (4H, m, 2 × CH$_2$CH_2Cl); ^{13}C NMR: δ 155.5 (Ar*C*OBn), 152.4 (*C*=O), 137.9 (2 × Ar*C*N and Ar*C*OBn), 128.1–129.3 (5 × Ar*C*H), 126.0 (2 × Ar*C*NH), 124.5 (4 × Ar*C*H '*o*' to urea), 116.2 (2 × Ar*C*H '*o*' to OBn), 113.5 (2 × Ar*C*H '*o*' to NCH_2CH_2Cl), 71.0 (*C*H_2), 54.3 (2 × *C*H$_2$CH$_2$Cl), 41.1 (2 × *C*H$_2$Cl); IR (thin film) ν cm^{-1} 3321 s NH, 3035, 2686 s, 2531 s, 1707 s, 1503 s, 1240 s and 908; *m/z* (CI) 91 (100%), 200 (45%), 458 (M + H, ^{35}Cl, ^{35}Cl, 20%), 460 (M + H, ^{35}Cl, ^{37}Cl, 13%), 462 (M + H ^{37}Cl, ^{37}Cl, 2%); Found 458.1412. $C_{24}H_{25}{}^{35}Cl_2N_3O_2 + H^+$ requires 458.1403; Found C, 62.39; H, 5.52; N, 9.61. $C_{24}H_{25}Cl_2N_3O_2$ requires C, 62.89; H, 5.50; N, 9.16%.

1-{4-[Bis-(2-chloroethyl)-amino]-phenyl}-3-(4-hydroxyphenyl) urea (1)

Benzyl ether (**10**) (0.34 g, 0.742 mmol) was dissolved in EtOAc (40 mL). Pd/C (10%) (0.15 g) was added and the suspension degassed using a water aspirator. $H_{2\,(g)}$ was then added *via* balloons and the reaction was left to stir for 2 h. The reaction mixture was filtered through a pad of Celite® and the filtrate was concentrated *in vacuo* to dryness. The residue was purified

by column chromatography (DCM–MeOH, 96 : 4, v/v) to give (**1**) as a colourless powder (0.22 g, 81%). Mp 160–162 °C; R_f 0.38, (DCM–MeOH, 95 : 5, v/v); ^{1}H NMR (MeOH-d$_4$): δ 7.16–7.09 (4H, m, Ar*H* '*o*' to urea), 6.71 (2H, d, *J* 9.0, Ar*H* '*o*' to OH), 6.59 (2H, d, *J* 9.0, Ar*H* '*o*' to NCH$_2$CH$_2$Cl), 6.20 (1H, s, br, N*H*), 6.13 (1H, br s, N*H*), 5.15 (1H, br s, O*H*), 3.60–365 (4H, m, 2 × C*H*$_2$CH$_2$Cl), 3.53–3.57 (4H, m, 2 × CH$_2$C*H*$_2$Cl); ^{13}C NMR (MeOH-d$_4$): δ 157.2 (Ar*C*OH), 155.0 (*C*=O), 144.6 (Ar*C*N), 132.6 (Ar*C*NH), 131.1 (Ar*C*NH), 124.1 (2 × Ar*C*H '*o*' to urea), 123.8 (2 × Ar*C*H '*o*' to urea), 116.8 (2 × Ar*C*H '*o*' to ArCOH), 114.5 (2 × Ar*C*H '*o*' to NCH$_2$CH$_2$Cl), 55.1 (2 × *C*H$_2$CH$_2$Cl), 42.2 (2 × CH$_2$*C*H$_2$Cl); IR (thin film) ν cm^{-1} 3321 br (OH), 1700 s, 1575 s; *m*/*z* (CI) 260 (100%), 368 (M + H, ^{35}Cl, ^{35}Cl, 30%), 370 (M + H, ^{35}Cl, ^{37}Cl, 18%), 372 (M + H ^{37}Cl, ^{37}Cl 3%); Found 368.0924. [$C_{17}H_{19}{}^{35}Cl_2N_3O_2$ + H]$^+$ requires 368.0933; Found C, 54.45; H, 5.19; N, 10.91. $C_{17}H_{19}Cl_2N_3O_2$ requires C, 55.45; H, 5.20; N, 11.40%; HPLC: t_R 3.8 min (method 3).

4-Benzyloxyphenyl isothiocyanate (11)

Et$_3$N (0.3 mL, 2.12 mmol) was added to a solution of 4-benzyloxyaniline hydrochloride salt (0.5 g, 2.12 mmol) in EtOAc (40 mL). The mixture was kept at 0–5 °C, and thiophosgene (0.65 mL, 8.48 mmol, 4 eq.) was added. The reaction was stirred for 24 h at room temperature, filtered and concentrated *in vacuo* to obtain a peach oil. Flash chromatography (hexane–EtOAc, 1 : 1, v/v) afforded 4-benzyloxyphenyl isothiocyanate (**11**) as a pale peach crystalline solid (0.49 g, 96%). Mp 54–56 °C (lit.[18] 60 °C); R_f 0.75 (hexane–EtOAc, 1 : 1, v/v); ^{1}H NMR: δ 7.26–7.28 (5H, m, Ar*H*), 7.10 (2H, d, *J* 9.0 Ar*H*, '*o*' to NCS), 6.84 (2H, d, *J* 9.0, Ar*H*, '*m*' to NCS), 4.98 (2H, m, C*H*$_2$); ^{13}C NMR: δ 158.1 (Ar*C*OBn), 136.7 (Ar*C*Bn), 127.4–129.1 (8 × Ar*C*H), 124.2 (N*C*S), 116.2 (2 × Ar*C*H '*o*' to OBn), 70.7 (CH$_2$); IR (thin film) ν cm^{-1} 2176 (N*C*S); *m*/*z* (CI) 91 (100%), 241 (M$^+$, 40%); Found 241.0569. [$C_{14}H_{11}NOS$]$^+$ requires 241.0561.

