Dual aromatase–sulfatase inhibitors based on the anastrozole template: synthesis, *in vitro* SAR, molecular modelling and *in vivo* activity†

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The synthesis and biological evaluation of a series of novel Dual Aromatase–Sulfatase Inhibitors (DASIs) are described. It is postulated that dual inhibition of the aromatase and steroid sulfatase enzymes, both responsible for the biosynthesis of oestrogens, will be beneficial in the treatment of hormone-dependent breast cancer. The compounds are based upon the Anastrozole aromatase inhibitor template which, while maintaining the haem ligating triazole moiety crucial for enzyme inhibition, was modified to include a phenol sulfamate ester motif, the pharmacophore for potent irreversible steroid sulfatase inhibition. Adaption of a synthetic route to Anastrozole was accomplished *via* selective radical bromination and substitution reactions to furnish a series of aromatase inhibitory pharmacophores. Linking these fragments to the phenol sulfamate ester moiety employed S_N2, Heck and Mitsunobu reactions with phenolic precursors, from where the completed DASIs were achieved *via* sulfamoylation. *In vitro*, the lead compound, **11**, had a high degree of potency against aromatase (IC_{50} 3.5 nM), comparable with that of Anastrozole (IC_{50} 1.5 nM) whereas, only moderate activity against steroid sulfatase was found. However, *in vivo*, **11** surprisingly exhibited potent dual inhibition. Compound **11** was modelled into the active site of a homology model of human aromatase and the X-ray crystal structure of steroid sulfatase.

Introduction

In 2003 there were 36 509 new cases of breast cancer diagnosed in England. This represented 32% of all cancers in women and was the most common cause of female cancer related death. However, despite an increase in incidence, mortality rates are declining as a result of earlier detection and improved treatment.[1] Four in five cases of breast cancer are diagnosed in women over the age of 50 with the postmenopausal 50–64 age group the most at risk. In the majority of these cases breast tumours are found to be hormone-dependent, with oestrogens playing a key role in the growth and development of the disease. This has led to the development of endocrine therapies to remove the influence of oestrogen on breast cancer cells. Currently, the preferred strategy to tackle hormone-dependent breast cancer (HDBC) involves blocking oestrogens at the receptor level by using selective oestrogen receptor modulators (SERMs) such as (*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-*N*,*N*-dimethyl-ethanamine (Tamoxifen). Alternative approaches concentrate on restricting the availability of oestrogens by interfering with the steroid biosynthetic pathway, achievable *via* the inhibition of the enzymes involved in this process, *e.g.* aromatase and steroid sulfatase (STS). The inhibition of aromatase, which converts androgens to oestrogens in the final step of steroid biosynthesis (see Fig. 1), has been the focus of extensive research[2,3], which, in particular, has resulted in two significant clinical non-steroidal agents; 2-[3-(cyano-dimethyl-methyl)-5-[1,2,4]triazol-1-ylmethyl-phenyl]-2-methyl-propionitrile (Anastrozole) and 4-[(4-cyanophenyl)–(1,2,4-triazol-1-yl)methyl]benzonitrile (Letrozole) (Fig. 2), that show improved efficacies and superior toxicity profiles when compared to Tamoxifen.[4,5]

Most oestrogens that orginate from the aromatase pathway are stored in the body as steroid sulfates. It is now widely recognised that this store provides an important source of oestrogens in tumours when oestrone sulfate (E1S) is hydrolysed to oestrone (E1) by STS (see Fig. 1).[6–12] Considerable progress has been made in developing steroidal and non-steroidal STS inhibitors containing a phenol sulfamate ester pharmacophore.[13,14] This moiety effects highly potent irreversible STS inhibition. Indeed, STX64 (now BN83495) (Fig. 2) has become the first STS inhibitor to enter clinical trial and has shown highly promising Phase 1 results.[15]

We have previously reported and validated the concept of dual aromatase–sulfatase inhibitors (DASIs).[16–18] The rationale to this hypothesis is that concomitant inhibition of aromatase and STS should achieve a more effective oestrogen ablation than that affected by singular inhibition of either enzyme. Whereas this could be achieved by co-administrating two stand-alone drugs, or, by administrating a fixed-dose bi-component drug (one tablet-two agents), an alternative strategy is to design a dual-pharmacophore single agent to act against both aromatase and STS. Recently,

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† Electronic supplementary information (ESI) available: Synthesis and spectroscopic data for Anastrozole and preceding intermediates following two different routes, and experimental data for compounds **1**, **2**, **3(a–c)**, **4(a–c)**, **5(a–c)** and **20–23**. See DOI: 10.1039/b707768h

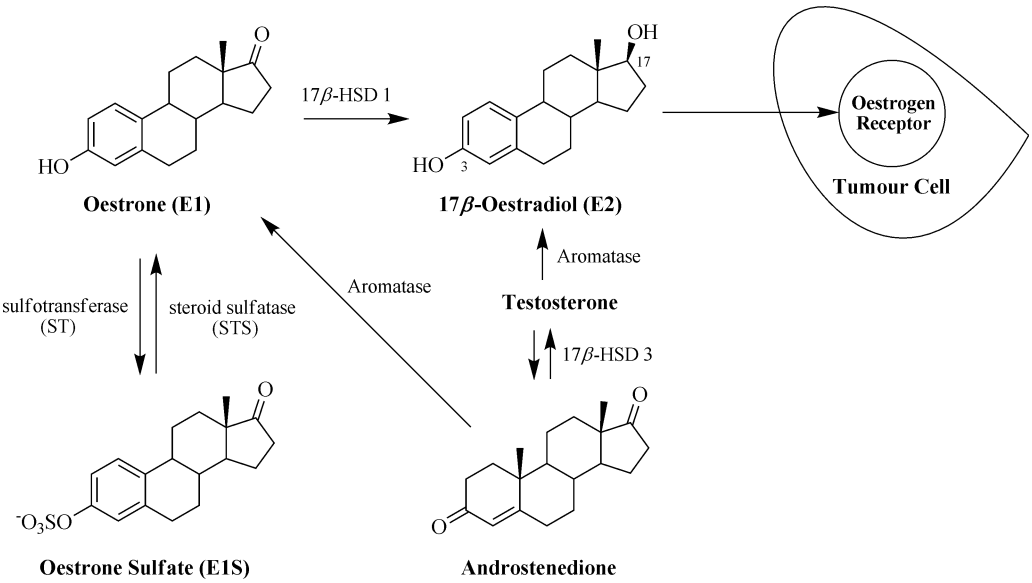


Fig. 1 The contribution of the aromatase and sulfatase pathways to oestrogen synthesis from androstenedione and oestrone sulfate in HDBC. 17β-HSDs are associated dehydrogenase/reductase enzymes.

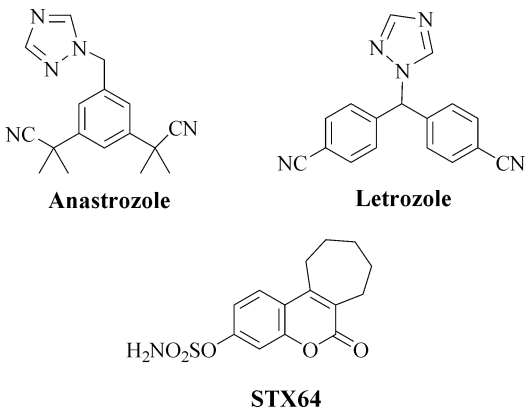


Fig. 2 Leading aromatase inhibitors (Anastrozole and Letrozole) and STS inhibitor (STX64).

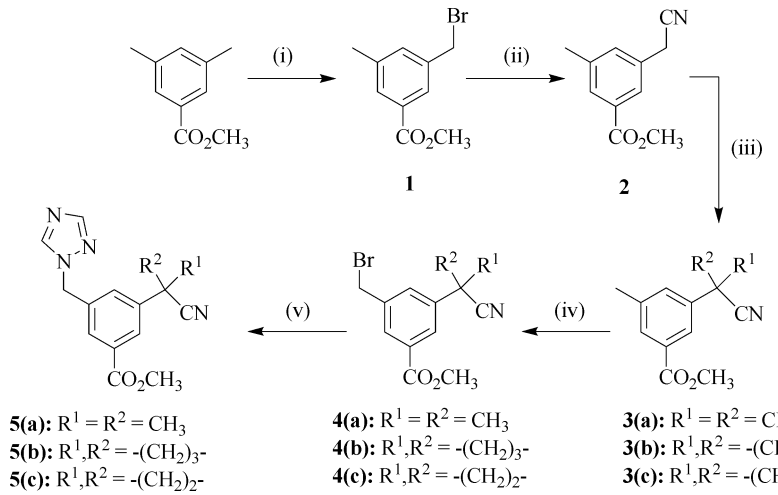
Morphy and Rankovic have reasoned that such a multiple-ligand strategy, as opposed to developing a multicomponent drug or drug cocktail, may provide significant pharmaceutical and development advantages.[19] Initial design of DASIs led to the successful incorporation of the phenol sulfamate ester moiety into the scaffold of potent aromatase inhibitors YM511,[16] and Letrozole.[17] These first examples of DASIs were shown to exhibit potent dual inhibition *in vivo*, introducing STS inhibition while preserving a high degree of the aromatase inhibition inherent in the original scaffold.

Adopting a similar strategy, we report here a new class of DASIs that is structurally based upon the Anastrozole template. Whilst retaining the haem-ligating triazole moiety key to the reversible inhibition of aromatase, the removal of one of the two dimethylpropionitrile side-arms of Anastrozole allows the incorporation of a phenol sulfamate ester group *via* linkers into the hybrid inhibitor. The use of ether, thioether and alkene linking units, as well as variation of the remaining dimethylpropionitrile side-arm to form cycloalkanecarbonitriles, generates a structurally diverse set of compounds. The *in vitro* biological activities of these compounds were evaluated in a JEG-3 cells assay from which extensive SAR information is derived. The leading compound from this series, compound **11** was studied *in vivo* alongside synthesised reference compounds. This compound was also docked into a homology model of the human aromatase enzyme[20] and into the crystal structure of STS[21] *in silico* in order to understand how it might interact with the individual enzymes.

