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Towards the synthesis of coumarin derivatives as potential dual-action HIV-1 protease and reverse transcriptase inhibitors

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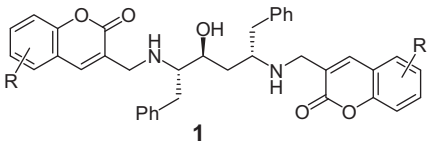
ABSTRACT

3-(Chloromethyl)coumarins, obtained via acid-catalysed cyclisation of salicylaldehyde-derived Baylis–Hillman adducts, have been treated with propargylamine; reaction of the resulting 3-alkynylmethylcoumarins with azidothymidine (AZT) in the presence of a Cu(I) catalyst has afforded a series of cycloaddition products for evaluation, in their own right, as potential dual-action HIV-1 protease and non-nucleoside reverse transcriptase inhibitors, and as scaffolds for further structural elaboration.

The development of novel therapeutics for the treatment of Acquired Immunodeficiency syndrome (AIDS) remains a major challenge. The genome of human immunodeficiency virus type 1 (HIV-1) encodes 15 distinct proteins,[1] including essential enzymes, such as reverse transcriptase (RT) and protease (PR), which have been identified as therapeutic targets. Problems associated with the development of drug-resistant viral variants[2–4] have led to the introduction of highly active antiretroviral therapy (HAART), typically involving concomitant treatment with a mixture of nucleoside and non-nucleoside HIV-1 RT inhibitors and an HIV-1 PR inhibitor.

As part of our research programme directed at the design, synthesis and evaluation of novel compounds as potential HIV-1 protease inhibitors, particular attention has been focused on the preparation of coumarin derivatives (2*H*-chromen-2-ones or 2*H*-1-benzopyran-2-ones), including ritonavir analogues (**1**).[5] Many coumarin-containing compounds are known to exhibit medicinally useful properties including anti-HIV, anti-cancer, anti-fungal, anti-bacterial and anti-coagulant activities.[6–11] Phenprocoumon (**2**), for example, is a competitive HIV-1 PR inhibitor and has served as a lead compound in the design of non-peptidic inhibitors,[12–17] while calanolide A has shown promise as an HIV-1 non-nucleoside RT inhibitor (NNRTI).[4] Azidothymidine (AZT), on the other hand, has found clinical use as a nucleoside analogue RT inhibitor. In continuation of research directed at: (i) exploring applications of Baylis–Hillman methodology in the construction of complex molecular targets and (ii) the development of novel HIV-1 PR inhibitors,[5,12] we now report the use of Baylis–Hillman-derived 3-(chloromethyl)coumarins to prepare a range of compounds, which contain *both* coumarin *and* triazolothymidine moieties, as potential dual-action HIV-1 PR and RT inhibitors, and as scaffolds for further structural elaboration.



DABCO-catalysed Baylis–Hillman reactions of the salicylaldehydes (**3a–e**; Scheme 1) with *t*-butyl acrylate (**4**) gave the corresponding adducts **5a–e**, cyclisation of which with HCl–AcOH afforded the 3-(chloromethyl)coumarins **6a–e**.[18] These compounds (**6a–e**) have been shown to undergo preferential S_N attack, by nitrogen nucleophiles, at the exocyclic 3-methylene centre[19] rather than attack at C-4 involving an S_N (or conjugate addition–elimination) mechanism. Reaction of each of the 3-(chloromethyl)coumarins **6a–e** with propargylamine (**7**) in THF also followed the direct S_N pathway to give the corresponding substitution products **8a–e** in yields ranging from 52% to 80% (Table 1). These alkynylated products were then subjected to Cu(I)-catalysed cycloaddition with azidothymidine (AZT; **9**) in the presence copper(II) sulfate and ascorbic acid—the latter to effect the reduction of Cu(II) to Cu(I). The resulting triazole derivatives **10a–e**, which were isolated in yields ranging from 64% to 76%. (Table 1) contain *both* a coumarin *and* an AZT moiety. Interestingly, the 1,2,3-triazole moiety has been shown to be an effective replacement for the peptide group in HIV-1 protease inhibitors, facilitating binding by hydrogen-bonding to structural water.[20]

A preliminary Saturation Transfer Difference (STD) NMR screening experiment, using a mixture of compounds **10a–e** as potential ligands, indicated that some of these compounds do, in fact, bind to HIV-1 subtype C PR (the subtype endemic to Sub-Saharan Africa).