1-(4-Benzyloxyphenyl)-3-{4-[bis-(2-chloroethylamino]-phenyl} thiourea (12)

The *N*-mustard salt (**7**) (0.5 g, 1.63 mmol) was dissolved in DCM (15 mL), and NEt$_3$ (0.46 mL, 3.28 mmol) and benzyl isothiocynate (**11**) (0.4 g, 1.63 mmol) were added. The reaction mixture was left to stir under $N_{2\ (g)}$ for 24 h. The reaction was partitioned between DCM–H$_2$O (1 : 1, 2 × 20 mL) and the aqueous layer was extracted with DCM (2 × 20 mL). The organic phases were collected and dried over MgSO$_4$ and concentrated *in vacuo* to give a pale brown solid which was purified by chromatography (hexane–EtOAc, 3 : 7, v/v) to afford (**12**) (0.37 g, 48%) as a pale pink powder. Mp 114–116 °C; R_f 0.67 (hexane–EtOAc, 3 : 7, v/v); ^{1}H NMR: δ 7.30–7.35 (7H, m, Ar*H*Bn, and '*o*' to OBn), 7.22–7.14 (4H, m, Ar*H* '*o*' to thiourea), 6.90 (2H, d, *J* 9.0 Ar*H* '*o*' to NCH$_2$CH$_2$Cl), 6.63 (1H, br s, N*H*), 6.60 (1H, br s, N*H*), 4.99 (2H, s, C*H*$_2$), 3.71–3.79 (4H, m, 2 × C*H*$_2$CH$_2$Cl), 3.58–3.62 (4H, m, 2 × CH$_2$C*H*$_2$Cl); ^{13}C NMR: δ 181.6 (*C*=S), 158.5 (Ar*C*O), 137.5 (2 × Ar*C*N and Ar*C*), 136.9 (2 × Ar*C*NH), 128.4–128.5 (5 × Ar*C*HBn), 127.8–127.9 (4 × Ar*C*H '*o*' to thiourea), 116.0 (2 × Ar*C*H '*o*' to OBn), 112.7 (2 × Ar*C*H '*o*' to NCH$_2$CH$_2$Cl), 70.7 (*C*H$_2$), 53.9 (2 × *C*H$_2$CH$_2$Cl), 40.6 (2 × CH$_2$*C*H$_2$Cl); IR (thin film) ν cm^{-1} 3351 s (NH), 2060, 1697, 1576, 1237, 1142, 967 and 919; *m*/*z* (CI) 475 (M + H, ^{35}Cl, ^{35}Cl 25%), 477 (M + H, ^{35}Cl, ^{37}Cl, 15%), 479 (M + H ^{37}Cl, ^{37}Cl 2%); Found 475.1168. [$C_{24}H_{25}{}^{35}Cl_2N_3OS$ + H]$^+$ requires 475.1208.

4-(*tert*-Butyldimethylsilanyloxy)-phenyl aniline (13)

To a solution of imidazole (2.0 g, 29 mmol) and 4-aminophenol (2.0 g, 18.3 mmol) in THF (50 mL) was added *tert*-butyldimethyl silyl chloride (3.6 g, 24 mmol) with rapid stirring at room temperature. A white precipitate formed immediately. After 30 min the reaction mixture was poured onto H$_2$O (150 mL) and extracted with Et$_2$O (2 × 50 mL). The organic extracts were combined, dried over MgSO$_4$, and concentrated *in vacuo* to give a dark yellow oil. This was purified by column chromatography (EtOAc–hexane, 1 : 1, v/v) to give the pure product (**13**) as a brown oil (3.7 g, 90%). R_f 0.38 (hexane–EtOAc, 1 : 1, v/v); ^{1}H NMR: δ 6.51 (2H, d, *J* 9.0, Ar*H* '*o*' to NH$_2$), 6.42 (2H, d, *J* 9.0, Ar*H* '*m*' to NH$_2$), 3.21 (2H, br s, N*H*$_2$), 0.81 (9H, s, ^{t}Bu), 0.10 (6H, s, 2 × C*H*$_3$); ^{13}C NMR: δ 148.6 (Ar*C*OTBDMS), 140.7 (Ar*C*NH$_2$), 121.1 (2 × Ar*C*H '*o*' to OTBDMS), 116.7 (2 × Ar*C*H '*m*' to OTBDMS), 26.1 (3 × *C*H$_3$), 18.5 (*C*CH$_3$), −4.0 (2 × CH$_3$); IR (thin film) ν cm^{-1} 3300 (NH$_2$), 2268 (NCO); *m*/*z* (CI) 224 (M + H, 100%); Found 224.1467. [$C_{12}H_{21}NOSi$ + H]$^+$ requires 224.1471.

4-(*tert*-Butyldimethylsilanyloxy)-phenyl isothiocyanate (14)

4-(*tert*-Butyldimethylsilanyloxy)-phenyl aniline (**13**) (0.5 g, 2.24 mmol) was dissolved in DCM (20 mL) and cooled in ice. Thiophosgene (0.7 mL, 8.96 mmol) was added dropwise whilst stirring then the solution was gradually brought to reflux at 37 °C under an inert atmosphere for 2 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*, to give a crude yellow product. Flash column chromatography (hexane–EtOAc, 7 : 3, v/v) yielded the pure product (**14**) as a pale yellow oil (0.52 g, 87%). R_f 0.39 (hexane–EtOAc, 7 : 3, v/v); ^{1}H NMR: δ 6.91 (2H, d, *J* 9.0, Ar*H* '*o*' to NCS), 6.60 (2H, d, *J* 9.0 Ar*H* '*m*' to NCS), 0.79 (9H, s, ^{t}*Bu*), 0.01 (6H, s, 2 × C*H*$_3$); ^{13}C NMR: δ 155.3 (N*C*S), 127.3 (2 × Ar*C*H '*o*' to NCS), 126.9 (2 × Ar*C*OTBDMS and Ar*C*NCS), 121.4 (2 × Ar*C*H '*m*' to NCS), 26.0 (3 × *C*H$_3$), 18.6 (*C*CH$_3$), −4.0 (2 × *C*H$_3$); IR (thin film) ν cm^{-1} 2106 s, 1501; *m*/*z* (CI) 265 (M$^+$ 100%); Found 265.0960. [$C_{13}H_{19}NOSSi$]$^+$ requires 265.0957.

1-{4-[Bis-(2-chloroethyl)-amino]-phenyl}-3-(4-hydroxyphenyl) thiourea (2)