Results and discussion

Chemistry

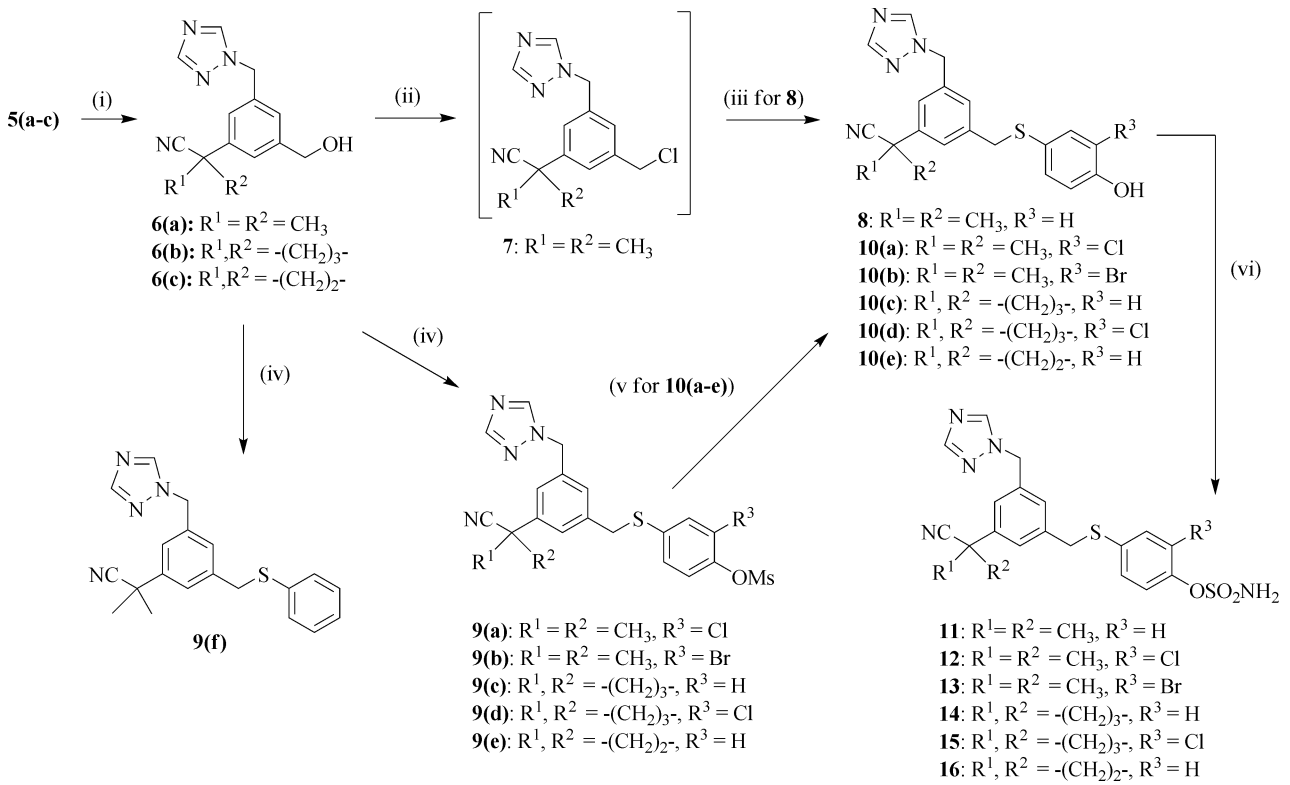
The synthesis of Anastrozole has been documented only in the patent literature.[22] We adapted two routes for the synthesis of Anastrozole and report for the first time full spectroscopic and analytical data (see ESI†). In contrast to Anastrozole the targeted DASI compounds are synthetically more demanding, largely owing to a loss of symmetry with three different substituents around the central aromatic ring as opposed to two. Whereas the preparation of Anastrozole involves the simultaneous construction of two dimethylpropionitrile side-arms, the DASI motif requires just one such side-arm with the second position left free to allow for the incorporation of the irreversible STS inhibiting pharmacophore. The synthetic strategy adopted for the synthesis of these Anastrozole-based DASIs (see Scheme 1) involved modification of one of the two routes to Anastrozole (see method B, reference 22 and ESI†). This was achieved by starting with the mono-bromination (instead of the di-bromination required for Anastrozole) of methyl 3,5-dimethylbenzoate, using the reaction conditions described by Kikuchi *et al.*,[23] to give the benzyl bromide **1**. Reaction of **1** with potassium cyanide gave the phenylacetonitrile **2** followed by *gem*-dimethylation to complete the carbonitrile side-arm in **3(a)**. Variation of the carbonitrile side-arm was possible by using 1,3-dibromopropane and 1,2-dibromoethane as alkylating species to form the cyclic side-arms in **3(b)** and **3(c)**



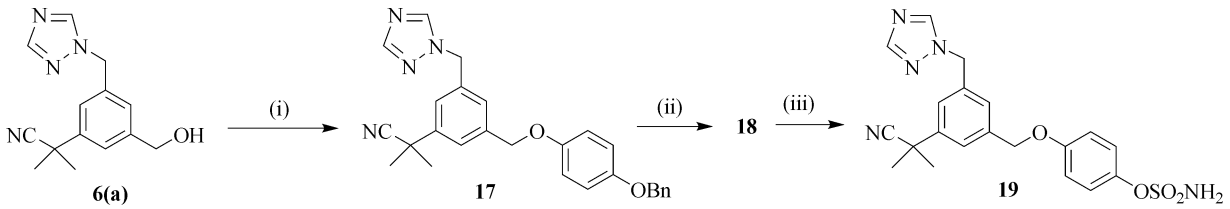
Scheme 1 *Reagents and conditions*: (i) $NaHSO_3$, $NaBrO_3$, H_2O–EtOAc, 85%; (ii) KCN, TBAB, H_2O–CH_2Cl_2, Δ, 75%; (iii) NaH, alkyl halide, THF, 35–78%; (iv) $NaHSO_3$, $NaBrO_3$, H_2O–EtOAc, 75–96%; (v) 1,2,4-triazole, K_2CO_3, KI, acetone, Δ, 43–59%.

respectively.[24] Incorporation of the haem-ligating heteroaryl unit was achieved *via* bromination of the remaining tolyl functionality of **3(a–c)** to give **4(a–c)** and then substitution with 1,2,4-triazole to complete the Anastrozole derivatives **5(a–c)** (Scheme 1).

Once these Anastrozole derivatives were complete the next task was to introduce the STS inhibiting pharmacophore in the form of phenol sulfamates (Scheme 2). Initially, the methyl ester groups in **5(a–c)** were reduced using sodium borohydride in PEG_{400}, a system which is inert towards nitrogen-containing functional groups.[25] Of the resulting benzyl alcohols, **6(a)** was converted to the unstable benzyl chloride **7**, which was used without purification or characterisation, and subsequently coupled with 4-hydroxythiophenol to form the thioether compound **8**. Sulfamoylation[26] of this compound afforded the first example of this DASI series, **11**. Derivatives of **11** were prepared by a modified strategy (Scheme 2). Thioether intermediates were prepared by an alternative transformation directly from the benzyl alcohols, **6(a–c)**, using (cyanomethyl)trimethylphosphonium iodide[27] and appropriately substituted thiophenols,[28] to form the mesylated compounds **9(a–e)** and **9(f)**. Deprotection of the mesylated phenols



Scheme 2 *Reagents and conditions*: (i) $NaBH_4$, PEG_{400}, 80 °C, 64–78%; (ii) $SOCl_2$, pyridine, DCM; (iii) 4-hydroxythiophenol, K_2CO_3, DMF, 26%; (iv) $(CH_3)_3P^+CH_2CN\ I^-$, DIPEA, EtCN, thiophenol or thiophenol mesylate, 30–92%; (v) 2 M $NaOH_{(aq)}$, THF, 68–99%; (vi) $ClSO_2NH_2$, DMA, 83–96%.



Scheme 3 *Reagents and conditions*: (i) DEAD, triphenylphosphine polystyrene, 4-(benzyloxy)phenol, 82%; (ii) 5% Pd/C, THF–MeOH, 76%; (iii) $ClSO_2NH_2$, DMA, 68%.

provided the free phenols **10(a–e)**. Incorporation of the phenol sulfamate ester moiety was completed *via* direct application of sulfamoyl chloride to the phenols (**10(a–e)**) to furnish the DASI compounds **12–16**.

In addition to the thioether linker between the Anastrozole fragment and the phenol sulfamate ester fragment in **11**, alternative linking groups were also introduced. An alkylether linked DASI was prepared *via* a Mitsunobu reaction between **6(a)** and 4-(benzyloxy)phenol to form **17** (Scheme 3). Debenzylation followed to give the free phenol **18** which was successfully converted to the sulfamate **19**.

