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Improved Photocontrol of α-Chymotrypsin Activity: Peptidomimetic Trifluoromethylketone Photoswitch Enzyme Inhibitors

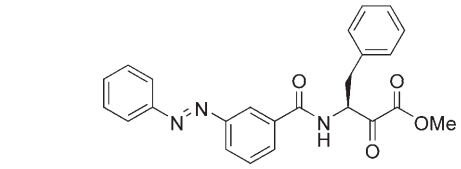
David Pearson,[b] Nathan Alexander,[b] and Andrew D. Abell*[a, b]

Abstract: A series of peptidomimetic trifluoromethylketones containing the photoisomerisable azobenzene group have been synthesised, photoisomerised and assayed against the serine protease α-chymotrypsin. All are inhibitors of the enzyme and exhibit up to a fivefold increase in activity on UV irradiation. Visible irradiation returns activity to close to that of the original sample. These results suggest the trifluoromethylketone group to be the best electrophilic enzyme binding group for use in photoswitch inhibitors of α-chymotrypsin.

Keywords: azo compounds · enzymes · molecular devices · peptidomimetics · photochromism

Introduction

A number of important biological processes can be optically controlled by incorporation of photochromic molecular switches into biomolecules or biological ligands.[1–5] This biological photoswitching has potential applications in advanced technologies such as reversible biosensors, photoactivated medicines and molecular devices. We[1,2] and others[4] have developed a range of photoswitch inhibitors of the serine protease α-chymotrypsin containing the photochromic azobenzene group. UV-visible irradiation of these inhibitors induces *E*–*Z* photoisomerisation of the azobenzene group, which results in a reversible change in inhibitor structure and hence enzyme affinity. For example, previously reported photoswitch inhibitor α-ketoester **1** exhibits greater activity as the *Z*-enriched state that results from UV irradiation (inhibition constant, K_i, of 40 nM, compared with 80 nM for the *E*-enriched state).[1] Boronate ester **2** was prepared in an attempt to increase the extent of photoswitching, but gave only semireversible photoswitching due to decomposition on irradiation with UV-visible light.[2]



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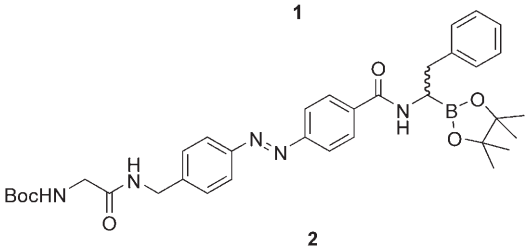

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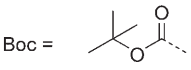

The present study reports synthesis, enzyme assay and photoisomerisation studies of trifluoromethylketone-based photoswitch enzyme inhibitors **3**–**9**. Compounds **3** and **4** are based on previously reported inhibitors **1** and **2**, respectively, but with an alternative trifluoromethylketone inhibitory group. This "warhead" is known to give potent inhibitors of α-chymotrypsin, but is as yet unstudied with regards to photoswitching, aside from a preliminary communication.[6] Compounds **5**–**9** contain a sulfonamide linker, rather than an amide, to provide improved stability towards enzyme-catalysed hydrolysis. In addition, a halide substituent was in-

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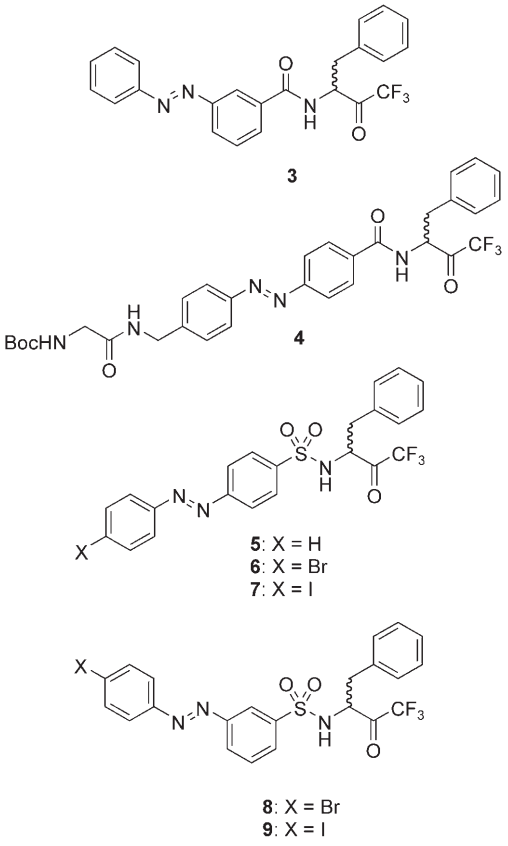
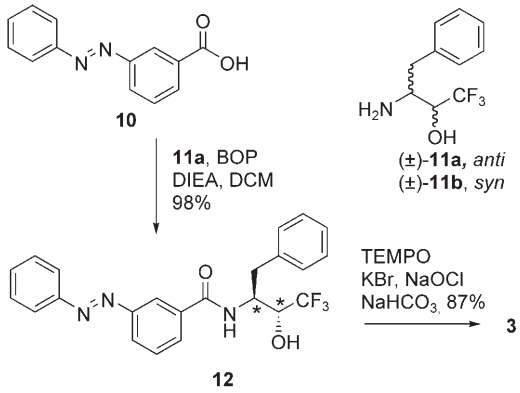
cluded in compounds **6–9** to increase the effective size of the photoswitch group and to provide a point for further structural elaboration by means of Pd-catalysed coupling reactions. It was anticipated that an increase in effective size of the photoswitch group would enhance enzyme photoswitching, due to a greater difference in structure and shape of the *E* and *Z* isomers. Furthermore, it was of interest to investigate the properties of photoswitch inhibitors containing 3′- and 4′-substituted azobenzene groups because related linker groups are required for the surface attachment of photoswitch inhibitors.[6] The substituents and substitution positions of these compounds were varied to investigate relationships between structure, activity and photoswitching, particularly the effect of molecular structure on the relative change in activity on photoisomerisation. Reported herein is the synthesis and assay of compounds **3–9** against α-chymotrypsin in the following forms: as the stable *E* isomer, as a *Z*-enriched photostationary state (PSS) resulting from UV irradiation and as an *E*-enriched PSS resulting from visible irradiation. Racemic samples of inhibitor are used for ease of synthesis and, given that one enantiomer is invariably significantly more active, to allow comparisons to be made.[2]

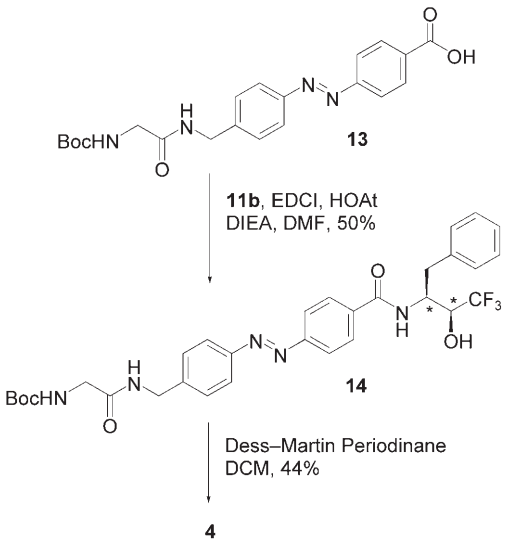


Results and Discussion

Trifluoromethylketones **3** and **4** were synthesised as racemic mixtures in a similar manner to the known inhibitors **1** and **2**, respectively. The monosubstituted azobenzene **3** was prepared by coupling carboxylic acid **10**[7,8] to racemic **11a**[9] using (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) to give **12**, followed by oxidation with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) to give **3** in a yield of 87% (Scheme 1). The disubstituted azobenzene **4** was prepared by coupling carboxylic acid **13**[2] to racemic **11b**[9] using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) to give **14**, followed by oxidation with Dess–Martin periodinane to give **4** (Scheme 2).