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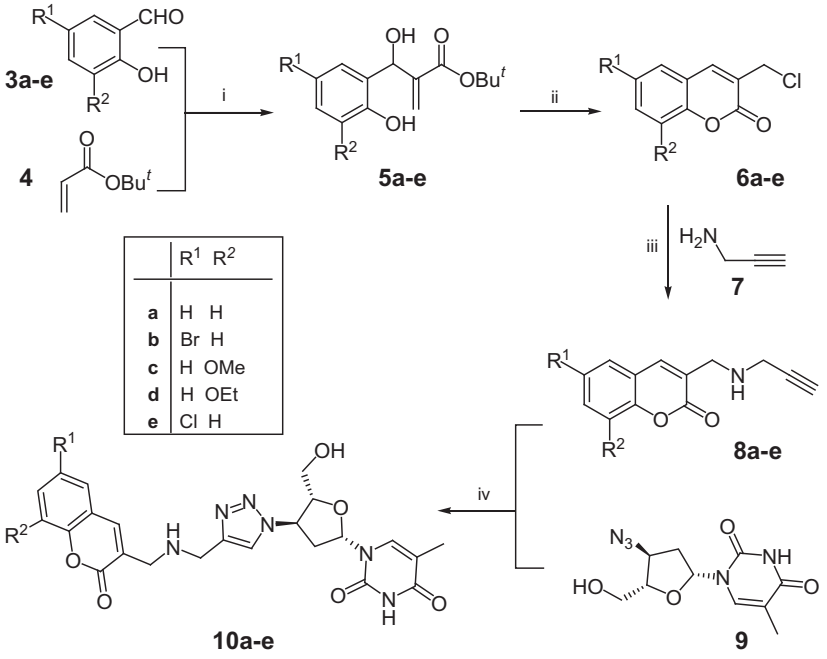

Scheme 1. Reagents and conditions: (i) DABCO, $CHCl_3$, rt; (ii) HCl, AcOH, reflux; (iii) propargylamine (**7**), THF; (vi) sodium ascorbate, $Cu_2SO_4{\cdot}5H_2O$, H_2O–THF.

Table 1
Yields obtained for the transformations **6** → **8** → **10** (Scheme 1)

Substrate	R^1	R^2	Yield of **8** (%)	Yield of **10** (%)
6a	H	H	80	65
6b	Br	H	52	76
6c	H	OMe	65	75
6d	H	OEt	70	70
6e	Cl	H	70	64

Computer modelling studies were also used to explore the in silico docking of these compounds in the HIV-1 PR and NNRT enzyme receptor sites.

Docking of the parent system **10a** into the receptor site of HIV-1 PR[21] revealed potential hydrogen-bonding interactions between proximate protein residues and *both* the coumarin *and* the AZT moieties (Fig. 1). Solvent (H_2O)-mediated hydrogen-bonding between the triazole moiety and the receptor-site residue Gly-27 would serve to enhance binding. Moreover, as is evident in Figure 2, there is close correspondence between the docked conformation of compound **10a** and the X-ray crystal structure[21] of the known inhibitor, ritonavir, in the active site of HIV-1 PR. While the ligands 10a–**e** lack a suitably located lipophilic group to occupy the P1 pocket[22]—a feature of most active PR inhibitors—the synthetic methodology reported herein is currently being extended to permit early attachment of such a group.

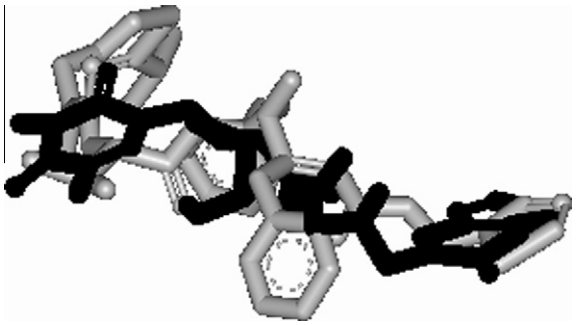

Figure 2. Overlay of the docked conformations of compound **10a** (in black) and ritonavir (in grey) in the HIV-1 PR active site, the latter ligand as it is found in the crystal structure of HIV-1 (PDB 1HXW).[21]