To the silyl ether thiourea (**15**) (210 mg, 0.422 mmol) in anhydrous THF (10 mL) was added Bu$_4$NF (1 M in THF, 0.42 mL). After 45 min, the solution was concentrated and purified by flash chromatography (hexane–EtOAc, 5 : 2, v/v) to afford the desired product (**2**) as a pale peach crystalline solid (100 mg, 62%). Mp 104–105 °C; R_f 0.25 (hexane–EtOAc, 5 : 2, v/v); ^{1}H NMR (MeOH-d$_4$): δ 7.75 (2H, br s, 2 × N*H*), 7.12–6.92 (4H, m, Ar*H* '*o*' to thiourea), 6.69 (2H, d, *J* 9.0, Ar*H* '*o*' to OH), 6.51 (2H, d, *J* 9.0, Ar*H* '*o*' to NCH$_2$CH$_2$Cl), 5.04 (1H, br s, O*H*), 3.60–3.69 (4H, m, 2 × C*H*$_2$CH$_2$Cl), 3.49–3.54 (4H, m, 2 × CH$_2$C*H*$_2$Cl); ^{13}C NMR (MeOH-d$_4$): δ 182.6 (*C*=S), 157.6 (Ar*C*OH), 146.8 (Ar*C*N), 132.5 (2 × Ar*C*NH), 129.0 (4 × Ar*C*H '*o*' to thiourea), 116.9 (2 × Ar*C*H '*o*' to OH), 113.9 (2 × Ar*C*H '*o*' to ArCNCH$_2$CH$_2$Cl), 54.9 (2 × *C*H$_2$CH$_2$Cl), 42.0 (2 × *C*H$_2$CH$_2$Cl); IR (thin film) ν cm^{-1} 3351 br (OH), 1697, 1576; *m*/*z* (CI) 384 (M + H, ^{35}Cl, ^{35}Cl, 100%), 386 (M + H, ^{35}Cl, ^{37}Cl, 65%), 388 (M + H, ^{37}Cl, ^{37}Cl, 10%); Found 384.3229. [$C_{17}H_{19}{}^{35}Cl_2N_3OS$ + H]$^+$ requires 384.3238; Found C, 52.98; H, 5.35; N, 10.46. $C_{17}H_{19}Cl_2N_3OS$ requires C, 53.13; H, 4.98; N, 10.93%; HPLC: t_R 4.0 min (method 3).

3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl amine (17)

5-Amino-2-methoxyphenol (**16**) (2.0 g, 14.37 mmol) was dissolved in DCM (50 mL) and added to a solution of *tert*-butyldimethyl silyl chloride (2.2 g, 14.37 mmol) in DCM (10 mL). NEt$_3$ (2 mL, 14.37 mmol) was added, followed by a catalytic amount of DMAP and the solution was stirred rapidly. A white precipitate formed immediately. After 30 min the reaction mixture was poured onto H$_2$O (150 mL) and extracted with Et$_2$O (2 × 50 mL). The organic extracts were combined, dried over MgSO$_4$, and concentrated *in vacuo* to give a dark yellow oil. This was purified by column chromatography (EtOAc–hexane, 1.5 : 1, v/v) to give the pure product (**17**) as a brown oil (2.38 g, 65%). R_f 0.67 (hexane–EtOAc, 2 : 8, v/v);

^1H NMR: δ 6.52 (1H, d, J 8.0 ArH '*o*' to OCH_3), 6.10–6.13 (2H, m, ArH '*m*' to OCH_3), 3.57 (3H, s, OCH_3), 3.08 (2H, br, s, NH_2), 0.84 (9H, s, ^tBu), 0.01 (6H, s, CH_3); ^{13}C NMR: δ 146.4 (*C*OMe), 144.7 (*C*OTBDMS), 141.0 (CNH_2), 114.7 (Ar*C*H '*o*' to OMe), 109.9 (*C*H '*m*' to OMe), 108.6 (Ar*C*H '*m*' to OMe), 56.9 (OCH_3), 26.1 (3 × CH_3), 18.8 (CCH_3), −4.3 (2 × CH_3); IR (thin film) ν cm^{-1} 3367 (NH), 2955, 2858 m (OMe), 1711; m/z (CI) 254 (M + H, 100%); Found 254.1576. $[C_{13}H_{23}NO_2Si + H]^+$ requires 254.1577.

tert-Butyl-(5-isocyanato-2-methoxyphenoxy)-dimethylsilane (18)

Amine (**17**) (0.5 g, 1.98 mmol) was dissolved in EtOAc (20 mL) and triphosgene (2.3 g, 7.90 mmol, 4 eq.) was added. The reaction was gradually brought to reflux at 77 °C and was monitored at this temperature for 2 h. The resultant mixture was concentrated *in vacuo* and purified by column chromatography (EtOAc–hexane, 7 : 3, v/v) to obtain the pure isocyanate (**18**) (0.51 g, 93%) as a dark brown oil. R_f 0.77 (EtOAc–hexane, 7 : 3, v/v); ^1H NMR: δ 6.61–6.78 (3H, m, ArH), 3.63 (3H, s, OCH_3), 0.84 (9H, s, ^tBu), 0.02 (6H, s, 2 × CH_3); ^{13}C NMR: δ 149.7 (*C*OMe), 145.9 (*C*OTBDMS), 131.0 (Ar*C*N), 126.3 (N*C*O), 118.2–118.0 (3 × Ar*C*H), 56.1 (OCH_3), 26.0 (3 × CH_3), 18.8 (CCH_3), −4.3 (2 × CH_3); IR (thin film) ν cm^{-1} 3325, 2859 (OCH_3), 2273 (NCO), 1520, and 841.

1-{4-[Bis-(2-chloroethyl)-amino]-phenyl}-3-[3-*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl] urea (19)

N-Mustard (**7**) (0.5 g, 1.63 mmol) was dissolved in DCM (15 mL) and NEt_3 (0.34 mL, 2.45 mmol) was added. The reaction mixture was stirred for 5 min, then the protected isocyanate (**18**) (0.45 g, 1.63 mmol) was added and the mixture stirred for 24 h. The resulting mixture was partitioned between H_2O–DCM and then purified by chromatography (EtOAc–hexane, 7 : 3, v/v) to obtain (**19**) (0.6 g, 71%) as a very pale yellow liquid. R_f 0.65 (hexane–EtOAc, 3 : 7, v/v); ^1H NMR: δ 7.03 (2H, d, J 9, ArH '*m*' to NCH_2CH_2Cl), 6.61–6.71 (3H, m, 1 × ArH '*o*' to OCH_3 and 2 × ArH '*o*' to NCH_2CH_2Cl), 6.50 (2H, d, J 9, ArH '*m*' to OCH_3), 6.05 (1H, br s, NH), 6.04 (1H, br s NH), 3.62 (3H, s, OCH_3), 3.59–3.40 (8H, m, 2 × CH_2CH_2Cl), 0.83 (9H, s, ^tBu), 0.01 (6H, s, 2 × CH_3); ^{13}C NMR: δ 150.1 (*C*=O), 149.5 (*C*OMe), 141.1 (Ar*C*OTBDMS), 140.0 (Ar*C*N), 131.1 (Ar*C*NH), 126.5 (Ar*C*NH), 120.5 (2 × Ar*C*H), 113.2 (2 × Ar*C*H), 112.9 (3 × Ar*C*H), 56.2 (2 × CH_2CH_2Cl), 54.1 (OCH_3), 40.8 (2 × CH_2CH_2Cl) 26.1 (3 × CH_3), 18.8 (CCH_3), −4.2 (2 × CH_3); IR (thin film) ν cm^{-1} 3327 br, 1500 s, 1223 s, 898; m/z (CI) 254 (100%), 512 (M + H, ^{35}Cl, ^{35}Cl, 25%), 514 (M + H, ^{35}Cl, ^{37}Cl, 15%), 516 (M + H, ^{37}Cl, ^{37}Cl, 3%); Found 512.1911. $[C_{24}H_{35}{}^{35}Cl_2N_3O_3Si + H]^+$ requires 512.1904.