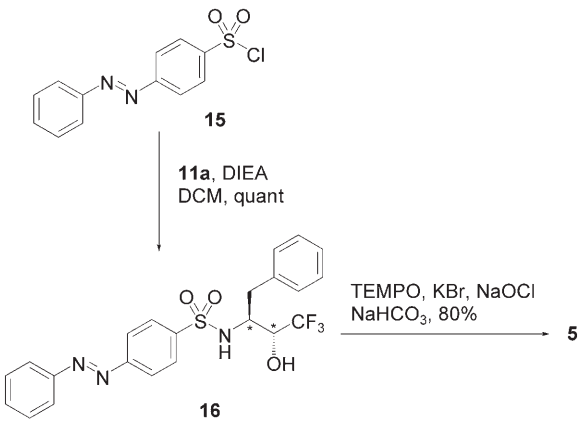
Scheme 1. Synthesis of **3** (DIEA: *N*,*N*-diisopropylethylamine).



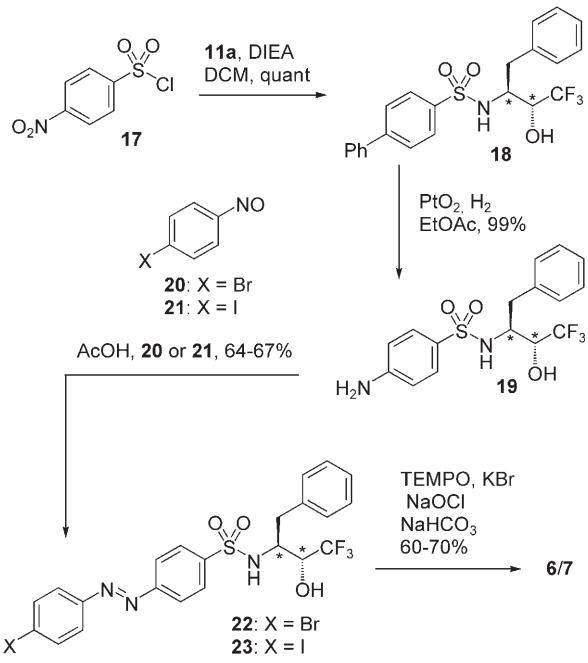
Scheme 2. Synthesis of **4**.

Racemic sulfonamide **5** was prepared by coupling azobenzenesulfonyl chloride **15** to racemic **11a** to give sulfonamide **16**, followed by oxidation using TEMPO to give ketone **5** in a yield of 80% (Scheme 3).

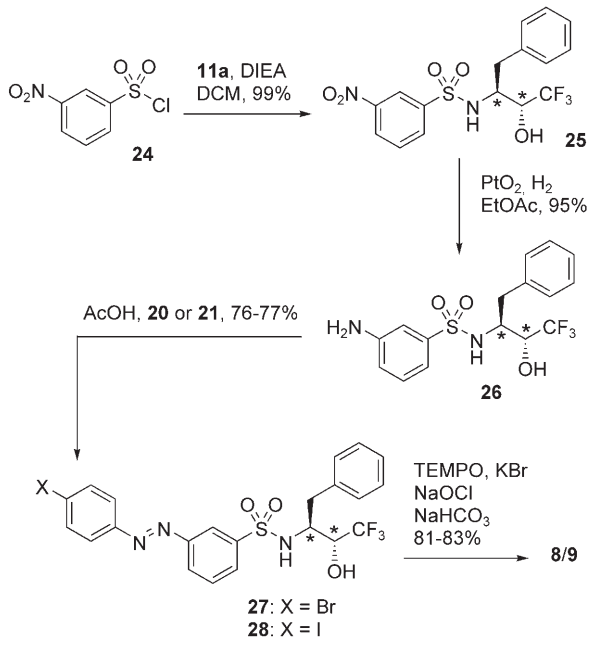
The 4-sulfonyl azobenzenes **6** and **7** (Scheme 4) and 3-sulfonyl azobenzenes **8** and **9** (Scheme 5) were synthesised using an alternative approach. This involved coupling of racemic **11a** to an aryl amine, followed by condensation with a



Scheme 3. Synthesis of **5**.



Scheme 4. Synthesis of **6** and **7**.



Scheme 5. Synthesis of **8** and **9**.

Table 1. *E*/*Z* compositions of azobenzenes **3**–**9** before and after irradiations.

Cmpd	% *Z* isomer before irradiation	% *Z* isomer after UV irradiation	% *Z* isomer after visible irradiation	Decrease in *Z* isomer after visible irradiation
3	<5	78	18	4.3-fold
4	<5	93	16	5.8-fold
5	<5	68	17	4.0-fold
6	7	77	14	5.5-fold
7	11	90	20	4.5-fold
8	8	89	18	4.9-fold
9	<5	86	12	7.2-fold

nitrosobenzene to form the azobenzene moiety and oxidation to give the trifluoromethylketone. In particular, compounds **6** and **7** were prepared by coupling of 4-nitrosulfonyl chloride **17** to **11a** to give sulfonamide **18**, catalytic reduction to **19**, condensation with nitrosobenzenes **20**[10,11] and **21**[12,13] to give the azobenzenes **22** and **23**, respectively, and finally oxidation using TEMPO to give ketones **6** and **7**, respectively. Compounds **8** and **9** were similarly prepared, using 3-nitrosulfonyl chloride **24** as starting material instead of **17**. All trifluoromethylketones were characterised as hydrates by using NMR spectroscopy in acetone containing 5% D_2O (see the Experimental Section for details).

Next, azobenzenes **3**–**9** were photoisomerised by UV-visible irradiation. A solution of each compound was irradiated with UV light ($\lambda = 320$–380 nm) to give the *Z*-enriched PSS (see Table 1 for *E*/*Z* compositions). The *Z*-enriched solutions were then irradiated with visible light ($\lambda > 360$ nm) to give the *E*-enriched PSS (see Table 1). ^{1}H NMR spectroscopy was used to determine the *E*/*Z* compositions of samples obtained from these irradiations. In all cases, the azobenzenes were initially (after synthesis and storage in the dark) predominantly present as the *E* isomer. After UV irradiation, compositions ranging from 68 to 93% *Z* isomer were obtained (see Table 1). Visible irradiation gave PSS compositions of 12 to 20% *Z* isomer.

The practical application of azobenzene photoswitches of the type presented herein requires consideration of two different approaches to photoswitching:

1) UV photoisomerisation of a purified *E* isomer to a *Z*-enriched PSS. This approach normally produces the largest possible increase in the proportion of *Z* isomer, and hence the largest change of activity on photoswitching, since the starting *E* isomer can be obtained in a relatively pure state (often <5% *Z* by chromatography or thermal isomerisation in the dark). However, this approach is not fully reversible over a short time period because

visible irradiation does not usually fully reconvert the *Z* isomer to the *E* isomer. Over a longer timescale, the *E* isomer can be thermally regenerated and can undergo photoswitching of this type again.

2) Reversible photoisomerisation between the UV and visible PSSs. Although this approach does not modulate activity as fully as irradiation of the purified *E* isomer, it does provide potential for fast reversible photoswitching.

The second approach is likely to be the most useful in practical switching applications. We describe the development of the method used herein with an initial goal of defining those factors that maximise the relative difference (in *E*/*Z* composition or enzyme activity) between the *E*- and *Z*-enriched PSSs of the inhibitors, something that is currently lacking in the literature. Photoisomerisation of the inhibitors is carried out prior to addition of the protease given that in situ photoswitching is known to result in poorer switching and protease instability.[4]

Of the compounds tested (Table 1), azobenzene **9** gave the best photoswitching between UV and visible PSSs, with 7.2 times more *Z* isomer obtained after UV irradiation. Compounds **4**, **7** and **8** gave the largest proportion of *Z* isomer after UV irradiation, but retained the largest proportion of *Z* isomer after visible irradiation (see Table 1). Either the *Z* isomers of these compounds are (relatively) thermodynamically stable, or the $\pi\rightarrow\pi^*$ electronic transition involved in $E\rightarrow Z$ photoisomerisation[14] is relatively favoured at both the UV and visible wavelengths used in this study. It is unclear why this is the case, since the closely related compounds **6** and **9** do not similarly favour the *Z* isomer.