The *non-nucleoside binding pocket* of HIV-1 RT appears to be quite forgiving in the variety of structural motifs it can accommodate.[23] Docking of ligands **10a–e** into this pocket reveals that: (i) they exhibit potential hydrogen-bonding interactions with amino acid residues that line the pocket (Fig. 3) and (ii) the coumarin moiety occupies the same cavity as efavirenz[24]—observations which

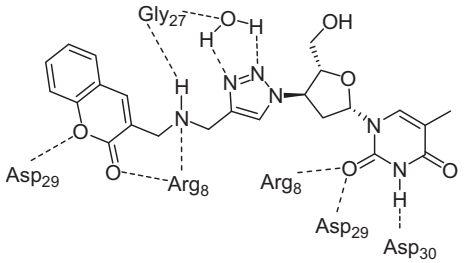

Figure 1. Schematic representation of potential hydrogen-bonding interactions (<4 Å) between compound **10a** and residues in the receptor cavity of HIV-1 protease (PDB 1HXW)[21] as determined using AUTODOCK 4.2.

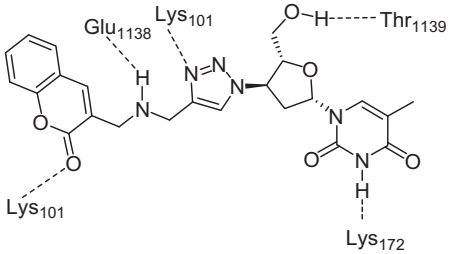

Figure 3. Schematic representation of hydrogen-bonding interactions (<4 Å) between AZT-coumarin product **10a** and residues in the non-nucleoside pocket of HIV-1 reverse transcriptase (PDB 1IKW)[24] as determined using AUTODOCK 4.2.

indicate the possibility of their acting as non-nucleoside RT inhibitors (NNRTIs). The nucleoside analogue (NRTI) pro-drug AZT **9**, however, is incorporated, following in vivo triphosphorylation,[25] into the transcribed DNA being generated in the *RT active site*. It has been suggested that the azido group in AZT **9** may interfere sterically with the phosphorylation process,[26] and it is possible that similar steric constraints may preclude the ligands **10a–e** from acting as NRTI pro-drugs. However, prior in vitro phosphorylation [as effected in the nucleotide analogue (NtRTI) tenofovir[27]] might well permit phosphorylated derivatives of compounds **10a–e** to act at the *RT active site*. Ongoing studies are focusing on: functional elaboration of the coumarin-AZT scaffold in compounds of type **10**; enzyme–inhibition assays of the synthetic ligands; and use of the resulting SAR data in the development of novel, dual-action HIV-1 PR and RT inhibitors.

In conclusion, the 3-(chloromethyl)coumarins **6a–e**, obtained via Baylis–Hillman methodology, have been successfully reacted with propargylamine (**7**) to afford compounds **8a–e**, which have been used, in turn, as substrates for 'Click Chemistry' cycloaddition with azidothymidine (AZT).[28] This four-step sequence, using readily available reactants, provides convenient access to the structurally complex coumarin-AZT conjugates **10a–e**, STD NMR binding- and in silico modelling studies of which encourage exploration of their potential as dual-action HIV-1 PR/RT inhibitors.

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28. *General procedures for the synthesis and characterisation of compounds* **8a–e** *and* **10a–e** are as follows.