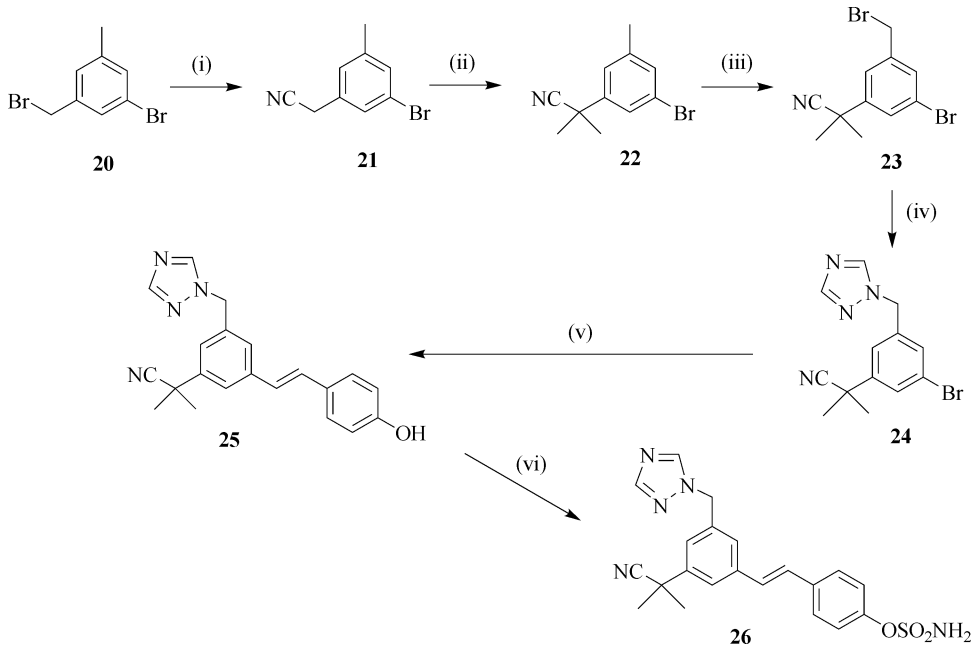
An alkene linked DASI, compound **26**, was also prepared (Scheme 4). Starting with 5-bromo-*m*-xylene, mono-bromination was undertaken to form the benzyl bromide **20**. Substitution with potassium cyanide gave **21** and the Anastrozole side-arm was completed *via* dimethylation to give **22**. Further bromination of the remaining tolyl group yielded **23** and the Anastrozole derivative **24** was completed *via* substitution with 1,2,4-triazole. A Heck reaction between this fragment and 4-vinylphenol gave the phenolic precursor **25** which was sulfamoylated to furnish **26**. Proton NMR confirms that **26** exists as one geometrical isomer with the coupling constant between the signals for the vinyl protons (^{1}H NMR δ_H 7.35 and ^{1}H NMR δ_H 7.38) being consistent with that expected for a *trans* stilbene ($J_{H-H} = 16.6$ Hz).

In relation to compound **11**, two reference compounds were prepared to assess the role of each pharmacophore on both target enzymes. The synthesis of **9(f)**, which does not contain a phenol sulfamate ester and therefore would not be expected to exhibit any significant STS inhibitory activity, has been previously described (see Scheme 2). The preparation of a control compound that does not contain a triazole moiety and hence would be expected to have relatively insignificant aromatase inhibition was also undertaken (see Scheme 5). To this end, commercially available 2-(3,5-dimethylphenyl)acetonitrile was dimethylated to give the *gem*-dimethyl compound **27** and then mono-brominated to yield the benzyl bromide **28**. Reaction with 4-hydroxythiophenol in the presence of base furnished the phenol **29** which was then sulfamoylated to give compound **30**.

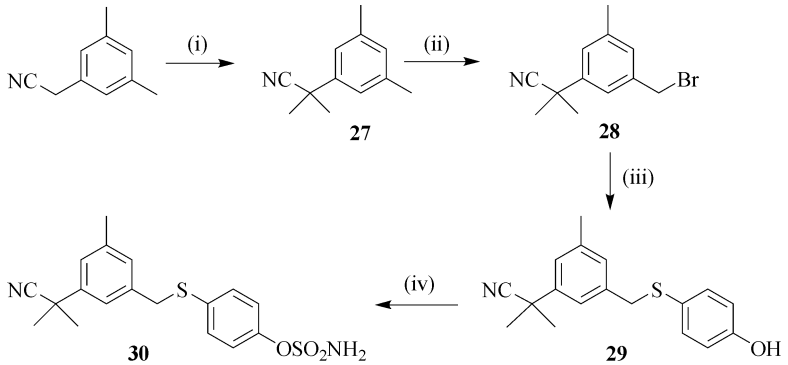
Inhibition of aromatase and sulfatase *in vitro*

The *in vitro* inhibitory activities of compounds **9(f)**, **11–16**, **19**, **26** and **30** against aromatase and STS in a JEG-3 cells preparation are shown in Table 1. Anastrozole, synthesised as described,[22] and STX64,[29] established potent aromatase and STS inhibitors respectively, are included in the same assay for reference.

All the compounds evaluated (except compound **30**) show potent inhibition of aromatase with IC_{50} values ranging from

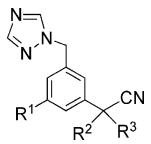


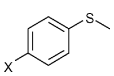
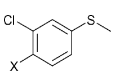
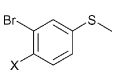
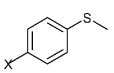
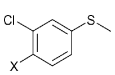
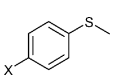
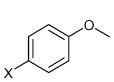
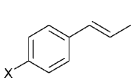
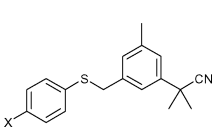
Scheme 4 *Reagents and conditions*: (i) KCN, TBAB, H_2O–DCM, Δ, 74%; (ii) NaH, CH_3I (2 eq.), THF, 83%; (iii) $NaHSO_3$, $NaBrO_3$, H_2O–EtOAc, 55%; (iv) 1,2,4-triazole, K_2CO_3, KI, acetone, Δ, 64%; (v) $Pd(OAc)_2$, PPh_3, NEt_3, 4-vinylphenol, THF, Δ, 71%; (vi) $ClSO_2NH_2$, DMA, 36%.



Scheme 5 *Reagents and conditions*: (i) $((CH_3)_3Si)_3NLi$, −78 °C, CH_3I (2 eq.), THF, 77%; (ii) NBS, Bz_2O_2, CCl_4, Δ, 66%; (iii) 4-hydroxythiophenol, K_2CO_3, DMF, 85%; (iv) $ClSO_2NH_2$, DMA, 74%.

Table 1 *In vitro* inhibition of aromatase and STS activity in JEG-3 cells by anastrozole, STX64 and compounds **9(f)**, **11–16**, **19**, **26** and **30**



Compound	R^1 (X = OSO_2NH_2)	R^2	R^3	Aromatase IC_{50} or % inhibition @ 1 μM	STS IC_{50} or % inhibition @ 10 μM
Anastrozole	—	—	—	1.5 ± 0.5 nM	<10%
STX64	—	—	—	300 ± 42 nM	1.5 ± 0.3 nM
9(f)		CH_3	CH_3	2.3 ± 0.09 nM	—
11		CH_3	CH_3	3.5 ± 1.5 nM	10.7 ± 0.8%
12		CH_3	CH_3	4.2 ± 0.10 nM	3000 ± 200 nM
13		CH_3	CH_3	5.0 ± 0.43 nM	73.6 ± 0.1%
14		$-CH_2CH_2CH_2-$		20.7 ± 4.2 nM	21.4 ± 7.9%
15		$-CH_2CH_2CH_2-$		97.1 ± 0.3%	75.3 ± 0.6%
16		$-CH_2CH_2-$		3.4 ± 0.28 nM	<10%
19		CH_3	CH_3	5.5 ± 0.2 nM	<10%
26		CH_3	CH_3	25.5 ± 2.7 nM	21.5 ± 4.7%
30		—	—	<10%	38.5 ± 2%

3.5–25.5 nM. The best aromatase inhibitor is the non-sulfamoylated compound **9(f)** (IC_{50} 2.3 nM), although the leading DASI **11** (3.5 nM) is similarly potent. Given the IC_{50} for Anastrozole is 1.5 nM the introduction of the phenol sulfamate ester STS inhibiting pharmacophore at the expense of a dimethylpropionitrile side-arm is well tolerated in this series of DASI compounds. With the exception of compounds **14** and **26**, all the other DASI compounds maintain aromatase inhibition within an order of magnitude of that of Anastrozole. The lack of a triazole group in compound **30** results in a complete loss of aromatase inhibitory activity. This confirms that the nitrogen-containing heterocycle triazole is crucial for potent aromatase inhibition in these non-steroidal inhibitors. Although our previous DASI work[16] has shown the introduction of a halogen *ortho* to the sulfamate group to be beneficial to aromatase inhibition, possibly due to steric and/or lipophilic factors, no significant effect is observed between the unsubstituted compound **11**, the chloro compound **12** (IC_{50} 4.2 nM) and the bromo compound **13** (IC_{50} 5.0 nM). Converting the dimethylpropionitrile side-arm in **11** to a cyclopropanecarbonitrile group in **16** (IC_{50} 3.4 nM) results in no significant change in activity. However, the introduction of a cyclobutanecarbonitrile side-arm in **14** (IC_{50} 20.7 nM) causes a six-fold reduction in potency against aromatase, presumably due to unfavourable steric interactions affecting the binding motif of the molecule in the aromatase active site. Replacement of the thioether in **11** with an ether linker in compound **19** (IC_{50} 5.5 nM) results in a minimal effect on activity despite the decrease in lipophilicity the introduction of an oxygen atom imparts. However, replacement of the thioether linker with a *trans* alkene group is clearly deleterious for aromatase inhibition, as borne out by the seven-fold difference in activity between **11** (IC_{50} 3.5 nM) and **26** (25.5 nM). It seems reasonable to suggest that the alkene linker of **26** restricts the conformational flexibility of the molecule between the two phenyl rings which, in turn, reduces the binding affinity of **26** to the aromatase active site.