1-{4-[Bis-(2-chloroethyl)-amino]-phenyl}-3-(3,4-dihydroxyphenyl) urea (3)

Urea (**19**) (0.6 g, 1.17 mmol) was dissolved in the minimum amount of conc. HCl and heated at reflux at 130 °C for 2 h. The product was washed with water and extracted with DCM (4 × 15 mL). The organic extracts were combined and dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue (0.35 g, 0.486 mmol) was dissolved in dry DCM and cooled to −78 °C (dry ice–acetone) under a stream of nitrogen. Boron tribromide (1.3 mL, 1 M solution in dichloromethane) was added slowly. After the addition of boron tribromide, the pale yellow/green solution was allowed to warm to room temperature over 4 h. The reaction mixture was quenched by the addition of $NaHCO_3$ (10%), extracted into dichloromethane (3 × 30 mL), dried over $MgSO_4$ and the solvents removed *in vacuo* to produce a light brown oil. This was purified by column chromatography (DCM–MeOH, 9 : 1, v/v) to afford prodrug (**3**) as a pale mustard solid (170 mg, 70% over 2 steps). Mp 112 °C; R_f 0.37 (DCM–MeOH, 9 : 1, v/v); ^1H NMR (MeOH-d_4): δ 7.69 (2H, d, J 9.0, ArH), 7.58 (2H, d, J 9.0, ArH), 7.01 (1H, t, J 2.0 ArH), 6.74 (2H, d, J 2.0, ArH), 4.04 (4H, t, J 13.0, 2 × CH_2CH_2Cl), 3.32 (4H, t, J 13.0, CH_2CH_2Cl); ^{13}C NMR (MeOH-d_4): δ 155.3 (*C*=O), 146.2 (Ar*C*OH), 142.5 (Ar*C*OH), 142.2 (Ar*C*N), 132.0 (Ar*C*NH), 131.1 (Ar*C*NH), 123.1 (2 × Ar*C*H), 121.3 (Ar*C*H), 116.3 (Ar*C*H), 113.1 (2 × Ar*C*H), 109.9 (Ar*C*H), 59.7 (2 × CH_2CH_2Cl), 38.3 (2 × CH_2CH_2Cl); IR (thin film) ν cm^{-1} 3422 br, 1642; m/z (CI) 384 (M + H, ^{35}Cl, ^{35}Cl, 55%), 386 (M + H, ^{35}Cl, ^{37}Cl, 33%), 388 (M + H, ^{37}Cl, ^{37}Cl, 5%); Found 384.0881. $[C_{17}H_{19}{}^{35}Cl_2N_3O_3S + H]^+$ requires 384.0882; HPLC: t_R 4.0 min (method 3).

1-{4-[Bis-(2-Chloroethyl)-amino]-phenyl-3-(3,4-dimethoxyphenyl)-thiourea (21)

To a stirred solution of *N*-mustard salt (**7**) (0.5 g, 1.63 mmol) and NEt_3 (0.5 mL, 3.26 mmol) in DCM was added 3, 4-dihydroxyphenyl isocyanate (**20**) (0.22 g, 1.63 mmol). The reaction was left to stir under an inert atmosphere until complete disappearance of the NCO stretch in the IR spectrum was observed, and total consumption of the *N*-mustard (**7**) was evident by TLC analysis. After 1.5 days, the reaction was washed with water, and extracted with DCM (30 mL). The organic extracts were combined and dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude mixture was purified by column chromatography (DCM–acetone, 7 : 1, v/v) to yield a colourless powder which was crystallised from hot toluene to give (**21**) as pale yellow crystals (0.63 g, 93%). Mp 104–107 °C; R_f 0.4 (EtOAc–hexane, 6 : 4, v/v); ^1H NMR (DMSO-d_6): δ 7.62 (2H, br s, 2 × NH), 7.25 (2H, d, J 9.0, ArH), 6.98 (1H, br s, ArH), 6.86 (2H, br s, ArH), 6.69 (2H, d, J 9.0, ArH), 3.88 (6H, s, 2 × OCH_3), 3.72–3.80 (4H, m, 2 × CH_2CH_2Cl), 3.44–3.52 (4H, m, 2 × CH_2CH_2Cl); ^{13}C NMR (DMSO-d_6): δ 180.9 (*C*=S), 148.2 (Ar*C*OMe), 145.4 (Ar*C*OMe), 127.9 (Ar*C*N and 2 × Ar*C*NH), 118.2 (Ar*C*H), 112.3 (Ar*C*H), 109.9 (4 × Ar*C*H), 56.1 (2 × CH_2CH_2Cl), 53.5 (2 × OCH_3), 40.3 (2 × CH_2CH_2Cl); IR (thin film) ν cm^{-1} 1513; m/z (CI) 428 (M + H, ^{35}Cl, ^{35}Cl, 60%), 430 (M + H, ^{35}Cl, ^{37}Cl, 35%), 432 (M + H, ^{37}Cl, ^{37}Cl, 6%); Found 428.0971. $[C_{19}H_{23}{}^{35}Cl_2N_3O_2S + H]^+$ requires 428.0967; Found C, 53.16; H, 5.40; N, 9.75. $C_{19}H_{23}Cl_2N_3O_2S$ requires C, 53.27; H, 5.41; N, 9.80%.