3-[(2-Propynylamino)methyl]coumarin (**8a**). Propargylamine (**7**) (0.6 mL, 8.8 mmol) was added to a solution of 3-(chloro-methyl)coumarin (**5a**) (0.80 g, 4.1 mmol) in dry THF (5 mL). After stirring at room temperature for 48 h, the reaction mixture was concentrated in vacuo and purified using flash chromatography [on silica gel; elution with hexane/EtOAc (3:2)] to afford *3-[(2-propynylamino)methyl]coumarin* (**8a**) as an off-white solid (0.7 g, 80%), mp 107–108 °C [HRMS: *m*/*z* calculated for $C_{13}H_{12}NO_2$ (MH^+): 214.0868. Found: 214.0860]; ν_{max}/cm^{-1} (neat) 1694 (C=O); δ_H (400 MHz, $CDCl_3$) 1.78 (1H, s, NH), 2.24 (1H, t, *J* = 2.4 Hz, C≡CH), 3.48 (2H, d, *J* = 2.4 Hz, CH_2–C≡CH), 3.82 (2H, s, CH_2NH), 7.26 (1H, m, ArH), 7.32 (1H, d, *J* = 8.1 Hz, ArH), 7.48 (2H, m, ArH) and 7.73 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 38.1 and 48.2 (CH_2N), 72.4 and 82.0 (C≡CH), 117.0, 119.6, 124.9, 127.3, 128.1, 131.6, 139.9 and 153.7 (Ar–C) and 161.8 (C=O).

6-Bromo-3-[(2-propynylamino)methyl]coumarin (**8b**) (0.55 g, 52%) as an off-white solid, mp 136–137 °C [HRMS: *m*/*z* calculated for $C_{13}H_{11}BrNO_2$ (MH^+) 291.9973. Found: 291.9966]; ν_{max}/cm^{-1} (neat) 1712 (C=O); δ_H (400 MHz, $CDCl_3$) 1.76 (1H, br s, NH), 2.23 (1H, s, C≡CH), 3.46 (2H, s, CH_2–C≡CH), 3.80 (2H, s, CH_2NH), 7.18 (1H, d, *J* = 8.7 Hz, ArH), 7.54 (1H, d, *J* = 8.8 Hz, ArH), 7.58 (1H, s, ArH) and 7.64 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 38.1 and 48.0 (CH_2N), 72.5 and 81.9 (C≡CH), 117.4, 118.6, 121.2, 128.8, 130.3, 134.2, 138.3 and 152.5 (Ar–C) and 161.0 (C=O).

8-Methoxy-3-[(2-propynylamino)methyl]coumarin (**8c**) (0.70 g, 65%) as a pale yellow solid, mp 89–91 °C [HRMS: *m*/*z* calculated for $C_{14}H_{14}NO_3$ (MH^+) 244.0974. Found: 244.0973]; ν_{max}/cm^{-1} (neat) 1702 (C=O); δ_H (400 MHz, $CDCl_3$) 1.83 (1H, br s, NH), 2.21 (1H, s, C≡CH), 3.45 (2H, s, CH_2–C≡CH), 3.78 (2H, s, CH_2NH), 3.91 (3H, s, OCH_3), 7.01 (2H, d, *J* = 7.9 Hz, ArH), 7.15 (1H, t, *J* = 7.9 Hz, ArH) and 7.67 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 38.0 and 48.2 (CH_2N), 56.7 (OCH_3), 72.4 and 82.0 (C≡CH), 113.5, 119.5, 120.3, 124.7, 127.5, 139.9, 143.3 and 147.5 (Ar–C) and 161.2 (C=O).

8-Methoxy-3-[(2-propynylamino)methyl]coumarin (**8d**) (0.75 g, 70%) as a pale yellow solid, mp 120–121 °C [HRMS: *m*/*z* calculated for $C_{15}H_{16}NO_3$ (MH^+) 258.1130. Found: 258.1124]; ν_{max}/cm^{-1} (neat) 1699 (C=O); δ_H (400 MHz, $CDCl_3$) 1.46 (3H, t, *J* = 7.0 Hz, OCH_2CH_3), 1.85 (1H, br s, NH), 2.23 (1H, t, *J* = 2.4 Hz, C≡CH), 3.46 (2H, d, *J* = 2.4 Hz, CH_2–C≡CH), 3.79 (2H, s, CH_2NH), 4.15 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 7.01 (2H, 2 × overlapping d, ArH), 7.15 (1H, dd, ArH) and 7.68 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 15.2 (CH_3), 38.0 and 48.2 (CH_2N), 65.4 (CH_2O), 72.4 and 82.0 (C≡CH), 114.8, 119.5, 120.4, 124.7, 127.4, 140.1, 143.6 and 146.8 (Ar–C) and 161.4 (C=O).