A molecular modelling study was performed to identify possible ligand–enzyme interactions by which Anastrozole and compound **11** might exert inhibitory effects on aromatase. Both were docked into the human aromatase homology model[20] using GOLD.[30] The second highest scoring modes of both inhibitors demonstrate a satisfactory representation of triazole ligation to the haem and are illustrated in Fig. 3 with Anastrozole depicted in pink, compound **11** in green and the haem iron atom as a purple ball. The substructures common to both ligands, the central phenyl ring, the triazolylmethyl moiety and one dimethylpropionitrile group, occupy the same space in the enzyme active site. Potential hydrogen bonding interactions are observed between the main chain nitrogen atom of Leu479 and the cyano component of the dimethylpropionitrile groups in both inhibitors. A number of lipophilic residues lining the enzyme active site (Val370, Leu477, Val373, Phe134, Ile305) form contacts with the common aromatic ring and the nitrile groups. The Ser478 side chain, which has been implicated in the binding of the non-steroidal inhibitor Vorozole,[20] is in close proximity (4.6 Å) to the cyano group of the dimethylpropionitrile side arm of **11**. With induced fit these groups may form a hydrogen bond in the enzyme active site. It is interesting to note there is the possibility for both the dimethylpropionitrile groups in Anastrozole to form hydrogen bonds with Ser478 (5.1 Å and 3.0 Å) with the group replaced

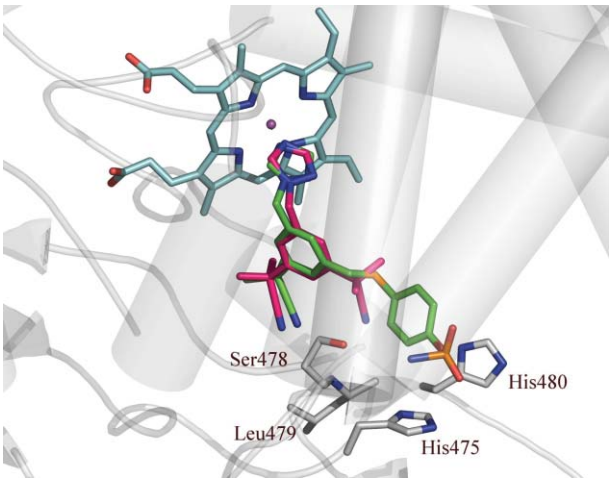


Fig. 3 Docking of Anastrozole (pink) and **11** (green) into the human aromatase homology model of Favia *et al.*[20] using the GOLD docking program version 3.1.1.[30] The haem iron atom is represented by a purple ball and active site amino acid residues are indicated.

in compound **11** appearing to be in the closest proximity (3.0 Å) to this residue. The phenol sulfamate ester group of **11** is in close proximity to His475, His480 and Trp224 and there is a potential for stabilizing interactions of this group with one or more of these amino acids.

From the set of compounds tested there are no examples of strong STS inhibitors *in vitro*. Of the best examples the *ortho* chlorinated derivative **12** has an IC_{50} of 3000 nM which is significantly less potent than STX64 (1.5 nM). Some trends are apparent, however. The presence of the triazole group introduces increased possibility of steric constraints for effective binding within the STS active site. Indeed, the comparative activities of **30**, and its triazole substituted analogue **11** (38.5% *versus* 10.7% inhibition at 10 μM) appear to bear this out. Secondly, as anticipated, and in line with previous observations,[16] the introduction of a halogen atom *ortho* to the sulfamate group increases the ability of the resulting compounds to inhibit STS (*cf.* **12** and **13** with **11** and also **14** *versus* **15**). The electron-withdrawing effect produced by the halogen atom improves the leaving group ability of the corresponding phenol which, *inter alia*, facilitates the inactivation of STS through sulfamoylation. Finally, *trans*-stilbenes are known to structurally mimic oestrogens[31] and hence, their sulfamate ester derivatives may mimic oestrogen sulfamates. However, although oestrone 3-*O*-sulfamate is a potent STS inhibitor, the stilbene diethylstilboestrol monosulfamate is relatively much less active.[32] Similarly, **26**, a congener of diethylstilboestrol monosulfamate, is also a weak inhibitor of STS *in vitro*.

Compounds **11**, together with the STS inhibitor STX64, were docked into the crystal structure of STS.[21] As illustrated in Fig. 4, the DASI compound occupies the same region of the active site as STX64. The sulfamate groups lie in close proximity to the Ca^{2+} ion (purple ball) and the *gem*-diol form of the formylglycine residue in the catalytic cavity. However, it is likely that, compared to STX64, compound **11** with a larger more flexible motif, particularly taking into account the protruding triazolylmethyl moiety and the thioether linker, does not interact as efficiently with the amino acid residues in the hydrophobic tunnel approaching the active

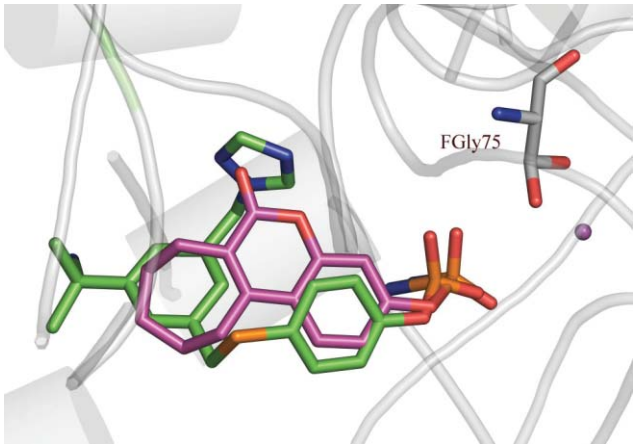


Fig. 4 Docking of **11** (green) and STX64 (purple) into the active site of STS. The Ca^{2+} ion is depicted as a purple ball. Hydrated FGly75 is the crucial mechanistic residue for desulfation.

site. The modes shown represent the binding event before any irreversible interaction takes place.

Inhibition of aromatase and sulfatase *in vivo*

Compounds **11**, **30** and Anastrozole were studied *in vivo* (Fig. 5). Female Wistar rats pre-treated with 200 IU/0.1 mL s.c. of PMSG (pregnant mares' serum gonadotropin) were treated 3 days later with individual agent at a single 10 mg kg^{-1} p.o. dose. Plasma oestradiol (E2) levels and liver STS activity were determined 3 hours after dosing.

At 10 mg kg^{-1} p.o. **11** and **30** were found to be highly active against STS, with near complete inhibition observed for **30** (98.5%) and 83% inhibition evident for **11**. These are encouraging results given that these compounds were observed to be only weak STS inhibitors *in vitro* (Table 1). However, this phenomenon is not unprecedented and the inhibitory activity difference of phenol sulfamate esters *in vitro* and *in vivo* has been previously observed.[16,17] Although the explanation for such a phenomenon is yet to be elucidated, other DMPK (Drug Metabolism and Pharmacokinetics) factors could be involved in transforming the inhibitor and/or delivering the active species to the target enzyme in the *in vivo* setting. Whereas, and as expected, Anastrozole, without a phenol sulfamate ester moiety, was completely ineffective in suppressing STS activity, complete inhibition (100%) of aromatase, shown as a measure of plasma E2 levels, was achieved at 10 mg kg^{-1} p.o. The DASI compound **11** showed only 70% inhibition against aromatase, which is a low figure in contrast to Anastrozole when taking into consideration the similarity of the *in vitro* IC_{50} values for these two compounds. It is possible that other factors such as DMPK issues could be responsible for this result. Compound **30**, however, in agreement with the *in vitro* results (Table 1), showed no significant inhibition against aromatase due to the absence of a haem-ligating triazole group.

Conclusions

A series of novel dual enzyme inhibitors has been synthesised, based upon the clinically potent aromatase inhibitor Anastrozole. These compounds include not only a haem-ligating triazole group important for aromatase inhibition, but also a phenol sulfamate ester group to act against STS activity. Evaluation *in vitro* shows these compounds to be highly potent towards aromatase with inhibitory activities close to the Anastrozole benchmark (IC_{50} = 1.5 nM). In contrast, the level of STS inhibition *in vitro* is not as potent across the series of compounds tested when compared to the non-steroidal STS inhibitor STX64. However, when compound **11** was tested *in vivo*, both the plasma E2 levels and liver STS activity were inhibited potently (STS: 83% and Aromatase: 70%) confirming that **11** is indeed a DASI. Analysis of the structure activity relationship for these compounds suggests that the incorporation of the second pharmacophore in the DASI compounds results in a slight impediment to the activity against both enzymes when compared to the (pharmacophores working as) independent entities. However, any decrease in inhibitory activity against one enzyme is expected to be largely compensated by the dual inhibition provided by these DASIs, which may strengthen the overall effectiveness of this type of endocrine therapy. Optimisation of this new structural class of DASI is still required to maximise the potencies against both aromatase and STS, but this series is clearly worthy of further development. With

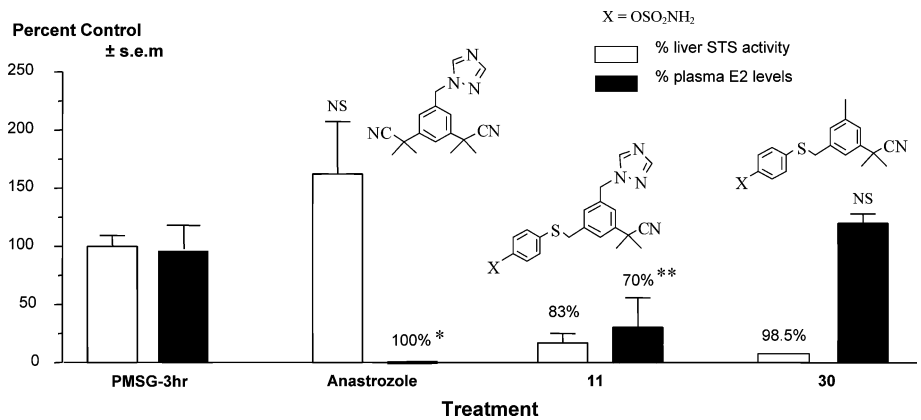


Fig. 5 % Inhibition of aromatase and STS activities in ♀ Wistar rats. Results are expressed as the % of PMSG stimulated E2 levels or % of liver STS activity in untreated rats (means ± se, n = 3). NS, not significant. * Figures above bars represent % inhibition. ** 2 rats gave >80% inhibition, 1 rat gave ~50%.

the DASI concept validated, the development of a novel effective treatment for HDBC is a realistic possibility.