1-{4-[Bis-(2-chloroethyl)-amino]-phenyl-3-(3,4-dimethoxyphenyl)-thiourea (4)

The dimethoxy urea (**21**) (200 mg, 0.147 mmol) was dissolved in dry DCM (6 mL) and cooled to −78 °C (dry ice–acetone) under a stream of nitrogen. Boron tribromide (1.2 mL, 1 M solution in dichloromethane) was added slowly. After the addition of boron tribromide the pale yellow/green solution was allowed to warm to room temperature over 4 h. The reaction mixture was quenched with brine (10%), extracted into dichloromethane (3 × 30 mL), dried over $MgSO_4$ and the solvents removed *in vacuo* to produce a light brown oil (184 mg, 98%). This was purified by column chromatography (DCM–MeOH, 9 : 1, v/v) to afford (**4**) as a pale mustard solid (174 mg, 92%). Mp 212–220 °C; R_f 0.33 (DCM–MeOH, 9 : 1, v/v); ^1H NMR (MeOH-d_4): δ 9.23 (1H, br s, OH), 9.16 (1H, br s, OH), 9.06 (1H, br s, NH), 8.80 (1H, br s, NH), 7.32 (2H, d, J 9.0, ArH), 6.84 (1H, d, J 2.0, ArH), 6.56–6.74 (4H, m, ArH), 3.79 (8H, br s, 2 × CH_2CH_2Cl); ^{13}C NMR (MeOH-d_4): δ 179.9 (*C*=S), 155.3 (Ar*C*OH), 144.1 (Ar*C*OH), 143.2 (Ar*C*N), 131.0 (Ar*C*NH), 129.5 (Ar*C*NH), 126.7 (2 × Ar*C*H), 115.9 (Ar*C*H), 115 (Ar*C*H), 113.1 (Ar*C*H), 111.9 (2 × Ar*C*H), 55.3 (2 × CH_2CH_2Cl), 41.5 (2 × CH_2CH_2Cl); IR (thin film) ν cm^{-1} 3449 br, 1620; m/z (CI) 400 (M + H, ^{35}Cl, ^{35}Cl, 40%), 402 (M + H, ^{35}Cl, ^{37}Cl, 27%), 404 (M + H, ^{37}Cl, ^{37}Cl, 4%); Found 400.0661. $[C_{17}H_{19}{}^{35}Cl_2N_3O_2S + H]^+$ requires 400.0654; Found C, 49.53; H, 4.90; N, 9.75. $C_{17}H_{19}Cl_2N_3O_2S{\cdot}H_2O$ requires C, 48.81; H, 5.06; N, 10.04%; HPLC: t_R 3.5 min (method 3).

N-tert-Butoxycarbonyl-6-nitrodopamine (24)

6-Nitrodopamine hydrogen sulfate salt (**23**)[9] (5.4 g, 18.2 mmol) was suspended in THF–H_2O (5 : 1 v/v, 60 mL) with triethylamine (6.4 mL, 45.6 mmol). Boc–ON (5.39 g, 22.0 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was then removed under reduced pressure and to the residue was added EtOAc (200 mL) and MeOH (50 mL). This was then dried ($MgSO_4$), filtered and reduced to dryness. The crude product was purified by column chromatography (DCM–MeOH, 96 : 4, v/v) to yield amine (**24**) as a yellow solid (3.19 g, 59%). Mp 169–171 °C; R_f 0.45 (hexane–EtOAc, 1 : 1, v/v); ^{1}H NMR (MeOH-d_4): δ 7.54 (1H, s, H5), 6.72 (1H, s, H2), 3.32 (2H, t, *J* 6.8, C*H*$_2$), 2.98 (2H, t, *J* 6.9, C*H*$_2$), 1.41 (9H, s, 3 × C*H*$_3$); ^{13}C NMR: δ 158.9 (NH*C*OO), 152.6 (Ar*C*), 145.6 (Ar*C*), 142.1 (Ar*C*), 130.0 (Ar*C*), 119.9 (Ar*C*H), 113.8 (Ar*C*H), 80.4 (*C*[CH$_3$]$_3$), 42.2 (*C*H$_2$), 35.4 (*C*H$_2$), 29.2 (C[*C*H$_3$]$_3$); IR (thin film) ν cm^{-1} 3401, 1674, 1455, 1394, 1368, 1329, 1286, 1161, 1018; Found C, 52.13; H, 6.03; N, 9.03. $C_{13}H_{18}N_2O_6$ requires C, 52.35; H, 6.08; N, 9.39%.

2-Acetoxy-5-(2-*tert*-butoxycarbonylaminoethyl)-4-nitrophenyl acetate (25)

N-tert-Butoxycarbonyl-6-nitrodopamine (**24**) (0.75 g, 2.51 mmol) was solubilised in pyridine (15 mL) and acetic anhydride (0.52 mL, 5.53 mmol) was added. The reaction mixture was stirred at room temperature for 16 h. DCM (20 mL) and water (20 mL) were added to the reaction mixture and the two phases were partitioned. The organic phase was washed with water (20 mL), dried ($MgSO_4$), filtered and reduced to dryness. The product (**25**) was isolated as a waxy yellow solid (834 mg, 88%). Mp 89–92 °C; R_f 0.57 (hexane–EtOAc, 1 : 1, v/v); ^{1}H NMR: δ 7.84 (1H, s, H5), 7.17 (1H, s, H2), 4.71 (1H, bs, N*H*Boc), 3.38 (2H, q, *J* 6.8, C*H*$_2$), 3.04 (2H, t, *J* 6.9, C*H*$_2$), 2.49 (6H, s, 2 × OCOC*H*$_3$), 1.36 (9H, s, 3 × C*H*$_3$); ^{13}C NMR: δ 169.0 (O*C*OCH$_3$), 167.7 (O*C*OCH$_3$), 156.3 (NH*C*OO), 146.6 (Ar*C*), 146.1 (Ar*C*), 141.1 (Ar*C*), 133.9 (Ar*C*), 127.7 (Ar*C*H), 121.3 (Ar*C*H), 79.9 (*C*[CH$_3$]$_3$), 41.1 (*C*H$_2$), 33.8 (*C*H$_2$), 28.7 (C[*C*H$_3$]$_3$), 21.0 (OCO*C*H$_3$), 20.9 (OCO*C*H$_3$); IR (thin film) ν cm^{-1} 1778, 1649, 1530, 1436, 1371, 1273, 1199, 1145; *m/z* (FAB) 405 (45%, M + Na), 327 (90), 283 (100); Found 405.1262. $C_{17}H_{22}N_2O_8$Na requires 405.1274.