6-Chloro-3-[(prop-2-ynylamino)methyl]coumarin (**8e**) (0.76 g, 70%) as a pale yellow solid, mp 116–117 °C [HRMS: *m*/*z* calculated for $C_{13}H_{11}ClNO_2$ (MH^+) 248.0478. Found: 248.0460]; ν_{max}/cm^{-1} (neat) 1702 (C=O); δ_H (400 MHz, $CDCl_3$) 1.76 (1H, br s, NH), 2.27 (1H, t, *J* = 2.4 Hz, C≡CH), 3.50 (2H, d, *J* = 2.4 Hz, CH_2–C≡CH), 3.84 (2H, s, CH_2NH), 7.28 (1H, d, *J* = 8.5 Hz, ArH), 7.45 (2H, m, ArH) and 7.69 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 38.1 and 48.0 (CH_2N), 72.6 and 81.8 (C≡CH), 118.4, 120.7, 127.3, 128.7, 130.1, 131.5, 138.5 and 152.0 (Ar–C) and 161.2 (C=O).

4-{[(2H-1-Benzopyran-2-one-3-yl)methylamino]methyl}-1-[(2S,3R,5R)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-1-yl)-2-(hydroxymethyl)tetrahydrofuran-3-yl]-1H-1,2,3-triazole (**10a**). 3′-Azido-3′-deoxythymidine (**9**) (0.61 g, 2.3 mmol) was dissolved in H_2O/THF (1:1; 12 mL) and 3-[(2-propynylamino)methyl]coumarin (**8a**) (0.49 g, 2.3 mmol), sodium ascorbate (96 mg, 0.49 mmol) and $Cu_2SO_4{\cdot}5H_2O$ (17 mg, 68 μmol) were added to the solution. After stirring for 24 h at room temperature, the mixture was extracted with CH_2Cl_2 (2 × 100 mL) and washed sequentially with H_2O (50 mL) and brine (30 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The crude material was purified by flash chromatography [on silica gel; elution with EtOAc and then with MeOH/EtOAc (2:3)] to afford the *coumarin-AZT conjugate* (**10a**) (0.72 g, 65%) as a light brown solid, mp 125–127 °C [HRMS: *m*/*z* calculated for $C_{23}H_{25}N_6O_6$ (MH^+) 481.1836. Found: 481.1823]; ν_{max}/cm^{-1} (neat) 3291 (OH) and 1684 (C=O); δ_H (400 MHz, methanol-d_4) 1.90 (3H, s, CH_3), 2.70 and 2.85 (2H, m, CH_2CHN), 3.72 and 3.95 (4H, s, 2 × NCH_2), 3.76 (1H, dd, *J* = 3.2, 12.3 Hz, CH_aOH), 3.89 (1H, dd, *J* = 2.9, 12.2 Hz, CH_bOH), 4.30–4.34 (1H, m, $OCHCH_2N$), 5.37–5.42 (1H, m, $OCHCH_2OH$), 6.47 (1H, t, *J* = 6.5 Hz, OCHN), 7.32–7.35 (2H, m, ArH), 7.54–7.59 (1H, m, ArH), 7.63 (1H, dd, *J* = 1.3, 7.9 Hz, ArH), 7.90 (1H, s, ArH), 7.93 (1H, s, ArH) and 8.05 (1H, s, ArH); δ_C (100 MHz, methanol-d_4) 10.0 (CH_3), 36.5 (CH_2CHN), 41.8 and 46.4 (CH_2N), 58.5 (CHN), 59.6 (CH_2O), 83.9

($HOCH_2CHO$), 84.2 (NCHO), 109.2, 114.8, 118.2, 121.6, 123.3, 125.0, 126.8, 130.1, 135.7, 139.3, 144.9, 150.0, 152.1, 160.6 and 164.2 (Ar–C and C=O).