Experimental

Chemistry

Unless otherwise stated HPLC grade solvents were used and commercial reagents and starting materials were used without further purification. Nuclear magnetic resonance spectra were recorded on either a Jeol Delta 270 MHz or Varian Mercury VX 400 MHz spectrometer. ^{1}H NMR spectra were recorded at 270 MHz or 400 MHz with shifts reported in parts per million (ppm, δ) relative to residual chloroform ($\delta_H = 7.26$ ppm) or residual DMSO ($\delta_H = 2.50$ ppm). Coupling constants, J, are reported in Hertz. ^{13}C NMR spectra were recorded at either 67.9 MHz or 100.6 MHz with the central peak of chloroform ($\delta_C = 77.16$ ppm) or DMSO ($\delta_C = 39.52$ ppm) as internal standard. Low resolution mass spectra were obtained from a Micromass platform LCZ ($APCI^+$). FAB high-resolution mass spectra were determined by the EPSRC mass spectrometry centre (Swansea, UK). HPLC analyses were performed on a Waters 996 PDA detector using a Symmetry® C18 column (4.6 × 150 mm) eluting with MeCN–H_2O at 1.0 mL min^{-1}. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Stanford Research Systems Optimelt MPA100 automated melting point system and are uncorrected. Thin layer chromatography was undertaken using Kieselgel 60 F_{254} plates (Merck). Flash column chromatography was performed on silica gel (Sorbsil/Matrex C60) or using Argonaut pre-packed columns with FlashMaster II. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger,[33] and was stored as a solution in toluene as described by Woo *et al.*[34] The experimental data for **1**, **2**, **3(a–c)**, **4(a–c)**, **5(a–c)**, **20–23** are available as ESI.†

Biology

The extent of *in vitro* inhibition of aromatase and STS activities was assessed using intact monolayers of JEG-3 cells. Cells were seeded into 24-well culture plates and maintained in MEM (Flow Laboratories, Irvine, UK) containing supplements and used when 80% confluent. To determine STS activity, cells were incubated for 1 h with [6,7–^{3}H]E1S (5 pmol, 7 × 10^{5} dpm, 60 Ci/mmol; Perkin Elmer LS, Wellesley, MA) in the presence or absence of (0.001–10 000 nmol L^{-1}) inhibitor. The product E1 was separated from E1S by a toluene partition using [4–^{14}C]E1 to monitor procedural losses, and the radioactivity was measured by scintillation spectrometry. Similarly, for aromatase activity, [1β-^{3}H]androstenedione (5 pmol, 30 Ci/mmol, Perkin Elmer LS, MA) was incubated with JEG-3 cells for 1 h in the presence or absence of inhibitor. The product, $^{3}H_2O$, was separated from the substrates using dextran-coated charcoal at 4 °C for 2 h and remaining radioactivity measured by scintillation spectrometry. Each IC_{50} value represents the mean ± SE of triplicate measurements.

The *in vivo* inhibition of aromatase and STS activity by **11** and STX64 was assessed in female Wistar rats. Animals received a single subcutaneous injection of pregnant mare's serum gonadotropin (PMSG, 200 IU, Sigma). Three days later drugs (10 mg kg^{-1}) were administered orally in THF–propylene glycol (10 : 90) as a single dose. Blood and liver samples were obtained 3 h after drug administration. Plasma concentrations of oestradiol were measured using a radio-immunoassay kit (Diagnostic Products Corporation, CA) to monitor the extent of aromatase inhibition. Liver STS activity was determined to assess the extent of STS inhibition. Results are expressed as the percentage of PMSG stimulated oestradiol levels for aromatase activity or percentage of activity in untreated animals for STS activity (means ± se, $n = 3$).

Molecular modelling

i) Homology model of the human aromatase enzyme. Models of Anastrozole and **11** were built in the SYBYL7.1[35] molecular modelling program. To obtain low energy conformations of these models energy minimization was performed to convergence using the MMFF94s force field with MMFF94 charges.

A homology model of the human aromatase enzyme, which is based on the crystal structure of the human CYP2C9 metabolic enzyme,[36] as described by Favia *et al.*[20] (PDB accession code 1TQA) was read into using SYBYL7.1. Hydrogen atoms were built onto the model and all atoms except hydrogens were fixed in aggregates. Hydrogen atom positions were then optimized to convergence using the Tripos force field with Gasteiger–Hückel charges.

The GOLD[30] docking program v3.1.1 was used to dock the Anastrozole and **11** models to the aromatase model. The aromatase active site was defined as a 12 Å sphere around the haem group iron atom. A distance constraint (minimum = 2.00 Å, maximum = 2.30 Å) was applied between the ligating triazole nitrogen atom of the ligand to the haem iron atom. The coordination number of the iron atom was defined as 6. The ligands were then docked to the enzyme a total of 25 times each using the GOLDScore fitness function.

ii) Crystal structure of steroid sulfatase. The 1P49.pdb crystal structure of Human Placental Oestrone/DHEA Sulfatase was used for the building of the *gem*-diol form of STS.[21] This involved a point mutation of the ALS75 residue in the crystal structure to the *gem*-diol form of the structure using editing tools within SYBYL7.1. The resulting structure was then minimised with the backbone atoms fixed to allow the *gem*-diol and surrounding side chain atoms to adopt low energy confirmations. Minimisations were undertaken using SYBYL7.1 applying the AMBER7 99 forcefield with Gasteiger–Hückel charges as implemented within SYBYL7.1. In order to mimic the sulfamate group of E1S, all sulfamate based compounds are docked into the active site with their sulfamate group in its mono-anionic form (*i.e.* $-OSO_2NH^-$).[37]

Syntheses

2-(3-Hydroxymethyl-5-[1,2,4]triazol-1-ylmethylphenyl)-2-methyl-propionitrile 6(a). A mixture of compound **5(a)** (0.500 g, 1.76 mmol) and polyethylene glycol 400 (6.0 g) was heated to 80 °C with stirring until a clear solution had formed. $NaBH_4$ (0.200 g, 5.28 mmol) was added carefully resulting in evolution of gas. The reaction mixture was stirred vigorously at 80 °C for 16 h. An extremely viscous residue formed that gradually dissolved in CH_2Cl_2 (50 mL) with warming (30 °C). This solution was washed with 1 M $HCl_{(aq)}$ (10 mL) and then carefully neutralised with

$NaHSO_{3\,(aq)}$. Washed with distilled H_2O (50 mL × 4) and brine (50 mL) and dried ($MgSO_4$). Solvent was removed *in vacuo* to leave a yellow viscous oil. Column chromatography (EtOAc) eluted **6(a)** as a white crystalline solid (0.351 g, 78%), mp 114–116 °C. ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.70 (6H, s, $ArC(CH_3)_2CN$), 2.19–2.28 (1H, t, J = 5.5 Hz, $ArCH_2OH$), 4.69–4.71 (2H, d, J = 5.5 Hz, $ArCH_2OH$), 5.35 (2H, s, $ArCH_2N$), 7.17 (1H, s, ArH), 7.29 (1H, s, ArH), 7.44 (1H, s, ArH), 7.95 (1H, s, $C_2H_2N_3$) and 8.09 (1H, s, $C_2H_2N_3$); *m/z* (APCI$^+$) 257 ((M + H)$^+$, 100%), 188 (88); HRMS (ES$^+$) 257.1392. $C_{14}H_{17}N_4O$ requires 257.1397.

1-(3-Hydroxymethyl-5-[1,2,4]triazol-1-ylmethylphenyl)cyclobutanecarbonitrile 6(b). Compound **6(b)** was prepared from **5(b)** using similar conditions to those described for the synthesis of compound **6(a)**. Column chromatography (EtOAc) eluted **6(b)** as a colourless viscous oil (0.238 g, 64%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 2.08–2.15 (1H, m, CH_2), 2.20–2.23 (1H, t, J = 5.8 Hz, $ArCH_2OH$), 2.46–2.55 (1H, m, CH_2), 2.61–2.68 (2H, m, CH_2), 2.84–2.89 (2H, m, CH_2), 4.76–4.77 (2H, d, J = 5.8 Hz, $ArCH_2OH$), 5.41 (2H, s, $ArCH_2N$), 7.25 (1H, s, ArH), 7.28 (1H, s, ArH), 7.45 (1H, s, ArH), 8.02 (1H, s, $C_2H_2N_3$), and 8.15 (1H, s, $C_2H_2N_3$); *m/z* (APCI$^+$) 269 ((M + H)$^+$, 100%).