2-Acetoxy-5-(2-*tert*-butoxycarbonylaminoethyl)-4-aminophenyl acetate (26)

2-Acetoxy-5-(2-*tert*-butoxycarbonylaminoethyl)-4-nitrophenyl acetate (**25**) (1.40 g, 3.66 mmol) was solubilised in MeOH containing 10% palladium on carbon (90 mg). The mixture was stirred under an atmosphere of hydrogen for 2 h and then filtered through a short pad of Celite. The filtrate was reduced to dryness and the crude product was purified by column chromatography (hexane–EtOAc, 1 : 1). The product (**26**) was obtained as a yellow oil that crystallised on standing (0.9 g, 70%). Mp 140–142 °C; R_f 0.37 (hexane–EtOAc, v/v); ^{1}H NMR (MeOH-d_4): δ 6.80 (1H, s, H5), 6.53 (1H, s, H2), 3.21 (2H, t, *J* 7.5 C*H*$_2$), 2.66 (2H, t, *J* 7.5, C*H*$_2$), 2.23 (3H, s, OCOC*H*$_3$), 2.22 (3H, s, OCOC*H*$_3$), 1.45 (9H, s, 3 × C*H*$_3$); ^{13}C NMR (MeOH-d_4): δ 173.9 (O*C*OCH$_3$), 173.2 (O*C*OCH$_3$), 161.6 (NH*C*OO), 148.6 (Ar*C*), 145.4 (Ar*C*), 137.3 (Ar*C*), 128.2 (Ar*C*), 125.6 (Ar*C*H), 113.6 (Ar*C*H), 83.0 (*C*[CH$_3$]$_3$), 43.5 (*C*H$_2$), 35.3 (*C*H$_2$), 31.7 (C[*C*H$_3$]$_3$), 23.4 (2 × OCO*C*H$_3$); IR (thin film) ν cm^{-1} 2526, 1762, 1646, 1508, 1448, 1376, 1216, 1098; *m/z* (FAB) 375 (25%, M + Na), 352 (100), 310 (42), 297 (27); Found 352.1634. $C_{17}H_{24}N_2O_6$ requires 352.1634; Found C, 57.91; H, 6.84; N, 7.87. $C_{17}H_{24}N_2O_6$ requires C, 57.94; H, 6.86; N, 7.95%.

2-Acetoxy-4(3-{4-[bis-(2-chloroethyl) amino]phenyl}ureido)-5-(2-*tert*-butoxycarbonylaminoethyl)phenyl acetate (29)

Amine (**26**) (150 mg, 0.43 mmol) was solubilised in DCM (4 mL) and cooled to 0 °C. Triphosgene (0.27 mL, 0.57 mmol of a 20% solution in toluene) was added dropwise, followed by the addition of triethylamine (0.13 mL, 0.94 mmol). The reaction mixture was stirred at 0 °C for 1 hour before the addition of aniline mustard dihydrochloride salt (**7**) (143 mg, 0.47 mmol) and pyridine (2 mL). The reaction was stirred at room temperature for 16 h, diluted with DCM (10 mL), dried over $MgSO_4$, filtered and the filtrate reduced to dryness. The crude residue was purified by column chromatography (hexane–EtOAc, 1 : 1, v/v) to yield the pure product (**29**) as a brown foam (163 mg, 63%). R_f 0.56 (Hexane–EtOAc, 1 : 1, v/v); ^{1}H NMR (MeOH-d_4): δ 8.76 (1H, bs, N*H*-urea), 8.30 (1H, s, H6), 7.73 (1H, bs, N*H*-urea), 7.40 (2H, d, *J* 9.0, *2ArH*), 6.92 (1H, s, H3), 6.75 (2H, d, *J* 9.0, 2Ar*H*), 5.35 (1H, t, *J* 6.0, N*H*Boc), 3.74–3.60 (8H, m, 4 × C*H*$_2$), 3.20–3.11 (2H, m, C*H*$_2$), 2.78 (2H, t, *J* 8.0, C*H*$_2$), 2.28 (3H, s, OCOC*H*$_3$), 2.27 (3H, s, OCOC*H*$_3$), 1.56 (9H, s, 3 × C*H*$_3$); ^{13}C NMR (MeOH-d_4): δ 169.1 (O*C*OCH$_3$), 168.9 (O*C*OCH$_3$), 158.4 (NH*C*OO), 153.4 (NH*C*OO), 141.4 (Ar*C*), 137.1 (Ar*C*), 136.3 (Ar*C*), 132.4 (Ar*C*), 124.3 (Ar*C*H), 123.8 (Ar*C*H), 121.6 (Ar*C*H), 114.6 (Ar*C*H), 114.1 (Ar*C*H), 81.5 (*C*[CH$_3$]$_3$), 54.6 (2 × *C*H$_2$), 40.7 (2 × *C*H$_2$ + Ar*C*H$_2$), 33.1 (*C*H$_2$N), 29.0 (C[*C*H$_3$]$_3$), 21.1 (2 × OCO*C*H$_3$); IR (thin film) ν cm^{-1} 3380, 2977, 1769, 1705, 1661, 1597, 1516, 1421, 1367, 1201, 1104, 1013; *m/z* (FAB) 633 (15%, M + Na), 610 (21), 511 (7), 154 (100); Found 610.1979. $C_{28}H_{36}N_4O_7Cl_2$ requires 610.1961; Found C, 54.01; H, 6.07; N, 9.11. $C_{28}H_{36}N_4O_7Cl_2$ requires C, 54.98; H, 5.95; N, 9.16%.

2-Acetoxy-4-(3-{4-[bis-(2-chloroethyl) amino]phenyl}thioureido)-5-(2-*tert*-butoxycarbonylaminoethyl)phenyl acetate (30)

Amine (**26**) (200 mg, 0.57 mmol) was solubilised in DCM (4 mL) and cooled to 0 °C. Thiophosgene (43 µL, 0.57 mmol) was added dropwise, followed by the addition of triethylamine (0.17 mL, 1.25 mmol). The reaction mixture was stirred at 0 °C for 1 hour before the addition of aniline mustard dihydrochloride salt (**7**) (191 mg, 0.62 mmol) and pyridine (2 mL). The reaction was stirred at room temperature for 16 h, diluted with DCM (10 mL), dried over $MgSO_4$, filtered and the filtrate reduced to dryness. The crude residue was purified by column chromatography (DCM–MeOH, 96 : 4, v/v) to yield the pure product (**30**) as a red/brown foam (252 mg, 71%). Mp 89–94 °C; R_f 0.78 (DCM–MeOH, 9 : 1, v/v); ^{1}H NMR (MeOH-d_4): δ 8.20 (1H, bs, N*H*-urea), 8.30 (1H, s, H6), 7.49 (1H, bs, N*H*-urea), 7.25 (2H, d, *J* 9.0, 2Ar*H*), 7.20 (1H, s, H3), 6.60 (2H, d, *J* 9.0, 2Ar*H*), 4.84 (1H, bs, N*H*Boc), 3.64 (4H, 2 × t, *J* 6.5, 2 × C*H*$_2$), 3.54 (4H, 2 × t, *J* 7.0, 2 × C*H*$_2$), 3.24 (2H, q, *J* 7.0, C*H*$_2$), 2.70 (2H, t, *J* 7.0, C*H*$_2$), 2.20 (6H, 2 × s, 2 × OCOC*H*$_3$), 1.32 (9H, s, 3 × C*H*$_3$); ^{13}C NMR: δ 181.9 (NH*C*OO), 168.6 (O*C*OCH$_3$), 168.5 (O*C*OCH$_3$), 158.3 (Ar*C*), 146.2 (Ar*C*), 134.9 (Ar*C* + NH*C*SNH), 129.0 (Ar*C*), 125.3 (Ar*C*H), 124.0 (Ar*C*), 112.6 (Ar*C*H), 80.4 (*C*[CH$_3$]$_3$), 53.9 (2 × *C*H$_2$), 40.7 (2 × *C*H$_2$ + Ar*C*H$_2$), 34.0 (*C*H$_2$N), 28.7 (C[*C*H$_3$]$_3$), 21.1 (2 × OCO*C*H$_3$); IR (thin film) ν cm^{-1} 3344, 2977, 1772, 1695, 1613, 1517, 1455, 1367, 1207, 1013; *m/z* (FAB) 649 (30%, M + Na), 625 (95), 571 (55); Found 626.1742. $C_{28}H_{36}N_4O_6SCl_2$ requires 626.1733; Found C, 52.92; H, 5.73; N, 9.03. $C_{28}H_{36}N_4O_6SCl_2$ requires C, 53.79; H, 5.78; N, 8.92%.