Separate aliquots from each of three solutions (the initial solution and the *E*- and *Z*-enriched PSSs) of racemic **3**–**9** were assayed for inhibition of α-chymotrypsin. All compounds were inhibitors of α-chymotrypsin with micromolar inhibition constants (see IC_{50} values in Table 2), and all showed reversible photoswitching of enzyme activity. All compounds were observed to initially exhibit weak inhibition, which subsequently increased over several minutes to reach a higher steady-state level. This 'slow-tight' binding has been previously observed for trifluoromethylketone inhibitors, and is considered to occur due to an equilibrium between the active ketone and a less-active hydrated form.[15] The IC_{50} values were calculated using the final steady-state rates of enzymatic reaction (after 8 min of incubation of enzyme, substrate and inhibitor) since the initial inhibition was weak. In all cases the *Z* isomer is the more potent inhibitor, as has been observed previously for α-ketoester inhibitors of α-chymotrypsin.[1,16] This differs from boronic acid based inhibitors of α-chymotrypsin and aldehyde inhibitors of calpain, which usually exhibit greater activity as the *E* isomer.[2,4,17]

The solubility of inhibitors **3**, **6**, **7** and **9** in water was insufficient to obtain an accurate IC_{50} value for the least active composition(s) before irradiation; however, an IC_{50} could be obtained for the active *Z*-enriched sample of each compound and for **3** and **7** after visible irradiation, which exhibit 50% inhibition below the solubility limit. This solubility problem limits to some extent the amount of enzyme photoswitching that can be measured by assay for these compounds. For example, inhibitor **9**, which is likely to give good enzyme photoswitching based on its excellent photoisomerisation and its structural similarity to good photoswitch inhibitors **6**–**8**, can only be accurately stated as having >2.2-fold change in activity on photoisomerisation.

The best enzyme photoswitch is **6**, which exhibits a >4.7-fold change in activity after UV or visible irradiation. Assuming that the enzyme inhibition occurs by formation of a 1:1 enzyme–inhibitor complex (as expected for these electrophilic transition-state mimics[18]), the observed change in the IC_{50} value of such inhibitors on photoisomerisation is limited to the relative change in the amount of the active *Z* isomer. In cases in which the *E* isomer also inhibits the enzyme, the actual change in the IC_{50} value will be less than this maximum. Therefore, the maximum possible change in activity for **6** is 5.5 times (see Table 1). The observed change in activity (>4.7-fold) is close to this limit, which shows the *Z* isomer to be significantly more active than the *E* isomer. Therefore, the major limitation to enzyme photoswitching of this compound is the incomplete isomerisation process between the *E* and *Z* forms.

Trifluoromethylketone **3** exhibited a 3.5-fold change in enzyme activity between its *E*- and *Z*-enriched PSSs. In comparison, 2-fold photoswitching was reported for **1**, which contains the same skeleton, but a different warhead group.[1] Trifluoromethylketone **4** exhibited a 2.2-fold change in activity between the *E*- and *Z*-enriched PSSs. This represents a significant improvement on the related **2**, which is reported to exhibit a less than 1.5-fold change in activity on photoswitching and partially decomposed on irradiation.[2] In addition, the trifluoromethylketones presented herein generally exhibit improved photoswitching (up to 5-fold changes in IC_{50} value) compared with α-ketoester and boronate ester based inhibitors of α-chymotrypsin, which exhibit at best only 3-fold changes in IC_{50} values on photoswitching.[1,2] These favourable comparisons suggest the trifluoromethylketone to be a superior warhead

Table 2. α-Chymotrypsin inhibition constants (IC_{50}) for compounds **3**–**9**.

Cmpd	IC_{50} before irradiation [μM]	IC_{50} after UV irradiation [μM]	IC_{50} after visible irradiation [μM]	Increase in activity after UV irradiation	Decrease in activity after visible irradiation
3	>65	16	56	>4.1-fold	3.5-fold
4	32	13	28	2.5-fold	2.2-fold
5	46	10	31	4.6-fold	3.1-fold
6	>108	23	>108	>4.7-fold	>4.7-fold
7	>86	17	64	>5.0-fold	3.8-fold
8	46	8.5	31	5.4-fold	3.6-fold
9	>60	27	>60	>2.2-fold	>2.2-fold

for photoswitch inhibitor design. Further studies are required to directly compare structurally analogous α-ketoesters and trifluoromethylketones.

A number of significant structure–activity photoswitching relationships are evident on comparison of the structurally related inhibitors **5**–**9**. Firstly, the halide-substituted derivatives **6**–**8** all exhibit improved photoswitching relative to the unsubstituted analogue **5**, with **6** being the best reversible photoswitch inhibitor of α-chymotrypsin reported to date (see above for discussion). This may be partially attributable to an increase in the effective bulk of these photoswitching groups leading to a greater change in molecular structure on photoisomerisation. It is expected that halide **9** would also give good photoswitching, but an accurate analysis was not possible here due to its poor solubility. The 4-sulfonamide series (**5**–**7**) allows comparison of the effect of different groups at the 4′-position (denoted X; **5**: X = H; **6**: X = Br; **7**: X = I). Interestingly both PSSs of **5** are significantly more potent than those of halides **6** and **7**, which suggests that a lack of steric bulk on the azobenzene provides a better fit to the active site. However, the greatest change in enzyme activity on photoswitching is exhibited by halides **6** and **7**. This is predominantly due to a greater amount of the more active *Z* isomer in the irradiated PSS in these cases (compare Tables 1 and 2). This is likely to be due to the electron-withdrawing properties of the halide, which are known to affect the energy levels involved in UV-visible absorbances.[14] It is interesting to note that the smaller substituent of **8** (X = Br) relative to that of **9** (X = I) also results in improved potency (see Table 2). However, the observed greater affinity of the bromide relative to the iodide contrasts with the results obtained for **6** and **7**, in which the larger iodide **7** gives the greater potency. Photoswitching of **8** and **9** cannot be compared accurately due to the poor solubility of **9**.

A comparison of 4-substituted **6** and **7** with 3-substituted **8** and **9** also provides some interesting comparisons with regards to enzyme affinity and photoswitching. In particular, the 4-sulfonamido-4′-bromoazobenzene **6** is significantly less potent than 3-sulfonamido-4′-bromoazobenzene **8**, but exhibits a larger change in activity on photoisomerisation. Both bromides give similar changes in the proportion of *Z* isomer on irradiation (see Table 1). Therefore, the difference in enzyme photoswitching is attributable to differences in the "fit" of the inhibitors into the enzyme active site. Interestingly, the 4-sulfonamido-4′-iodoazobenzene **7** is more potent than 3-sulfonamido-4′-iodoazobenzene **9**, which differs from the results obtained for bromides **6** and **8**. However, again photoswitching cannot be compared due to the poor solubility of **9**.

Conclusion

A series of trifluoromethylketone photoswitch inhibitors of α-chymotrypsin has been developed and tested. The best photoswitch, bromide **6**, exhibits significantly improved enzyme photoswitching (>4.7-fold) compared with previously reported reversible photoswitch inhibitors of α-chymotrypsin. Comparisons with known and related α-ketoesters and boronate esters suggest the trifluoromethylketone warhead to be the best for photoswitching in such inhibitors. In addition, the 4′-halides **6**–**8** are better photoswitches than the simple **5** that lacks a 4′-substituent. This implies that photoswitching can be enhanced by increasing the effective bulk of the photoswitch group. This is an important observation for the surface attachment of photoswitchable inhibitors using a bulky linker substituent.[6] However, although the surface attachment of inhibitors by means of a linker group in the 3′- or 4′-position is expected to be generally favourable for photoswitching, no clearly favoured substitution pattern or substituent is evident. Ongoing studies are focused on improving photoswitching by optimising the size and character of 4′ linker groups. Our findings provide an important lead for the ongoing development of photoswitchable inhibitors of proteases for use in reversible biosensors, photoactivated medicines and molecular devices.