1-(3-Hydroxymethyl-5-[1,2,4]triazol-1-ylmethyl-phenyl)-cyclopropanecarbonitrile 6(c). Compound **6(c)** was prepared from **5(c)** using similar conditions to those described for the synthesis of compound **6(a)**. Chromatography (EtOAc) eluted **6(c)** as a colourless viscous oil (0.274 g, 78%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.36–1.40 (2H, dd, J = 2.5 & 5.3 Hz, CH_2), 1.70–1.75 (2H, dd, J = 2.5 & 5.3 Hz, CH_2), 1.89–1.94 (1H, t, J = 5.8 Hz, $ArCH_2OH$), 4.66–4.68 (2H, d, J = 5.8 Hz, $ArCH_2OH$), 5.31 (2H, s, $ArCH_2N$), 7.12 (1H, s, ArH), 7.14 (1H, s, ArH), 7.24 (1H, s, ArH), 7.95 (1H, s, $C_2H_2N_3$), and 8.08 (1H, s, $C_2H_2N_3$); *m/z* (APCI$^+$) 255 ((M + H)$^+$, 100%); HRMS (ES$^+$) 255.1237. $C_{14}H_{15}N_4O$ requires 255.1240

2-[3-(4-Hydroxyphenylsulfanylmethyl)-5-[1,2,4]triazol-1-ylmethyl-phenyl]-2-methylpropionitrile 8. To a solution of **6(a)** (0.322 mg, 1.26 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0 °C under inert conditions was added anhydrous pyridine (0.15 mL, 1.88 mmol) followed by $SOCl_2$ (0.14 mL, 1.88 mmol). The reaction mixture was stirred at room temperature for 2 h and then refluxed for 1 h. After cooling, solvent was removed *in vacuo* to obtain **7** as a brown oil. Compound **7** was dissolved in anhydrous DMF (5 mL) at room temperature and under inert conditions and treated with K_2CO_3 (1.74 g, 12.6 mmol) followed by a solution of 4-hydroxythiophenol (0.196 g, 1.51 mmol) in anhydrous DMF (0.5 mL). The resulting dark yellow suspension was heated at 50 °C for 18 h and then diluted with EtOAc (40 mL). The organic layer was washed with brine (50 mL), dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give dark brown residues. Column chromatography (EtOAc) eluted **8** as a light brown viscous oil (0.090 g, 20%). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.66 (6H, s, $ArC(CH_3)_2CN$), 3.87 (2H, s, $ArCH_2S$), 5.25 (2H, s, $ArCH_2N$), 6.68 (2H, m, ArH), 6.74 (1H, s, ArH), 7.04 (2H, m, ArH), 7.19 (1H, m, ArH), 7.24 (1H, m, ArH), 7.95 (1H, s, $C_2H_2N_3$) and 7.99 (1H, s, $C_2H_2N_3$) and 8.70 (1H, br s, OH); *m/z* (ES$^+$) 365 ((M + H)$^+$, 100%), 296 (7), 269 (8); HRMS (FAB$^+$) 365.1441 $C_{20}H_{21}N_4OS$ requires 365.1436.

Methanesulfonic acid 2-chloro-4-[3-(cyanodimethylmethyl)-5-[1,2,4]triazol-1-ylmethylbenzylsulfanyl]phenyl ester 9(a). (Cyanomethyl)trimethylphosphonium iodide (0.114 g, 0.470 mmol) was added to a mixture of **6(a)** (0.100 g, 0.390 mmol), methanesulfonic acid-2-chloro-4-mercaptophenyl ester (0.136 g, 0.570 mmol), diisopropylethylamine (88.0 μL, 0.510 mmol) and propionitrile (1.0 mL) under inert conditions. The mixture was then set to stir at 92 °C. After 17 h the reaction was allowed to cool. CH_2Cl_2 (20 mL) and distilled H_2O (20 mL) were added and the aqueous layer separated and extracted with CH_2Cl_2 (20 mL × 2). The organic fractions were combined and washed with brine (20 mL), dried ($MgSO_4$) and solvent removed *in vacuo* to leave yellow residues. Column chromatography (EtOAc) eluted **9(a)** as a yellow viscous oil (0.114 g, 61%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.60 (6H, s, $ArC(CH_3)_2CN$), 3.19 (3H, s, $ArOSO_2CH_3$), 4.01 (2H, s, $ArCH_2S$), 5.26 (2H, s, $ArCH_2N$), 6.96 (1H, s, ArH), 7.07–7.12 (1H, dd, J = 2.3 & 6.3 Hz, ArH), 7.19–7.26 (4H, m, ArH), 7.92 (1H, s, $C_2H_2N_3$) and 8.04 (1H, s, $C_2H_2N_3$); *m/z* (APCI$^+$) 479 ((^{37}ClM + H)$^+$, 45%), 477 ((^{35}ClM + H)$^+$, 100).

Methanesulfonic acid 2-bromo-4-[3-(cyanodimethylmethyl)-5-[1,2,4]triazol-1-ylmethylbenzylsulfanyl]phenyl ester 9(b). Compound **9(b)** was prepared from **6(a)** and 2-bromo-4-mercaptophenyl methanesulfonate using similar conditions to those described for the synthesis of compound **9(a)**. Column chromatography (EtOAc) eluted **9(b)** as a yellow viscous oil (0.152 g, 75%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.62 (6H, s, $ArC(CH_3)_2CN$), 3.25 (3H, s, $ArOSO_2CH_3$), 4.04 (2H, s, $ArCH_2SAr$), 5.31 (2H, s, $ArCH_2N$), 7.00 (1H, s, ArH), 7.16–7.31 (4H, m, ArH), 7.45–7.46 (1H, d, J = 2.2 Hz, ArH), 7.97 (1H, s, $C_2H_2N_3$) and 8.10 (1H, s, $C_2H_2N_3$); *m/z* (APCI$^+$) 523 ((^{81}BrM + H)$^+$, 100%), 521 ((^{79}BrM + H)$^+$, 85).

Methanesulfonic acid 4-[3-(1-cyanocyclobutyl)-5-[1,2,4]triazol-1-ylmethylbenzylsulfanyl]phenyl ester 9(c). Compound **9(c)** was prepared from **6(b)** and methanesulfonic acid 4-mercaptophenyl ester using similar conditions to those described for the synthesis of compound **9(a)**. Column chromatography (EtOAc) eluted **9(c)** as a yellow viscous oil (0.151 g, 92%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.99–2.03 (1H, m, CH_2), 2.32–2.58 (3H, m, CH_2), 2.71–2.85 (2H, m, CH_2), 3.15 (3H, s, $ArOSO_2CH_3$), 4.06 (2H, s, $ArCH_2S$), 5.29 (2H, s, $ArCH_2N$), 6.97 (1H, s, ArH), 7.08–7.35 (6H, m, ArH), 7.97 (1H, s, $C_2H_2N_3$) and 8.08 (1H, s, $C_2H_2N_3$); *m/z* (APCI$^+$) 455 ((M + H)$^+$, 100%).

4-({3-[(1H-1,2,4-triazol-1-yl)methyl]-5-(1-cyanocyclobutyl)benzyl}sulfanyl)-2-chlorophenyl methanesulfonate 9(d). Compound **9(d)** was prepared from **6(b)** and methanesulfonic acid-2-chloro-4-mercaptophenyl ester using similar conditions to those described for the synthesis of compound **9(a)**. Column chromatography (EtOAc) eluted **9(d)** as a yellow viscous oil (0.054 g, 30%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.96–2.08 (1H, m, CH_2), 2.31–2.58 (3H, m, CH_2), 2.70–2.83 (2H, m, CH_2), 3.24 (3H, s, $ArOSO_2CH_3$), 4.05 (2H, s, $ArCH_2S$), 5.31 (2H, s, $ArCH_2N$), 7.00 (1H, s, ArH), 7.08–7.32 (5H, m, ArH), 7.96 (1H, s, $C_2H_2N_3$) and 8.10 (1H, s, $C_2H_2N_3$); *m/z* (APCI$^+$) 489 ((M + H)$^+$, 100%).

Methanesulfonic acid 4-[3-(1-cyanocyclopropyl)-5-[1,2,4]triazol-1-ylmethylbenzylsulfanyl]phenyl ester 9(e). Compound **9(e)** was prepared from **6(c)** and methanesulfonic acid 4-mercaptophenyl ester using similar conditions to those described for the synthesis

of compound **9(a)**. Column chromatography (EtOAc) eluted **9(e)** as a yellow viscous oil (0.134 g, 77%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.29–1.34 (2H, dd, J = 5.0 & 7.9 Hz, CH_2), 1.69–1.74 (2H, dd, J = 5.0 & 7.9 Hz, CH_2), 3.16 (3H, s, $ArOSO_2CH_3$), 4.01 (2H, s, $ArCH_2S$), 5.27 (2H, s, $ArCH_2N$), 6.94 (1H, s, ArH), 7.05 (1H, s, ArH), 7.08 (1H, s, ArH), 7.14–7.28 (4H, dd, J = 8.6 & 27.9 Hz, ArH), 7.96 (1H, s, $C_2H_2N_3$) and 8.07 (1H, s, $C_2H_2N_3$); m/z (APCI$^+$) 441 ((M + H)$^+$, 100%).

2-Methyl-2-(3-phenylsulfanylmethyl-5-[1,2,4]triazol-1-ylmethyl-phenyl)propionitrile 9(f). Compound **9(f)** was prepared from **6(a)** and thiophenol using similar conditions to those described for the synthesis of compound **9(a)**. Column chromatography (EtOAc) eluted **9(f)** as a yellow oil (0.047 g, 68%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.61 (6H, s, $ArC(CH_3)_2CN$), 4.04 (2H, s, $ArCH_2SAr$), 5.30 (2H, s, $ArCH_2N$), 7.05 (1H, s, ArH), 7.18–7.25 (7H, m, ArH), 7.97 (1H, s, $C_2H_2N_3$) and 8.01 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (100.6 MHz, $CDCl_3$) 29.1 (CH_3), 37.1 (C), 39.2 (CH_2), 53.3 (CH_2), 123.6 (CH), 124.1 (C), 125.9 (CH), 127.2 (CH), 127.9 (CH), 129.1 (CH), 131.1 (CH), 135.0 (C), 135.8 (C), 139.9 (C), 142.6 (C), 143.2 (CH) and 152.4 (CH); m/z (APCI$^+$) 349 ((M + H)$^+$, 100%); HRMS (ES$^+$) 349.1481.$C_{20}H_{21}N_4S$ requires 349.1481.