1-[2-(2-Aminoethyl)-4,5-dihydroxyphenyl]-3-{4-[bis-(2-chloroethyl) amino]-phenyl}urea (5)

Urea (**29**) (100 mg, 0.16 mmol) was solubilised in acetone (4 mL) and heated to reflux with 6 M HCl (2 mL) for 4 h. After removal of the solvent by lyophilisation, the pure product (**5**) was obtained as a red/brown solid (104 mg, 100%). Mp 98 °C;

R_f 0.19 (MeCN–H_2O, 94 : 6, v/v); ^{1}H NMR (MeOH-d_4): δ 7.62 (2H, d, J 9.0, 2ArH), 7.33 (2H, d, J 9.0, 2ArH), 6.84 (1H, s, H6), 3.99 (4H, t, J 6.5, 2 × CH_2), 3.74 (1H, s, H3), 3.66 (4H, 2 × t, J 6.5, 2 × CH_2), 3.15 (2H, t, J 7.5, CH_2), 2.89 (2H, t, J 7.5, CH_2); ^{13}C NMR (MeOH-d_4): δ 157.4 (NHCONH), 146.4 (ArC), 146.3 (ArC), 145.8 (ArC), 129.1 (ArC), 125.1 (ArC), 122.6 (ArCH), 122.1 (ArC), 117.8 (ArC), 116.1 (ArCH), 59.6 (2 × CH$_2$), 41.5 (2 × CH$_2$), 39.2 (ArCH$_2$), 30.4 (CH$_2$NH); IR (thin film) ν cm^{-1} 2531, 2361, 1645, 1558, 1515, 1456, 1319, 1221; m/z (FAB) 467 (54%, M + K), 427 (100), 233 (35), 209 (33); Found 427.1302. $C_{19}H_{25}N_4O_3Cl_2$ requires 427.1304; Found C, 42.76; H, 5.33; N, 9.92. $C_{19}H_{25}N_4O_3Cl_2\cdot 3HCl$ requires C, 42.52; H, 5.08; N, 10.44%; HPLC: t_R 21.1 min (method 1), 23.6 min (method 2).

1-[2-(2-Aminoethyl)-4,5-dihydroxyphenyl]-3-{4-[bis-(2-chloroethyl) amino]-phenyl}thiourea (6)

Thiourea (**30**) (200 mg, 0.36 mmol) was solubilised in acetone (4 mL) and heated to reflux with 6 M HCl (2 mL) for 4 h. After removal of the solvent by lyophilisation, the pure product (**6**) was purified by column chromatography (MeCN–H_2O, 94 : 6, v/v) to yield the pure product as a yellow solid (55 mg, 67%). Mp 226 °C; R_f 0.19 (MeCN–H_2O, 94 : 6, v/v); ^{1}H NMR (MeOH-d_4): δ 7.25 (2H, d, J 9.0, 2ArH), 6.79–6.77 (3H, m, H6 + 2ArH), 6.71 (1H, s, H3), 3.81–3.68 (8H, m, 4 × CH_2), 3.18 (2H, t, J 7.5, CH_2), 2.86 (2H, t, J 7.5, CH_2); ^{13}C NMR (MeOH-d_4): δ 183.7 (C=S), 147.0 (ArC), 146.4 (ArC), 141.8 (ArC), 129.1 (ArC), 127.3 (ArC), 118.1 (2 × ArCH), 117.9 (ArC), 117.6 (ArC), 113.9 (2 × ArCH), 54.9 (2 × CH$_2$), 42.1 (2 × CH$_2$), 41.6 (ArCH$_2$), 30.6 (CH$_2$NH); IR (thin film) ν cm^{-1} 2531, 2361, 1645, 1558, 1515, 1456, 1319, 1221; m/z (FAB) 523 (30%, M + 2H + 2K), 443 (75), 409 (20); Found 443.1070. $C_{19}H_{24}N_3O_2SCl_2$ requires 443.0997; HPLC: t_R 20.7 min (method 1), 22.9 min (method 2).

Oximetry studies. To a vigorously stirred solution of mushroom tyrosinase (2.2 mL, 300 units, Sigma mushroom tyrosinase, 2060 units mg^{-1}) in phosphate buffer, pH 7.2, were added 100 µL of a 10 mM solution of the compound under investigation. Oxygen uptake was monitored using a YSI 5300 biological oxygen monitor. Experiments were carried out at 37 °C in triplicate.

Drug release studies. Tyrosinase (300 µL of a 2500 units mL^{-1} solution in phosphate buffer) was diluted with phosphate buffer, pH 7.2 (700 µL) and incubated at 37 °C with the prodrug (100 µL of a 10 mM solution in DMSO–phosphate buffer (2 : 100, v/v; 700 µL)). At various intervals, the solution was analysed by HPLC.

Chemical stability studies. The prodrug (100 µL of a 10 mM solution in DMSO–phosphate buffer (2 : 100, v/v; 700 µL)) was incubated in phosphate buffer (900 µL, pH 7.2) at 37 °C. Aliquots (100 µL) were removed at various time intervals and diluted with MeCN (500 µL) and analysed by HPLC.

Serum stability studies. The prodrug (100 µL of a 10 mM solution in DMSO–phosphate buffer (2 : 100, v/v; 700 µL)) was incubated in phosphate buffer–adult bovine serum–RPMI growth media (900 µL, 1 : 1 : 1, v/v/v) at 37 °C. Aliquots (100 µL) were removed at various time intervals and diluted with MeCN (500 µL) and analysed by HPLC.