Experimental Section

General: NMR spectra were obtained on a Varian INOVA spectrometer operating at 500 MHz for ^{1}H NMR, 126 MHz for ^{13}C NMR and 282 MHz for ^{19}F NMR, or on a Varian UNITY 300 spectrometer operating at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR (selected NMR spectra are given in the Supporting Information). Two-dimensional NMR experiments including COSY and HSQC were used to assign the spectra, and were obtained on the Varian INOVA spectrometer operating at 500 MHz. For the racemic compounds, unless stated otherwise, the NMR spectroscopy data given are for the major *E* isomer. Compounds **3**–**9** were characterized in their hydrated forms. Electrospray ionisation mass spectrometry was performed on a Micromass LCT TOF mass spectrometer operating in electrospray (ES) mode with 50% acetonitrile/H_2O as solvent. Electron impact ionisation (EI) mass spectroscopy was performed on a Kratos MS80 mass spectrometer operating at 4 kV (accelerating potential) and 70 eV (ionising potential). Anhydrous DMF was purchased from Acros; anhydrous THF was distilled from sodium or potassium; anhydrous CH_2Cl_2 was distilled from CaH_2. EtOAc and AcOH were distilled before use. HPLC-grade acetonitrile was purchased from BDH. All other commercial reagents were used as received.

Photoisomerisation and enzyme assays: Photoisomerisation studies and enzyme assays were carried out as has been reported previously,[2] except that spectrophotometric assays were run for 10 min (instead of 5 min), and the final steady-state rates were used to find IC_{50} values rather than initial rates. *E*/*Z* ratios were determined based on the relative integrals of well-separated peaks in the ^{1}H NMR spectra, in particular those corresponding to NH and azobenzene aromatic protons.

Synthesis

(*E*)-[*N*-(Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-phenylazobenzamide] (3): A solution of **12** (43 mg, 0.10 mmol), KBr (0.2 equiv) and TEMPO (0.1 equiv) in CH_2Cl_2 (3.5 mL) was cooled to 0 °C. Buffered NaOCl (5.25%, adjusted to pH 9 by addition of $NaHCO_3$, 1.8 mL) was added dropwise and the reaction mixture was stirred rapidly for 2 h. The mixture was diluted with EtOAc, separated, washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography (EtOAc/CH_2Cl_2 1:9) followed by recrystallisation from MeOH/H_2O to afford **3** (37 mg, 87%, 88% *E* isomer) as an orange solid. M.p. 154–156 °C; ^{1}H NMR ([D_6]acetone containing 5% D_2O, 500 MHz): δ = 8.18 (s, 1H), 7.99 (d, J = 7.9 Hz, 1H), 7.90 (dd, J = 1.5, 8.2 Hz, 2H), 7.83 (d, J = 7.7 Hz, 1H), 7.64–7.53 (m, 4H), 7.32 (d, J =

7.2 Hz, 2H), 7.20 (t, J=7.5 Hz, 2H), 7.11 (t, J=7.4 Hz, 1H), 6.79 (d, J=8.5 Hz, 1H; N*H*), 4.61 (dd, J=2.6, 11.8 Hz, 1H; C*H*CH_2), 3.35 (dd, J=3.0, 14.1 Hz, 1H; CHC*H*H), 3.07 ppm (dd, J=12.3, 13.7 Hz, 1H; CHCH*H*); selected ^{1}H NMR peaks for the minor *Z* hydrate isomer: δ=7.58 (d, J=8.1 Hz, 1H), 6.93–6.89 (m, 2H), 4.64 (d, J=11.6 Hz, 1H), 3.44–3.39 (m, 1H), 3.12–3.06 ppm (m, 1H); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=168.5 (*C*ONH), 153.0, 139.3, 136.2, 132.4, 130.6, 130.2, 123.1, 128.9, 126.9, 126.0, 124.8 (q, J=289.7 Hz; *C*F$_3$), 123.5, 122.3, 94.7 (q, J=29.9 Hz; *C*(OH)$_2$), 56.8 (*C*HCH$_2$), 34.0 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−80.5 ppm (s, 3F); *m*/*z* (ES) calcd for $C_{23}H_{21}F_3N_3O_3$ [M^++H]: 444.1535; found: 444.1528.

(*E*)-*tert*-Butyl-2-oxo-2-(4-{[4-(4,4,4-trifluoro-3-oxo-1-phenylbutan-2-ylcarbamoyl)phenyl]diazenyl}benzylamino)ethyl carbamate (4): A solution of **14** (7.0 mg, 0.011 mmol) and Dess–Martin periodinane (34 mg, 7 equiv) in CH_2Cl_2 (0.5 mL) was stirred for 3 h, diluted with EtOAc (50 mL), washed with $Na_2S_2O_3$ (0.25 M in saturated $NaHCO_3$, 50 mL), saturated $NaHCO_3$ (2×50 mL), brine (50 mL), dried over $MgSO_4$ and concentrated. The crude material was purified by column chromatography (EtOAc/CH_2Cl_2 4:1) to give **4** (3.0 mg, 44%, >95% *E* isomer) as an orange solid. M.p. 122–125 °C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=7.99 (s, 1H), 7.89–7.85 (m, 6H), 7.52 (d, J=8.1 Hz, 2H), 7.33 (d, J=7.2 Hz, 2H), 7.24 (t, J=7.6 Hz, 2H), 7.16 (t, J=7.3 Hz, 1H), 6.41 (s, 1H), 4.55–4.46 (m, 3H), 3.80 (d, J=5.6 Hz, 2H), 3.37 (dd, J=14.1, 3.0 Hz, 1H), 3.21–3.15 (m, 1H), 1.40 ppm (s, C(C*H*$_3$)$_3$, 9H); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=170.6, 169.7, 155.1, 152.3, 144.8, 139.5, 136.7, 130.1, 129.5, 129.2, 129.0, 127.2, 123.8, 123.3, 95.4 (q, J=29.9 Hz, C(OH)$_2$), 79.4, 58.6, 44.8, 43.0, 33.7, 28.5 ppm; *m*/*z* (ES) calcd for $C_{32}H_{37}F_3N_5O_6$ [M^++CH_5O]: 644.2696; found: 644.2717.

(*E*)-[*N*-(Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-phenylazobenzenesulfonamide] (5): A solution of **15** (50 mg, 0.11 mmol), KBr (0.2 equiv) and TEMPO (0.1 equiv) in CH_2Cl_2 (4.0 mL) was cooled to 0 °C. Buffered NaOCl (5.25%, adjusted to pH 9 by addition of $NaHCO_3$, 2.0 mL) was added dropwise and the reaction mixture was stirred rapidly for 2 h. The mixture was diluted with EtOAc, separated, washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/petroleum ether 3:7) followed by recrystallisation from MeOH/H_2O to give **5** (40 mg, 80%, >95% *E* isomer) as fluffy orange needles. M.p. 99–110 °C (decomp); ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=8.01 (dd, J=1.5, 7.7 Hz, 2H), 7.73 (d, J=8.5 Hz, 2H), 7.65 (m, 3H), 7.59 (d, J=8.4 Hz, 2H), 7.23 (d, J=9.0 Hz, 1H; N*H*), 7.06 (m, 2H), 6.94 (m, 3H), 6.28 (s, 1H; O*H*), 6.23 (s, 1H; O*H*), 3.93 (m, 1H; C*H*CH$_2$), 3.24 (dd, J=2.3, 14.3 Hz, 1H; CHC*H*H), 2.74 ppm (dd, J=11.3, 14.3 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=154.6, 153.4, 143.7, 138.5, 132.8, 130.3, 130.0, 129.0, 128.3, 127.0, 124.7 (q, J=289.2 Hz; *C*F$_3$), 123.8, 123.6, 94.2 (q, J=29.4 Hz; *C*(OH)$_2$), 61.7 (*C*HCH$_2$), 36.2 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−78.9 ppm (s, 3F); *m*/*z* (EI) calcd for $C_{22}H_{18}F_3N_3O_3S$ ([M^+], ketone): 461.1021; found: 461.1031.