2-[3-(3-Chloro-4-hydroxyphenylsulfanylmethyl)-5-[1,2,4]triazol-1-ylmethylphenyl]-2-methylpropionitrile 10(a). Compound **9(a)** (0.100 g, 0.210 mmol) was dissolved in THF (2.5 mL) and MeOH (1.5 mL) to which 2 M $NaOH_{(aq)}$ (0.52 mL) was added. The mixture was set to stir at room temperature for 1 h. THF was removed *in vacuo* and the residues taken up in EtOAc (20 mL) and washed with 2 M $KHSO_{4\,(aq)}$ (20 mL), distilled H_2O (20 mL × 2) and brine (20 mL). The organic layer was then dried over $MgSO_4$ and solvent removed *in vacuo* to leave a colourless viscous oil. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **10(a)** as a colourless viscous oil that crystallised on standing to a white crystalline solid (0.071 g, 84%), mp 131–132 °C. ^{1}H NMR δ_H (400 MHz, DMSO-d_6) 1.59 (6H, s, $ArC(CH_3)_2CN$), 4.10 (2H, s, $ArCH_2SAr$), 5.43 (2H, s, $ArCH_2N$), 6.85–6.87 (1H, d, J = 8.6 Hz, ArH), 7.07–7.10 (1H, dd, J = 2.3 & 8.6 Hz, ArH), 7.14 (1H, s, ArH), 7.20 (1H, s, ArH), 7.26 (1H, s, ArH), 7.34 (1H, s, ArH), 8.00 (1H, s, $C_2H_2N_3$), 8.66 (1H, s, $C_2H_2N_3$) and 10.37 (1H, s, ArOH); m/z (APCI$^+$) 401 ((^{37}ClM + H)$^+$, 30%), 399 ((^{35}ClM + H)$^+$, 100).

2-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-[(3-bromo-4-hydroxyphenylthio)methyl]phenyl}-2-methylpropanenitrile 10(b). Compound **10(b)** was prepared from **9(b)** using similar conditions to those described for the synthesis of compound **10(a)**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **10(b)** as a colourless viscous oil (0.087 g, 68%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.63 (6H, s, $ArC(CH_3)_2CN$), 3.90 (2H, s, $ArCH_2SAr$), 5.29 (2H, s, $ArCH_2N$), 6.75 (1H, br s, ArOH), 6.83–6.87 (2H, m, ArH), 7.06–7.09 (1H, dd, J = 2.2 & 8.4 Hz, ArH), 7.14 (1H, s, ArH), 7.24 (1H, s, ArH), 7.30–7.31 (1H, d, J = 2.2 Hz, ArH), 7.99 (1H, s, $C_2H_2N_3$) and 8.04 (1H, s, $C_2H_2N_3$); m/z (APCI$^+$) 445 ((^{81}BrM + H)$^+$, 89%), 443 ((^{79}BrM + H)$^+$, 100).

1-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-[(4-hydroxyphenylthio)methyl]phenyl}cyclobutanecarbonitrile 10(c). Compound **10(c)** was prepared from **9(c)** using similar conditions to those described for the synthesis of compound **10(a)**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **10(c)** as a colourless viscous oil (0.091 g, 82%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.93–2.03 (1H, m, CH_2), 2.31–2.58 (3H, m, CH_2), 2.70–2.82 (2H, m, CH_2), 3.86 (2H, s, $ArCH_2S$), 5.23 (2H, s, $ArCH_2N$), 6.66–6.72 (3H, m, ArH), 7.01–7.08 (2H, dd, J = 2.2 & 6.7, ArH), 7.15 (2H, s, ArH), 7.92 (1H, s, $C_2H_2N_3$), 7.99 (1H, s, $C_2H_2N_3$) and 8.18 (1H, br s, ArOH); m/z (APCI$^+$) 377 ((M + H)$^+$, 100%).

1-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-[(3-chloro-4-hydroxyphenylthio)methyl]phenyl}cyclobutanecarbonitrile 10(d). Compound **10(d)** was prepared from **9(d)** using similar conditions to those described for the synthesis of compound **10(a)** and was obtained as a light yellow viscous oil (0.039 g, 99%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.92–2.12 (1H, m, CH_2), 2.31–2.62 (3H, m, CH_2), 2.65–2.88 (2H, m, CH_2), 3.89 (2H, s, $ArCH_2SAr$), 5.28 (2H, s, $ArCH_2N$), 6.81–7.28 (6H, m, ArH), 7.99 (1H, s, $C_2H_2N_3$), 7.99 (1H, s, $C_2H_2N_3$) and 8.06 (1H, br s, ArOH); m/z (APCI$^+$) 411 ((M + H)$^+$, 100%).

1-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-[(4-hydroxyphenylthio)methyl]phenyl}cyclopropanecarbonitrile 10(e). Compound **10(e)** was prepared from **9(e)** using similar conditions to those described for the synthesis of compound **10(a)**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **10(e)** as a white solid (0.076 g, 72%), mp 135–137 °C. ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.31–1.35 (2H, dd, J = 5.2 & 8.0 Hz, CH_2), 1.68–1.73 (2H, dd, J = 5.2 & 8.0 Hz, CH_2), 3.83 (2H, s, $ArCH_2S$), 5.21 (2H, s, $ArCH_2N$), 6.65–6.70 (3H, m, ArH), 6.99–7.04 (3H, m, ArH), 7.11 (1H, s, ArH), 7.91 (1H, s, $C_2H_2N_3$), 7.99 (1H, s, $C_2H_2N_3$) and 8.12 (1H, s, ArOH); m/z (APCI$^+$) 363 ((M + H)$^+$, 100%).

Sulfamic acid 4-[3-(cyanodimethylmethyl)-5-[1,2,4]triazol-1-ylmethylbenzylsulfanyl]phenyl ester 11. Sulfamoyl chloride in toluene (1.94 mL, 1.17 mmol) was transferred to a reaction vessel and the solvent removed under vacuum at 30 °C. On cooling a white solid formed to which was added a solution of **8** (0.085 g, 0.233 mmol) in *N,N*-dimethylacetamide (1.5 mL) at 0 °C to form a colourless solution. The reaction mixture was left to stir at room temperature under inert conditions for 20 h. The reaction mixture was then poured into EtOAc (30 mL) and washed with distilled H_2O (30 mL × 4) and brine (30 mL). Dried ($MgSO_4$) and solvent removed *in vacuo* to leave off white residues. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **11** as a colourless viscous oil (0.060 g, 58%). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.73 (6H, s, $ArC(CH_3)_2CN$), 4.04 (2H, s, $ArCH_2SAr$), 5.25 (2H, s, CH_2N), 6.58 (1H, s, ArH), 6.87 (2H, br s, $ArOSO_2NH_2$), 7.15 (4H, s, ArH), 7.23 (1H, s, ArH), 7.34 (1H, m, ArH), 7.57 (1H, s, $C_2H_2N_3$) and 7.85 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (100.6 MHz, DMSO-d_6) 28.2 (CH_3), 36.5 (C), 36.8 (CH_2), 51.8 (CH_2), 122.8 (C), 123.6 (CH), 124.4 (CH), 125.4 (CH), 127.5 (CH), 130.1 (CH), 133.8 (C), 137.3 (C), 138.8 (C), 142.0 (C), 144.4 (CH), 148.5 (C) and 151.9 (CH); m/z (ES$^+$) 444 ((M + H)$^+$, 100%); HRMS (FAB$^+$) 444.1170. $C_{20}H_{22}N_5O_3S_2$ requires 444.1164.

Sulfamic acid 2-chloro-4-[3-(cyanodimethylmethyl)-5-[1,2,4]-triazol-1-ylmethylbenzylsulfanyl]phenyl ester 12. Compound **12** was prepared from **10(a)** using similar conditions to those described for the synthesis of compound **11**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **12** as a white solid (0.051 g, 85%), mp 120–124 °C. ^{1}H NMR δ_H (270 MHz, DMSO-d_6) 1.62 (6H, s, $ArC(CH_3)_2CN$), 4.32 (2H, s, $ArCH_2S$), 5.44 (2H, s, $ArCH_2N$), 7.21 (1H, s, ArH), 7.34–7.39 (3H, m, ArH), 7.45 (1H, s, ArH), 7.53–7.54 (1H, d, J = 1.5 Hz, ArH), 7.99 (1H, s, $C_2H_2N_3$),

8.27 (2H, br s, $ArOSO_2NH_2$) and 8.66 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (100.6 MHz, DMSO-d_6) 28.3 (CH_3), 36.2 (CH_2), 36.5 (C), 51.8 (CH_2), 123.7 (CH), 124.1 (CH), 124.3 (C), 125.5 (CH), 127.0 (CH), 127.5 (CH), 128.3 (C), 129.6 (CH), 135.5 (C), 137.4 (C), 138.5 (C), 142.0 (C), 144.2 (C), 144.4 (CH) and 151.9 (CH); *m/z* (APCI$^-$) 478 ((^{37}ClM − H)$^-$, 18%), 476 ((^{35}ClM − H)$^-$, 40), 399 (40), 397 ((^{35}ClM − $C_2H_2N_3$)$^-$, 100). HRMS (ES$^+$) 478.0773. $C_{20}H_{21}ClN_5O_3S_2$ requires 478.0769.

Sulfamic acid 2-bromo-4-[3-(cyanodimethylmethyl)-5-[1,2,4]-triazol-1-ylmethylbenzylsulfanyl]phenyl ester 13. Compound **13** was prepared from **10(b)** using similar conditions to those described for the synthesis of compound **11**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **13** as a white solid (0.082 g, 96%), mp 111–115 °C. ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.63 (6H, s, $ArC(CH_3)_2CN$), 4.06 (2H, s, $ArCH_2SAr$), 5.28 (2H, s, $ArCH_2N$), 6.54 (1H, s, ArH), 6.97–7.01 (1H, dd, J = 2.2 & 8.6 Hz, ArH), 7.09 (2H, br s, $ArOSO_2NH_2$), 7.25–7.31 (2H, m, ArH), 7.35 (1H, s, ArH), 7.40–7.41 (1H, d, J = 2.2 Hz, ArH), 7.63 (1H, s, $C_2H_2N_3$) and 7.87 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (100.6 MHz, DMSO-d_6) 28.7 (CH_3), 36.3 (C), 36.5 (CH_2), 51.8 (CH_2), 116.5 (C), 123.6 (C), 123.7 (CH), 124.3 (CH), 125.4 (CH), 127.5 (CH), 129.0 (CH), 132.6 (CH), 135.6 (C), 137.4 (C), 138.5 (C), 142.0 (C), 144.4 (CH), 145.6 (C) and 151.9 (CH); *m/z* (APCI$^+$) 524 ((^{81}BrM + H)$^+$, 100%), 522 ((^{79}BrM + H)$^+$, 80). HRMS (ES$^+$) 522.0267. $C_{20}H_{21}BrN_5O_3S_2$ requires 522.0264.