Coumarin-AZT conjugate (**10b**) (0.51 g, 76%) as an off-white solid, mp 129–130 °C [HRMS: *m*/*z* calculated for $C_{23}H_{24}BrN_6O_6$ (MH^+) 559.0941. Found: 559.0922]; ν_{max}/cm^{-1} (neat) 3367 (OH) and 1674 (C=O); δ_H (400 MHz, DMSO-d_6) 1.82 (3H, s, CH_3), 2.60–2.72 (2H, m, CH_2CHN), 3.59–3.72 (4H, overlapping s and dd, CH_2OH and $NCH_2CC{=}O$), 3.84 (2H, s, NCH_2CN), 4.16–4.22 (1H, m, OCH*CH*N), 5.33–5.39 (1H, m, $OCHCH_2OH$), 6.42 (1H, t, *J* = 6.5 Hz, OCHN), 7.38 (1H, d, *J* = 8.8 Hz, ArH), 7.72 (1H, d, *J* = 8.8 Hz, ArH), 7.83 (1H, s, ArH), 7.98 (2H, overlapping s, ArH), 8.21 (1H, s, ArH) and 11.38 (1H, br s, NHC=O); δ_C (100 MHz, DMSO-d_6) 13.2 (CH_3), 38.0 (CH_2CHN), 44.4 and 48.0 (CH_2N), 60.0 (CHN), 61.6 (CH_2O), 84.8 ($HOCH_2CHO$), 85.4 (NCHO), 110.5, 117.0, 119.1, 122.0, 123.4, 129.5, 131.0, 134.2, 137.1, 138.1, 147.3, 151.4, 152.4, 160.9 and 164.7 (Ar–C and C=O).

Coumarin-AZT conjugate (**10c**) (0.45 g, 75%) as a yellow solid, mp 126–127 °C [HRMS: *m*/*z* calculated for $C_{24}H_{27}N_6O_7$ (MH^+) 511.1941. Found: 511.1940]; ν_{max}/cm^{-1} (neat) 3291 (OH) and 1671 (C=O); δ_H (400 MHz, DMSO-d_6) 1.81 (3H, s, CH_3), 2.58–2.75 (2H, m, CH_2CHN), 3.59–3.71 (4H, overlapping s and dd, CH_2OH and $NCH_2CC{=}O$), 3.83 (2H, s, NCH_2CN), 3.90 (3H, s, OMe), 4.16–4.21 (1H, m, OCH*CH*N), 5.27–5.37 (2H, m, $OCHCH_2OH$ and OH), 6.41 (1H, t, *J* = 5.6 Hz, OCHN), 7.23–7.32 (3H, m, ArH), 7.81 (1H, s, ArH), 7.95 (1H, s, ArH), 8.18 (1H, s, ArH) and 11.36 (1H, br s, NHC=O); δ_C (100 MHz, DMSO-d_6) 13.1 (CH_3), 38.0 (CH_2CHN), 44.4 and 48.0 (CH_2N), 56.9 (OCH_3), 59.9 (CHN), 61.6 (CH_2O), 84.7 ($HOCH_2CHO$), 85.4 (NCHO), 110.5, 114.2, 120.1, 120.6, 123.3, 125.3, 128.3, 137.1, 139.7, 142.7, 147.3, 147.4, 151.3, 161.1 and 164.7 (Ar–C and C=O).

Coumarin-AZT conjugate (**10d**) (0.44 g, 70%) as a yellow solid, mp 114–116 °C [HRMS: *m*/*z* calculated for $C_{25}H_{29}N_6O_7$ (MH^+) 525.2079. Found: 525.2077]; ν_{max}/cm^{-1} (neat) 3306 (OH) and 1679 (C=O); δ_H (400 MHz, DMSO-d_6) 1.40 (3H, t, *J* = 6.9 Hz, OCH_2CH_3), 1.81 (3H, s, CH_3), 2.58–2.73 (2H, m, CH_2CHN), 3.60–3.71 (4H, overlapping s and dd, CH_2OH and $NCH_2CC{=}O$), 3.83 (2H, s, NCH_2CN), 4.14–4.20 (3H, overlapping m and q, OCH*CH*N and OCH_2CH_3), 5.31–5.38 (2H, m, $OCHCH_2OH$ and OH), 6.41 (1H, t, *J* = 6.5 Hz, OCHN), 7.23–7.28 (3H, m, ArH), 7.82 (1H, s, ArH), 7.94 (1H, s, ArH), 8.19 (1H, s, ArH) and 11.43 (1H, br s, NHC=O); δ_C (100 MHz, DMSO-d_6) 13.1 and 15.5 (2 × CH_3), 38.0 (CH_2CHN), 44.5 and 48.0 (CH_2N), 59.9 (CHN), 61.6 and 65.2 (2 × CH_2O), 84.8 ($HOCH_2CHO$), 85.4 (NCHO), 110.5, 115.1, 120.1, 120.6, 123.3, 125.3, 128.2, 137.1, 139.8, 142.8, 146.5, 147.4, 151.3, 161.2 and 164.6 (Ar–C and C=O).