(*E*)-[*N*-(Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-(4-bromophenylazo)benzenesulfonamide] (6): A solution of **22** (81 mg, 0.15 mmol), KBr (0.2 equiv) and TEMPO (0.1 equiv) in EtOAc/CH_2Cl_2 (2:1, 15 mL) was cooled to 0 °C. Buffered NaOCl (5.25%, adjusted to pH 9 by addition of $NaHCO_3$, 7.5 mL) was added dropwise and the reaction mixture was stirred rapidly for 3 h. The biphasic mixture was diluted with EtOAc, separated, washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was recrystallised from MeOH/H_2O to give **6** (57 mg, 70%, >95% *E* isomer) as an orange solid. M.p. 140–141 °C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=7.96 (d, J=8.7 Hz, 2H), 7.84 (d, J=8.7 Hz, 2H), 7.74 (d, J=8.6 Hz, 2H), 7.59 (d, J=8.6 Hz, 2H), 7.23 (br s, 1H; N*H*), 7.05 (dd, J=1.9, 7.1 Hz, 2H), 6.93 (m, 3H), 6.30–6.20 (m, 2H), 3.93 (d, J=9.9 Hz, 1H; C*H*CH$_2$), 3.25 (dd, J=2.4, 14.3 Hz, 1H; CHC*H*H), 2.73 ppm (dd, J=11.3, 14.3 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=154.2, 152.1, 144.4, 138.6, 133.5, 130.1, 128.9, 128.2, 126.9, 126.6, 125.5, 124.6 (q, J=289.1 Hz; *C*F$_3$), 123.7, 94.2 (q, J=29.9 Hz; *C*(OH)$_2$), 61.5 (*C*HCH$_2$), 36.1 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−79.0 ppm (s; 3F); *m*/*z* (ES) calcd for $C_{22}H_{20}{}^{79}BrF_3N_3O_4S$ [M^++H]: 558.0310; found: 558.0311.

(*E*)-[*N*-(Benzyl-3,3,3-trifluoro-2-oxopropyl)-4-(4-iodophenylazo)benzenesulfonamide] (7): A solution of **23** (88 mg, 0.15 mmol), KBr (0.2 equiv) and TEMPO (0.1 equiv) in EtOAc/CH_2Cl_2 2:1 (15 mL) was cooled to 0 °C. Buffered NaOCl (5.25%, adjusted to pH 9 by addition of $NaHCO_3$, 7.5 mL) was added dropwise and the reaction mixture was stirred rapidly for 3 h. The biphasic mixture was diluted with EtOAc, separated, washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was recrystallised from MeOH/H_2O to afford the hydrate of **7** (53 mg, 60%, >95% *E* isomer) as an orange solid. M.p. 154–156 °C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=8.05 (d, J=8.5 Hz, 2H), 7.79 (d, J=8.5 Hz, 2H), 7.73 (d, J=8.6 Hz, 2H), 7.58 (d, J=8.6 Hz, 2H), 7.22 (br s, 1H; N*H*), 7.05 (dd, J=1.8, 7.0 Hz, 2H), 6.93 (m, 3H), 6.26 (br s, 2H), 3.93 (d, J=10.5 Hz, 1H; C*H*CH$_2$), 3.24 (dd, J=2.2, 14.3 Hz, 1H; CHC*H*H), 2.73 ppm (dd, J=11.3, 14.3 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=154.0, 152.4, 144.3, 139.4, 138.4, 129.9, 128.8, 128.0, 126.7, 125.2, 124.4 (q, J=289.2; *C*F$_3$), 123.5, 99.2, 94.0 (q, J=29.8 Hz; *C*(OH)$_2$), 61.2 (*C*HCH$_2$), 35.9 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−79.0 ppm (s, 3F); *m*/*z* (EI) calcd for $C_{22}H_{17}F_3IN_3O_3S$ [M^+]: 586.9987; found: 586.9992.

(*E*)-[*N*-(Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-(4-bromophenylazo)benzenesulfonamide] (8): A solution of **27** (22 mg, 40 μmol), KBr (0.2 equiv) and TEMPO (0.1 equiv) in CH_2Cl_2 (0.5 mL) was cooled to 0 °C. Buffered NaOCl (5.25%, adjusted to pH 9 by addition of $NaHCO_3$, 0.25 mL) was added dropwise and the reaction mixture was stirred rapidly for 2 h. The biphasic mixture was diluted with EtOAc, separated, washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/CH_2Cl_2 1:9) followed by recrystallisation from MeOH/H_2O to give the hydrate of **8** (18 mg, 83%, >95% *E* isomer) as an orange solid. M.p. 86–88 °C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=7.94 (d, J=7.9 Hz, 1H), 7.89 (d, J=8.7 Hz, 2H), 7.79 (d, J=8.8 Hz, 3H), 7.62 (d, J=7.9 Hz, 1H), 7.50 (t, J=7.9 Hz, 1H), 6.98 (d, J=7.2 Hz, 2H), 6.83 (t, J=7.4 Hz, 2H), 6.77 (t, J=7.3 Hz, 1H), 3.96 (dd, J=2.5, 11.0 Hz, 1H; C*H*CH$_2$), 3.19 (dd, J=2.6, 14.3 Hz, 1H; CHC*H*H), 2.64 ppm (dd, J=11.1, 14.3 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=152.4, 151.8, 143.8, 138.4, 133.3, 130.6, 129.8, 129.5, 128.7, 127.4, 127.2, 126.3, 125.3, 120.2, 94.1 (q, J=29.7 Hz; *C*(OH)$_2$), 61.4 (*C*HCH$_2$), 36.0 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−78.9 ppm (s, 3F); *m*/*z* (ES) calcd for $C_{22}H_{20}{}^{79}BrF_3N_3O_4S$ [M^++H]: 558.0310; found: 558.0302.

(*E*)-[*N*-(Benzyl-3,3,3-trifluoro-2-oxopropyl)-3-(4-iodophenylazo)benzenesulfonamide] (9): A solution of **28** (24 mg, 40 μmol), KBr (0.2 equiv) and TEMPO (0.1 equiv) in CH_2Cl_2 (0.5 mL) was cooled to 0 °C. Buffered NaOCl (5.25%, adjusted to pH 9 by addition of $NaHCO_3$, 0.25 mL CH_2Cl_2) was added dropwise and the reaction mixture was stirred rapidly for 2 h. The biphasic mixture was diluted with EtOAc, separated, washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/CH_2Cl_2 1:9), followed by recrystallisation from aqueous MeOH/H_2O to give the ketone/hydrate mixture **9** (19 mg, 81%, >95% *E* isomer) as an orange solid. M.p. 109–112 °C; ^{1}H NMR ([D$_6$]acetone containing 5% D_2O, 500 MHz): δ=8.02 (d, J=8.5 Hz, 2H), 7.95 (d, J=7.8 Hz, 1H), 7.80 (s, 1H), 7.75 (d, J=8.5 Hz, 2H), 7.61 (d, J=7.9 Hz, 1H), 7.51 (t, J=7.8 Hz, 1H), 6.98 (d, J=7.4 Hz, 2H), 6.84 (t, J=7.5 Hz, 2H), 6.77 (t, J=7.4 Hz, 1H), 3.96 (dd, J=2.2, 11.0 Hz, 1H; C*H*CH$_2$), 3.18 (dd, J=2.4, 14.2 Hz, 1H; CHC*H*H), 2.64 ppm (dd, J=11.3, 14.1 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=152.6, 152.5, 143.6, 139.5, 138.4, 130.7, 129.9, 129.6, 128.8, 127.6, 127.2, 125.3, 124.6 (q, J=289.2 Hz; *C*F$_3$), 120.4, 99.0, 94.4 (q, J=29.6 Hz; *C*(OH)$_2$), 61.6 (*C*HCH$_2$), 36.1 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−78.9 ppm (s, 3F); *m*/*z* (ES) calcd for $C_{22}H_{20}F_3IN_3O_4S$ [M^++H]: 606.0171; found: 606.0165.