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References

1 (*a*) A. M. Jordan, T. H. Khan, H. M. I. Osborn, A. Photiou and P. A. Riley, *Bioorg. Med. Chem.*, 1999, **7**, 1775–1780; (*b*) A. M. Jordan, T. H. Khan, H. Malkin, H. M. I. Osborn, A. Photiou and P. A. Riley, *Bioorg. Med. Chem.*, 2001, **9**, 1549–1558; (*c*) A. M. Jordan, T. H. Khan, H. Malkin and H. M. I. Osborn, *Bioorg. Med. Chem.*, 2002, **10**, 2625–2633.

2 For recent reviews see: (*a*) M. Rooseboom, J. N. M. Commandeur and N. P. E. Vermeulen, *Pharmacol. Rev.*, 2004, **56**, 53–102; (*b*) L. F. Tietze and T. Feverstein, *Curr. Pharm. Des.*, 2003, **9**, 2155–2175; (*c*) D. A. Goodwin and C. F. Meares, *Biotechnol. Adv.*, 2001, **19**, 435–450; (*d*) K. N. Syrigos and A. A. Epenetos, *Anticancer Res.*, 1999, **19**, 605–613.

3 (*a*) J. Pawelek, A. Korner, A. Bergstrom and J. Bologna, *Nature*, 1980, **286**, 617–619; (*b*) E. J. Land, C. A. Ramsden and P. A. Riley, *Methods Enzymol.*, 2004, **378**, 88–109; (*c*) P. A. Riley, *Pigm. Cell Res.*, 2003, **16**, 548–552.

4 (*a*) V. C. Pearson, J. Ferguson, P. M. Rogers, L. R. Kelland and D. J. Robins, *Oncol. Res.*, 2003, **13**, 503–512; (*b*) N. J. Lant, P. McKeown, M. C. Timoney, L. R. Kelland, P. M. Rogers and D. J. Robins, *Anti-Cancer Drug Des.*, 2001, **16**, 49–55; (*c*) N. J. Lant, P. McKeown, L. R. Kelland, P. M. Rogers and D. J. Robins, *Anti-Cancer Drug Des.*, 2000, **15**, 295–302; (*d*) A. Gali, P. D. Thomas, M. Ota and K. Jimbow, *Melanoma Res.*, 2000, **10**, 9–15; (*e*) M. Simoneva, A. Wall, R. Weissleder and A. Bogdanov, Jr., *Cancer Res.*, 2000, **60**, 6656–6662; (*f*) G. Prota, M. d'Ischia and D. Mascagna, *Melanoma Res.*, 1994, **4**, 351–358; (*g*) K. Jimbow, T. Iwashina, F. Alena, K. Yamada, J. Pankovich and T. Umemura, *J. Invest. Dermatol.*, 1993, **100**, 231S–238S; (*h*) P. A. Riley, C. J. Cooksey, C. I. Johnson, E. J. Land, A. M. Latter and C. A. Ramsden, *Eur. J. Cancer*, 1997, **33**, 135–143; (*i*) M. M. Wick, *J. Invest. Dermatol.*, 1980, **74**, 63–65; (*j*) M. E. Morrison, M. J. Yagi and G. Cohen, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 2960–2964; (*k*) S. Ito, T. Kato, K. Ishikawa, T. Kasuya and K. Jimbow, *Biochem. Pharmacol.*, 1987, **36**, 2007–2011.

5 For a recent example see: E. M. Noorda, B. C. Vrouenraets, O. E. Nieweg, J. M. Klasse, J. van der Zee and B. B. R. Kroon, *Melanoma Res.*, 2003, **13**, 395–399.

6 For recent examples see: (*a*) I. Niculescu-Duvaz, I. Scanlon, D. Niculescu-Duvaz, F. Friedlos, J. Martin, R. Marais and C. J. Springer, *J. Med. Chem.*, 2004, **47**, 2651–2658; (*b*) C. J. Springer and I. Niculescu-Duvaz, *Anti-Cancer Drug Des.*, 1995, **10**, 361–372.

7 E. Frei, B. A. Teicher, S. A. Holden, K. N. Cathcart and Y. Young, *Cancer Res.*, 1988, **48**, 6417–6423.

8 H. M. I. Osborn and N. A. O. Williams, *Org. Lett.*, 2004, **6**, 3111–3113.

9 A. Napolitano, M. d'Ischia, C. Constantini and G. Prota, *Tetrahedron*, 1992, **48**, 8515–8522.

10 (*a*) M. H. Benn, A. M. Creighton, L. N. Owen and G. R. White, *J. Chem. Soc.*, 1961, 2365–2375; (*b*) R. Everett, *J. Chem. Soc.*, 1949, 1972–1980; (*c*) Y. H. Cho and J. C. Park, *Tetrahedron Lett.*, 1997, **38**, 8331–8334.

11 O. Yuji and T. Tatsuyoshi, *Tetrahedron Lett.*, 1984, **25**, 5397–5400.

12 A. C. Cheng and N. Castagnoli, Jr., *J. Med. Chem.*, 1984, **27**, 513–520.

13 For example see: (*a*) S. Y. Seo, V. K. Sharma and N. Sharma, *J. Agric. Food Chem.*, 2003, **51**, 2837–2853; (*b*) G. H. Müller, A. Lang, D. R. Seithel and H. Waldmann, *Chem.–Eur. J.*, 1998, **4**, 2513–2522; (*c*) A. J. M. Schoot-Uiterkamp and H. S. Mason, *Proc. Natl. Acad. Sci. USA*, 1973, **70**, 993–996; (*d*) K. Nishioka, *Eur. J. Biochem.*, 1978, **85**, 137–146.

14 J. A. Prezioso, M. W. Epperly, N. Wang and W. B. Bloomer, *Cancer Lett.*, 1992, **63**, 73–79.

15 Tyrosinase activity in the cell lines was determined according to the method of Pifferi *et al.*: P. G. Pifferi and L. Baldassari, *Anal. Biochem.*, 1973, **52**, 325.

16 Cytotoxicity assays were performed in triplicate using the Sulforhodamine B assay and are reported as the mean of the three values according to: P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107.

17 C. R. Rasmussen, J. F. Gardocki, J. N. Plampin, B. L. Twardzik, B. E. Reynolds, A. J. Molinari, N. Schwartz, W. W. Bennets, B. E. Price and J. Marakouski, *J. Med. Chem.*, 1978, **21**, 1044–1054.

18 J. K. Wilson, R. C. Illing, N. Subusinghe, J. B. Hoffmann, M. J. Rudolph, R. Soll, C. J. Molley, R. Bone, D. Green, T. Randall and M. Zhang, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 915–918.