Coumarin-AZT conjugate (**10e**) (0.40 g, 64%) as an off-white solid, mp 103–105 °C [HRMS: *m*/*z* calculated for $C_{23}H_{24}ClN_6O_6$ (MH^+) 515.1446. Found: 515.1435]; ν_{max}/cm^{-1} (neat) 3260 (OH) and 1679 (C=O); δ_H (400 MHz, DMSO-d_6) 1.80 (3H, s, CH_3), 2.58–2.74 (2H, m, CH_2CHN), 3.60–3.71 (4H, overlapping s and dd, CH_2OH and $NCH_2CC{=}O$), 3.82 (2H, s, NCH_2CN), 4.16 (1H, dd, *J* = 3.5, 8.2 Hz, OCH*CH*N), 5.37–5.43 (1H, m, $OCHCH_2OH$), 5.46 (1H, t, *J* = 4.60 Hz, OH), 6.41 (1H, t, *J* = 6.6 Hz, OCHN), 7.43 (1H, d, *J* = 8.8 Hz, ArH), 7.59 (1H, dd, *J* = 2.4, 8.8 Hz, ArH), 7.86 (2H, d, *J* = 2.6 Hz, ArH), 7.98 (1H, s, ArH), 8.23 (1H, s, ArH) and 11.34 (1H, br s, NHC=O); δ_C (100 MHz, DMSO-d_6) 13.1 (CH_3), 38.0 (CH_2CHN), 44.4 and 48.0 (CH_2N), 60.0 (CHN), 61.6 (CH_2O), 84.8 ($HOCH_2CHO$), 85.4 (NCHO), 110.5, 118.8, 121.5, 123.5, 128.0, 129.1, 129.4, 131.5, 137.1, 138.3, 147.2, 151.3, 151.9, 160.9 and 164.6 (Ar–C and C=O).

Saturation Transfer Difference (STD) NMR studies using HIV-1 subtype C protease.[29] The over-expression and purification of the wild-type PR has been described previously.[30] Briefly, plasmid DNA encoding the PR was transformed into *Escherichia coli* BL21 (DE3) pLysS cells. The protease was over-expressed and purified from inclusion bodies. The inclusion bodies were dissolved in 8 M urea and refolded by extensive dialysis into protease storage buffer (10 mM sodium acetate, 1 mM NaCl and 1 mM DTT, pH 5.0). The protease was estimated to be ⩾99% pure with an apparent oligomeric molecular mass of 22 kDa. The protein concentration was determined spectrophotometrically using an extinction coefficient $E_{1\%}$ of 11.8 at 280 nm. A buffered, aqueous solution of HIV-1 subtype C protease (0.35 mL) was freeze-dried and then re-constituted in an equal volume of D_2O. The five ligands **10a**–**e** (2 mg of each) were dissolved together in D_2O (0.25 mL) and the resulting solution was added to the protein solution. Magnetic resonance saturation of the protein was achieved using a train of four Guassian bell-shaped pulses separated by a 1 ms delay at a power level of 40 dB. The frequency of the saturating on-resonance pulse was at 1.64 ppm and the off-resonance pulse at 20 ppm. No spin-lock filter was used.

Computer modelling studies. The structures of the coumarin derivative **10a** and the protein were prepared using Discovery Studio Visualiser.[31] The protease and reverse transcriptase structures were obtained using the HIV-1-PR and -RT coordinates taken from the RCSB Protein Data Base (PDB entry code 1HXW[21] and 1IKW,[24] respectively); all water molecules were removed from the original PDB file, hydrogen atoms were added and each atom assigned an AUTODOCK Type using AUTODOCK Tools (ADT). The AUTODOCK 4.2 programme[32] was used to explore the binding mode of compound **10a** when docked in the protease binding site. For docking calculations, Gasteiger partial charges[33] were assigned to the coumarin derivatives and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking.

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