(*E*)-[*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-phenylazobenzamide] (12): A suspension of 3-phenylazobenzoic acid **10**[7,8] (88 mg, 0.39 mmol), **11a**[9] (100 mg, 0.40 mmol) and BOP (180 mg, 0.40 mmol) in CH_2Cl_2 (5.0 mL) was stirred at RT under an inert atmosphere. After 5 min, DIEA (140 μL, 0.80 mmol) was added dropwise. The resulting solution was stirred for a further 16 h then diluted with EtOAc (20 mL), washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated. Recrystallisation from aqueous

acetone gave **12** (160 mg, 98%, >95% *E* isomer) as an orange powder. M.p. 187–188°C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=8.28 (t, J=1.6 Hz, 1H), 8.03 (dd, J=1.7, 7.8 Hz, 2H), 7.95 (dd, J=1.2, 8.0 Hz, 2H), 7.92 (d, J=7.8 Hz, 1H), 7.64–7.57 (m, 4H), 7.36 (d, J=7.4 Hz, 2H), 7.27 (t, J=7.6 Hz, 2H), 7.17 (t, J=7.4 Hz, 1H), 4.67 (m, 1H; C*H*CH$_2$), 4.44 (m, 1H; C*H*OH), 3.26 (dd J=3.6, 14.2 Hz, 1H; CHC*H*H), 3.16 ppm (dd, J=10.8, 14.2 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone): δ=167.0 (*C*O), 153.2, 139.2, 136.9, 132.5, 130.6, 130.2, 129.1, 127.1, 126.4 (q, J=283.2 Hz; *C*F$_3$), 125.9, 123.6, 122.4, 72.0 (q, J=28.6 Hz; *C*HOH), 52.8 (*C*HCH$_2$), 35.6 ppm (CH*C*H$_2$); ^{19}F NMR (CDCl$_3$, 282 MHz): δ=−74.4 ppm (d, J=7.4 Hz, 3F); *m*/*z* (ES) calcd for $C_{23}H_{21}F_3N_3O_2$ [M^++H]: 428.1586: found: 428.1595.

(*E*)-*tert*-Butyl-2-oxo-2-(4-{[4-(4,4,4-trifluoro-3-hydroxy-1-phenylbutan-2-ylcarbamoyl)phenyl]diazenyl}benzylamino)ethyl carbamate (14): DIEA (9.0 μL, 6.7 mg, 1 equiv) was added to a solution of **13**[2] (20 mg, 0.049 mmol), **11b**[9] (11 mg, 1 equiv), EDCI (10 mg, 1 equiv) and HOAt (7.0 mg, 1 equiv) in DMF (3.0 mL), and the resulting mixture was stirred for 16 h, diluted with EtOAc (50 mL), washed with H_2O (50 mL×2), brine (50 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (EtOAc/CH_2Cl_2 3:2) to give **14** (15 mg, 50%, >95% *E* isomer) as an orange solid. M.p. 182–185°C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ?7.98 (d, J=8.5 Hz, 2H), 7.83–7.96 (m, 6H), 7.50 (d, J=8.0 Hz, 2H), 7.37 (d, J=7.4 Hz, 2H; C*H*CHCHCHC*H*), 7.30 (t, J=7.4 Hz, 2H; CHC*H*CHC*H*CH), 7.21 (t, J=7.4 Hz, 1H; CHCHC*H*CHCH), 4.72 (m, 1H), 6.35–6.40 (m, 2H), 4.53 (d, J=6.0 Hz, 2H), 4.23 (m, 1H), 3.84 (d, J=5.7 Hz, 2H), 3.17 (m, 2H; CHC*H*$_2$), 1.42 ppm (s, 9H; C(C*H*$_3$)$_3$); ^{13}C NMR ([D$_6$]acetone, 126 MHz): δ=170.7, 167.3, 154.9, 152.3, 144.5, 138.9, 137.4, 130.2, 129.3, 129.2, 128.9, 127.4, 123.8, 123.3, 79.4, 70.5 (q, J=29.5 Hz; CHOH), 52.2, 44.8, 43.0, 37.9, 28.5 ppm; *m*/*z* (ES) calcd for $C_{31}H_{35}F_3N_5O_5$ [M^++H]: 614.2602; found: 614.2590.

(*E*)-[*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-phenylazobenzenesulfonamide] (16): DIEA (0.39 mL, 2.2 mmol) was added to a stirred suspension of **15** (290 mg, 1.0 mmol) and **11a** (290 mg, 1.1 mmol) in CH_2Cl_2 (10 mL). The resulting solution was heated at reflux for 4 h, cooled and then concentrated. The residue was dissolved in EtOAc, washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated to give **16** (47 mg, quantitative, >95% *E* isomer) as an orange solid. M.p. 143–144°C (MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ=8.12 (dd, J=1.7, 7.9 Hz, 2H), 7.90 (d, J=8.5 Hz, 2H), 7.74 (m, 5H), 7.18 (m, 3H), 7.14 (m, 3H), 6.02 (d, J=6.9 Hz, 1H; O*H*), 4.57 (m, 1H; *C*HOH), 3.93 (m, 1H; C*H*CH$_2$), 3.08 (dd, J=3.1, 14.4 Hz, 1H; CHC*H*H), 2.96 ppm (dd, J=10.8, 14.4 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=154.7, 153.3, 143.3, 138.1, 132.9, 130.2, 130.0, 129.0, 128.3, 127.1, 126.0 (q, J=283.5 Hz; *C*F$_3$), 123.8, 123.7, 73.6 (q, J=28.8 Hz; *C*HOH), 56.7 (*C*HCH$_2$), 34.8 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−74.2 ppm (d, J=8.0 Hz, 3F); *m*/*z* (EI) calcd for $C_{22}H_{20}F_3N_3O_3S$ [M^+]: 463.1178; found: 463.1191.

***N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-nitrobenzenesulfonamide (18)**: DIEA (0.38 mL, 2.2 mmol) was added to a stirred suspension of 4-nitrobenzenesulfonyl chloride (230 mg, 1.00 mmol) and **11a**[9] (280 mg, 1.10 mmol) in CH_2Cl_2 (10 mL). The resulting solution was heated at reflux under an inert atmosphere for 4 h, cooled and concentrated. The residue was redissolved in EtOAc, successively extracted with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. Flash chromatography (CH_2Cl_2) of the crude material gave **18** (400 mg, quantitative) as a pale yellow solid. M.p. 129–131°C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=8.05 (d, J=8.9 Hz, 2H), 7.61 (d, J=8.9 Hz, 2H), 7.28 (d, J=8.3 Hz, 1H; N*H*), 7.01–6.95 (m, 5H), 5.93 (d, J=6.9 Hz, 1H; O*H*), 4.44 (m, 1H; *C*HOH), 3.80 (m, 1H; C*H*CH$_2$), 2.94 (dd, J=2.9, 14.4 Hz, 1H; CHC*H*H), 2.78 ppm (dd, J=11.2, 14.4 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=150.2, 147.0, 138.0, 130.0, 129.0, 128.3, 126.9, 125.8 (q, J=283.5; *C*F$_3$), 124.8, 73.9 (q, J=29.0 Hz; *C*HOH), 56.1 (*C*HCH$_2$), 34.7 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−74.2 ppm (d, J=8.0 Hz, 3F).

4-Amino-*N*-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)benzenesulfonamide (19): PtO_2 (catalytic amount) was added to a solution of **18** (1.3 g, 3.6 mmol) in EtOAc (50 mL). The suspension was rapidly stirred under vacuum for 5 min then stirred under a hydrogen atmosphere for a further 16 h, filtered through a mixture of Celite and $MgSO_4$ and concentrated to give **19** (1.2 g, 99%) as a pale yellow solid. M.p. 175–180°C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=7.17–7.12 (m, 5H), 7.05 (dd, J=3.0, 6.5 Hz, 2H), 6.51 (d, J=8.7 Hz, 2H), 6.20 (d, J=7.8 Hz, 1H; N*H*), 5.73 (d, J=6.9 Hz, 1H; O*H*), 5.38 (s, 2H; ArN*H*$_2$), 4.40 (m, 1H; *C*HOH), 3.65 (m, 1H; C*H*CH$_2$), 2.92 (dd, J=3.5, 14.5 Hz, 1H; CHC*H*H), 2.80 ppm (dd, J=10.2, 14.5 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=153.1, 138.2, 130.0, 129.4, 129.0, 127.2, 127.0, 126.1 (q, J=283.3 Hz; *C*F$_3$), 113.9, 71.7 (q, J=28.8 Hz; *C*HOH), 55.8 (*C*HCH$_2$), 34.9 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−74.1 ppm (d, J=8.1 Hz, 3F); *m*/*z* (EI) calcd for $C_{16}H_{17}F_3N_2O_3S$ [M^+]: 374.0912; found: 374.0900.

(*E*)-[*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-(4-bromophenylazo)-benzenesulfonamide] (22): A suspension of **19** (200 mg, 0.60 mmol) and 4-bromonitrosobenzene **20**[10,11] (110 mg, 0.60 mmol) in AcOH (2.4 mL) was stirred at 100°C for 4 h. The reaction mixture was cooled to RT then diluted with H_2O/CH_2Cl_2 (1:1, 50 mL). Solid Na_2CO_3 was added until effervescence ceased and the aqueous layer was basic to litmus. The aqueous layer was back-extracted with CH_2Cl_2 and the combined organics were washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$ then brine, dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography (EtOAc/CH_2Cl_2 1:9) gave **22** (210 mg, 64%, >95% *E* isomer) as an orange solid. M.p. 135–138°C (MeOH); ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=7.94 (d, J=8.6 Hz, 2H), 7.83 (d, J=8.6 Hz, 2H), 7.78 (d, J=8.5 Hz, 2H), 7.60 (d, J=8.5 Hz, 2H), 7.04 (m, 3H), 7.00 (m, 3H), 5.91 (d, J=6.9 Hz, 1H; O*H*), 4.44 (m, 1H; *C*HOH), 3.81 (m, 1H; C*H*CH$_2$), 2.95 (dd, J=3.0, 14.4 Hz, 1H; CHC*H*H), 2.89 (s, 2H; *H*$_2$O), 2.83 ppm (dd, J=10.9, 14.6 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=154.6, 152.2, 143.7, 138.1, 133.5, 130.0, 129.1, 128.2, 126.1, 126.8, 126.0 (q, J=283.5 Hz; *C*F$_3$), 125.6, 123.9, 73.6 (q, J=28.9 Hz; *C*HOH), 56.7 (*C*HCH$_2$), 34.8 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−74.2 ppm (d, J=8.0 Hz, 3F); *m*/*z* (EI) calcd for $C_{22}H_{19}{}^{79}BrF_3N_3O_3S$ [M^+]: 541.0283; found: 541.0280.

(*E*)-[*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-4-(4-iodophenylazo)-benzenesulfonamide] (23): A suspension of **19** (130 mg, 0.34 mmol) and 4-iodonitrosobenzene **21**[12,13] (79 mg, 0.34 mmol) in AcOH (1.5 mL) was stirred at 100°C for 4 h. The reaction mixture was cooled to RT then diluted with water/CH_2Cl_2 (1:1, 50 mL). Solid Na_2CO_3 was added until effervescence ceased and the aqueous layer was basic to litmus. The aqueous layer was back-extracted with CH_2Cl_2 and the combined organics washed successively with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography (EtOAc/CH_2Cl_2 1:9) gave **23** (130 mg, 67%, >95% *E* isomer) as an orange solid. M.p. 149–150°C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): δ=8.05 (d, J=8.5 Hz, 2H), 7.79 (d, J=8.5 Hz, 2H), 7.78 (d, J=8.4 Hz, 2H), 7.60 (d, J=8.4 Hz, 2H), 7.04 (m, 3H), 7.00 (m, 3H), 4.44 (m, 1H; *C*HOH), 3.80 (m, 1H; C*H*CH$_2$), 2.95 (dd, J=2.9, 14.4 Hz, 1H; CHC*H*H), 2.83 ppm (dd, J=10.9, 14.4 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): δ=154.5, 152.6, 143.7, 139.6, 138.1, 130.0, 129.0, 128.3, 127.1, 126.0 (q, J=283.4 Hz; *C*F$_3$), 125.5, 123.8, 99.3, 73.6 (q, J=28.7 Hz; *C*HOH), 56.7 (*C*HCH$_2$), 34.8 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): δ=−74.26ppm (d, J=7.9 Hz, 3F); *m*/*z* (EI) calcd for $C_{22}H_{19}F_3IN_3O_3S$ [M^+]: 589.0144; found: 589.0155.

***N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-nitrobenzenesulfonamide (25)**: DIEA (0.37 mL, 2.1 mmol) was added to a stirred suspension of 3-nitrobenzenesulfonyl chloride **24** (220 mg, 1.00 mmol) and **11a**[9] (270 mg, 1.1 mmol) in CH_2Cl_2 (10 mL). The resulting solution was heated at reflux for 4 h, cooled and then concentrated. The residue was redissolved in EtOAc, washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. Flash chromatography (CH_2Cl_2) of the crude material gave **25** (400 mg, 99%) as a pale yellow solid. M.p. 116–118°C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ=8.26 (d, J=8.2 Hz, 1H), 8.12 (s, 1H), 7.78 (d, J=7.9 Hz, 1H), 7.48 (t, J=8.0 Hz, 1H), 6.97–6.94 (m, 3H), 6.85 (dd, J=2.8, 6.3 Hz, 2H), 5.44 (d, J=8.1 Hz, 1H; N*H*), 4.55 (m, 1H; C*H*OH), 3.62–3.73 (m, 2H), 2.97 (dd, J=3.3, 14.4 Hz, 1H; CHC*H*H), 2.68 ppm (dd, J=11.6, 14.4 Hz, 1H; CHCH*H*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ=148.2, 140.6, 136.0, 132.1, 130.0, 128.8, 128.6, 127.0, 126.7, 124.1 (q, J=283.3 Hz; *C*F$_3$), 122.0, 72.7 (q, J=

30.1 Hz; *C*HOH), 56.3 (*C*HCH$_2$), 34.2 ppm (CH*C*H$_2$); ^{19}F NMR (CDCl$_3$, 282 MHz): $\delta = -74.7$ ppm (d, $J = 7.4$ Hz, 3F).

3-Amino-*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)benzenesulfonamide (26): PtO_2 (10 mg) was added to a solution of **25** (300 mg, 0.75 mmol) in EtOAc (50 mL). The suspension was rapidly stirred under vacuum for 3–5 min then stirred under a hydrogen atmosphere for a further 16 h, filtered through a mixture of Celite and $MgSO_4$ and concentrated to give **26** (270 mg, 95%) as a foamy off-white solid. M.p. 131–134 °C (EtOAc); ^{1}H NMR (CDCl$_3$, 500 MHz): $\delta = 7.15$–7.02 (m, 4H), 6.89 (dd, $J = 2.5$, 6.0 Hz, 2H), 6.85 (d, $J = 7.7$ Hz, 1H), 6.73 (d, $J = 8.0$ Hz, 1H), 6.67 (s, 1H), 5.37 (d, $J = 8.0$ Hz, 1H; N*H*), 4.46 (m, 1H; C*H*OH), 3.90 (br, 2H; ArNH_2), 3.68 (m, 1H; C*H*CH$_2$), 2.92 (dd, $J = 3.6$, 14.4 Hz, 1H; CHC*H*H), 2.68 ppm (dd, $J = 10.7$, 14.4 Hz, 1H; CHCH*H*); ^{13}C NMR (CDCl$_3$, 75 MHz): $\delta = 147.2$, 139.2, 136.2, 130.0, 129.2, 128.6, 126.9, 124.5 (q, $J = 283.6$ Hz; *C*F$_3$), 119.6, 116.5, 112.9, 72.0 (q, $J = 29.2$ Hz; *C*HOH), 55.7 (*C*HCH$_2$), 34.4 ppm (CH*C*H$_2$); ^{19}F NMR (CDCl$_3$, 282 MHz): $\delta = -74.1$ (d, $J = 8.0$ Hz, 3F); *m*/*z* (ES) calcd for $C_{16}H_{18}F_3N_2O_3S$ [M^++H]: 375.0990; found: 375.0991.

(*E*)-[*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-(4-bromophenylazo)-benzenesulfonamide] (27): A suspension of **26** (100 mg, 0.30 mmol) and **20** (56 mg, 0.30 mmol) in AcOH (1.2 mL) was stirred at 100 °C for 4 h. The reaction mixture was cooled to RT then diluted with water/CH_2Cl_2 (1:1, 50 mL). Solid Na_2CO_3 was added until effervescence ceased and the aqueous layer was basic to litmus. The aqueous layer was back-extracted with CH_2Cl_2 and the combined organics washed successively with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography (EtOAc/CH_2Cl_2 1:9) gave **27** (120 mg, 76%, >95% *E* isomer) as an orange solid. M.p. 140–141 °C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): $\delta = 8.02$ (d, $J = 7.9$ Hz, 1H), 7.93 (d, $J = 8.7$ Hz, 2H), 7.89 (s, 1H), 7.83 (d, $J = 8.7$ Hz, 2H), 7.61 (d, $J = 7.9$ Hz, 1H), 7.55 (t, $J = 6.1$ Hz, 1H), 7.05 (d, $J = 8.2$ Hz, 1H; N*H*), 7.01 (d, $J = 7.2$ Hz, 2H), 6.93 (t, $J = 7.3$ Hz, 2H), 6.88 (m, $J = 7.3$ Hz, 1H), 5.92 (d, $J = 6.9$ Hz, 1H; O*H*), 4.46 (m, 1H; C*H*OH), 3.82 (m, 1H; C*H*CH$_2$), 2.94 (dd, $J = 3.0$, 14.4 Hz, 1H; CHC*H*H) 2.82 ppm (dd, $J = 10.9$, 14.4 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): $\delta = 152.9$, 152.1, 143.0, 138.0, 133.5, 130.9, 129.9, 129.7, 128.9, 127.7, 127.3, 126.5, 126.0 (q, $J = 283.5$ Hz; *C*F$_3$), 125.5, 120.6, 73.6 (q, $J = 28.8$ Hz; *C*HOH), 56.8 (*C*HCH$_2$), 34.8 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): $\delta = -74.2$ ppm (d, $J = 8.0$ Hz, 3F); *m*/*z* (EI) calcd for $C_{22}H_{19}{}^{79}BrF_3N_3O_3S$ [M^+]: 541.0283; found: 541.0278.

(*E*)-[*N*-(1-Benzyl-3,3,3-trifluoro-2-hydroxypropyl)-3-(4-iodophenylazo)-benzenesulfonamide] (28): A suspension of **26** (100 mg, 0.30 mmol) and **21** (70 mg, 0.30 mmol) in AcOH (1.2 mL) was stirred at 100 °C for 4 h. The reaction mixture was cooled to RT then diluted with water/CH_2Cl_2 (1:1, 50 mL). Solid Na_2CO_3 was added until effervescence ceased and the aqueous layer was basic to litmus. The aqueous layer was back-extracted with CH_2Cl_2 and the combined organics washed successively with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography (EtOAc/CH_2Cl_2 1:9) gave **28** (140 mg, 77%, >95% *E* isomer) as an orange solid. M.p. 134–137 °C; ^{1}H NMR ([D$_6$]acetone, 500 MHz): $\delta = 8.04$ (d, $J = 8.7$ Hz, 2H), 8.02 (d, $J = 7.9$ Hz, 1H), 7.89 (s, 1H), 7.77 (d, $J = 8.6$ Hz, 2H), 7.61 (d, $J = 7.8$ Hz, 1H), 7.54 (t, $J = 7.8$ Hz, 1H), 7.05 (d, $J = 8.2$ Hz, 1H; N*H*), 7.01 (d, $J = 7.1$ Hz, 2H), 6.93 (t, $J = 7.3$ Hz, 2H), 6.87 (t, $J = 7.2$ Hz, 1H), 5.90 (d, $J = 6.9$ Hz, 1H; O*H*), 4.47 (m, 1H; C*H*OH), 3.82 (m, 1H; C*H*CH$_2$), 2.94 (dd, $J = 2.9$, 14.4 Hz, 1H; CHC*H*H), 2.82 ppm (dd, $J = 10.9$, 14.4 Hz, 1H; CHCH*H*); ^{13}C NMR ([D$_6$]acetone, 75 MHz): $\delta = 152.8$, 152.5, 143.0, 139.6, 138.0, 130.8, 129.8, 129.7, 128.9, 127.7, 127.2, 126.0 (q, $J = 283.4$ Hz; *C*F$_3$), 125.4, 120.6, 99.1, 73.6 (q, $J = 28.7$ Hz; *C*HOH), 56.8 (*C*HCH$_2$), 34.8 ppm (CH*C*H$_2$); ^{19}F NMR ([D$_6$]acetone, 282 MHz): $\delta = -74.2$ ppm (d, $J = 8.0$ Hz, 3F); *m*/*z* (EI) calcd for $C_{22}H_{19}F_3IN_3O_3S$ [M^+]: 589.0144; found: 589.0146.

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[1] A. J. Harvey, A. D. Abell, *Tetrahedron* **2000**, *56*, 9763.
[2] D. Pearson, A. D. Abell, *Org. Biomol. Chem.* **2006**, *4*, 3618.
[3] M. A. Wainberg, B. F. Erlanger, *Biochemistry* **1971**, *10*, 3816.
[4] P. R. Westmark, J. P. Kelly, B. D. Smith, *J. Am. Chem. Soc.* **1993**, *115*, 3416.
[5] J. Auernheimer, C. Dahmen, U. Hersel, A. Bausch, H. Kessler, *J. Am. Chem. Soc.* **2005**, *127*, 16107.
[6] D. Pearson, A. J. Downard, A. Muscroft-Taylor, A. D. Abell, *J. Am. Chem. Soc.* **2007**, *129*, 14862.
[7] P. Jacobson, *Justus Liebigs Ann. Chem.* **1909**, *367*, 329.
[8] C. J. Byrne, D. A. R. Happer, M. P. Hartshorn, H. K. J. Powell, *J. Chem. Soc. Perkin Trans. 2* **1987**, 1649.
[9] N. P. Peet, J. P. Burkhart, M. R. Angelastro, E. L. Giroux, S. Mehdi, P. Bey, M. Kolb, B. Neises, D. Schirlin, *J. Med. Chem.* **1990**, *33*, 394.
[10] S. Tollari, M. Cuscela, F. Porta, *J. Chem. Soc. Chem. Commun.* **1993**, 1510.
[11] A. Defoin, *Synthesis* **2004**, 706.
[12] D. A. Fletcher, B. G. Gowenlock, K. G. Orrell, *J. Chem. Soc. Perkin Trans. 2* **1997**, 2201.
[13] E. Bamberger, *Ber. Dtsch. Chem. Ges.* **1895**, 245.
[14] H. Rau in *Photochromism: Molecules and Systems* (Eds.: H. Dürr, H. Bouas-Laurent), Elsevier, Amsterdam, **1990**, p. 165.
[15] K. Brady, R. H. Abeles, *Biochemistry* **1990**, *29*, 7608.
[16] A. J. Harvey, A. D. Abell, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2441.
[17] A. D. Abell, M. A. Jones, A. T. Neffe, S. G. Aitken, T. P. Cain, R. J. Payne, S. B. McNabb, J. M. Coxon, B. G. Stuart, D. Pearson, H. Y.-Y. Lee, J. D. Morton, *J. Med. Chem.* **2007**, *50*, 2916.
[18] B. Imperiali, R. H. Abeles, *Biochemistry* **1986**, *25*, 3760.

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