Sulfamic acid 4-[3-(1-cyanocyclobutyl)-5-[1,2,4]triazol-1-ylmethylbenzylsulfanyl]phenyl ester 14. Compound **14** was prepared from **10(c)** using similar conditions to those described for the synthesis of compound **11**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **14** as a colourless viscous oil (0.058 g, 55%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 2.03–2.10 (1H, m, CH_2), 2.42–2.61 (3H, m, CH_2), 2.77–2.82 (2H, m, CH_2), 4.03 (2H, s, $ArCH_2SAr$), 5.27 (2H, s, $ArCH_2N$), 6.59 (1H, s, ArH), 6.88 (2H, br s, $ArOSO_2NH_2$), 7.11–7.33 (6H, m, ArH), 7.59 (1H, s, $C_2H_2N_3$) and 7.92 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (67.9 MHz, $CDCl_3$) 17.2 (C), 34.6 (CH_2), 38.2 (CH_2), 40.0 (CH_2), 53.2 (CH_2), 116.0 (C), 123.4 (CH), 124.0 (CH), 126.5 (CH), 126.8 (CH), 132.5 (CH), 132.7 (C), 135.6 (C), 140.1 (C), 141.2 (C), 143.9 (CH), 149.3 (C) and 151.2 (CH); *m/z* (APCI$^+$) 456 ((M + H)$^+$, 100%), 377 (20); HRMS (ES$^+$) 456.1159. $C_{21}H_{22}N_5O_3S_2$ requires 456.1159.

Sulfamic acid 2-chloro-4-[3-(1-cyanocyclobutyl)-5-[1,2,4]triazol-1-ylmethylbenzylsulfanyl]phenyl ester 15. Compound **15** was prepared from **10(d)** using similar conditions to those described for the synthesis of compound **11**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **15** as a white amorphous solid (0.037 g, 77%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 2.04–2.13 (1H, m, CH_2), 2.44–2.67 (3H, m, CH_2), 2.80–2.87 (2H, m, CH_2), 4.06 (2H, s, $ArCH_2SAr$), 5.28 (2H, s, $ArCH_2N$), 6.56 (1H, s, ArH), 6.89–6.97 (1H, dd, J = 2.2 & 8.6 Hz, ArH), 7.10 (2H, br s, $ArOSO_2NH_2$), 7.23–7.30 (4H, m, ArH), 7.62 (1H, s, $C_2H_2N_3$) and 7.86 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (67.9 MHz, DMSO-d_6) 17.3 (C), 34.0 (CH_2), 34.4 (CH_2), 36.8 (CH_2), 52.2 (CH_2), 124.6 (CH), 124.7 (CH), 126.4 (CH), 127.5 (C), 128.2 (CH), 128.8 (CH), 130.2 (CH), 136.0 (C), 138.1 (C), 139.2 (C), 140.8 (C), 144.7 (C), 144.9 (CH) and 152.4 (CH) (one overlapping resonance); *m/z* (APCI$^+$) 492 ((^{37}ClM + H)$^+$, 40%), 490 ((^{35}ClM + H)$^+$, 100); HRMS (ES$^+$) 490.0768. $C_{21}H_{21}ClN_5O_3S$ requires 490.0769.

Sulfamic acid 4-[3-(1-cyanocyclopropyl)-5-[1,2,4]triazol-1-ylmethylbenzylsulfanyl]phenyl ester 16. Compound **16** was prepared from **10(e)** using similar conditions to those described for the synthesis of compound **11**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **16** as a colourless viscous oil (0.055 g, 73%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.39–1.44 (2H, dd, J = 5.0 & 7.9 Hz, CH_2), 1.74–1.79 (2H, dd, J = 5.0 & 7.9 Hz, CH_2), 4.01 (2H, s, $ArCH_2SAr$), 5.21 (2H, s, $ArCH_2N$), 6.55 (1H, s, ArH), 6.88 (2H, br s, $ArOSO_2NH_2$), 7.03 (1H, s, ArH), 7.11–7.21 (5H, m, ArH), 7.54 (1H, s, $C_2H_2N_3$) and 7.85 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (67.9 MHz, $CDCl_3$) 13.7 (CH_2), 18.7 (C), 38.3 (CH_2), 53.2 (CH_2), 122.2 (C), 123.4 (CH), 123.9 (CH), 126.5 (CH), 126.6 (CH), 132.4 (CH), 132.9 (C), 135.6 (C), 137.6 (C), 140.1 (C), 142.9 (CH), 149.3 (C) and 151.1 (CH); *m/z* (APCI$^+$) 442 ((M + H)$^+$, 100%), 363 (35); HRMS (ES$^+$) 442.1002. $C_{20}H_{20}N_5O_3S_2$ requires 442.1003.

2-(3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-{[4-(benzyloxy)phenoxy]-methyl}phenyl)-2-methylpropanenitrile 17. Under inert conditions compound **6(a)** (0.150 g, 0.586 mmol) and 4-(benzyloxy)-phenol (0.098 g, 0.488 mmol) were dissolved in anhydrous CH_2Cl_2 (3 mL) to form a clear solution to which was added triphenylphosphine polystyrene (0.586 g, 0.586 mmol) and the mixture cooled to 0 °C. DEAD (92.3 μL, 0.586 mmol) was added drop-wise and the reaction allowed to warm to room temperature and left to stir for 16 h. The reaction mixture was then filtered to remove the polystyrene which was washed with CH_2Cl_2 (25 mL × 6). These washings were combined with the filtrate and washed with distilled H_2O (25 mL × 2) and brine (25 mL) then dried ($MgSO_4$) and solvent removed *in vacuo*. Column chromatography (EtOAc) eluted **17** as a light yellow solid (0.175 g, 82%), mp 119.3–120.5 °C. ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.70 (6H, s, $ArC(CH_3)_2CN$), 4.98 (2H, s, $ArCH_2O$), 5.01 (2H, s, $ArCH_2O$), 5.37 (2H, s, $ArCH_2N$), 6.84–6.92 (4H, m, ArH), 7.29–7.49 (8H, m, ArH), 7.99 (1H, s, $C_2H_2N_3$) and 8.10 (1H, s, $C_2H_2N_3$); *m/z* (APCI$^+$) 439 ((M + H)$^+$, 100%).

2-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-[(4-hydroxyphenoxy)-methyl]phenyl}-2-methylpropanenitrile 18. Compound **17** (0.100 g, 0.228 mmol) was dissolved in THF (2.5 mL) and MeOH (2.5 mL) to form a clear solution to which was added 5% Pd/C (0.010 g) to form a black suspension on vigorous stirring. The reaction vessel was degassed and the mixture set to stir under a hydrogen atmosphere for 16 h. The reaction mixture was filtered through Celite which was subsequently washed with THF (30 mL × 2). Solvent was removed *in vacuo* to leave brown residues. Column chromatography (EtOAc) eluted **18** as a colourless viscous oil (0.060 g, 76%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.70 (6H, s, $ArC(CH_3)_2CN$), 4.97 (2H, s, $ArCH_2O$), 5.17 (1H, s, ArOH), 5.37 (2H, s, $ArCH_2N$), 6.73–6.82 (4H, m, ArH), 7.22 (1H, s, ArH), 7.33 (1H, s, ArH), 7.49 (1H, s, ArH), 7.99 (1H, s, $C_2H_2N_2$) and 8.10 (1H, s, $C_2H_2N_2$); *m/z* (APCI$^+$) 349 ((M + H)$^+$, 100%).

Sulfamic acid 4-[3-(cyanodimethylmethyl)-5-[1,2,4]triazol-1-ylmethylbenzyloxy]phenyl ester 19. Compound **19** was prepared from **18** (0.057 g, 0.164 mmol) using similar conditions to those described for the synthesis of compound **11**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **19** as a white solid (0.049 g, 69%), mp 158–160 °C (found: C, 56.3; H, 5.2; N, 16.0. $C_{20}H_{21}N_5O_4S$ requires C, 56.2; H, 5.0; N, 16.4%). ^{1}H NMR

δ_H (270 MHz, DMSO-d_6) 1.68 (6H, s, $ArC(CH_3)_2CN$), 5.11 (2H, s, $ArCH_2OAr$), 5.48 (2H, s, $ArCH_2N$), 7.05–7.21 (4H, dd, $J = 8.8$ & 53.2 Hz, AA′BB′), 7.30 (1H, s, ArH), 7.46 (1H, s, ArH), 7.57 (1H, s, ArH), 7.91 (2H, br s, $ArOSO_2NH_2$), 8.00 (1H, s, $C_2H_2N_3$) and 8.69 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (100.6 MHz, DMSO-d_6) 28.3 (CH_3), 36.6 (C), 51.8 (CH_2), 69.3 (CH_2), 115.6 (CH), 123.4 (CH), 124.2 (CH), 124.4 (CH), 126.5 (CH), 137.4 (C), 138.2 (C), 142.2 (C), 143.8 (C), 144.4 (CH), 151.9 (CH) and 156.5 (C) (one overlapping resonance); m/z (APCI$^+$) 428 ((M + H)$^+$, 100%); HRMS (ES$^+$) 428.1385. $C_{20}H_{22}N_5O_4S$ requires 428.1387.

2-(3-Bromo-5-[1,2,4]-triazole-1-ylmethylphenyl)-2-methylpropionitrile 24. Compound **24** was prepared from **23** (3.20 g, 10.09 mmol) using similar conditions to those described for the synthesis of compound **5(a)**. Column chromatography (EtOAc) eluted **23** as a clear viscous oil that crystallised on standing to give a colourless crystalline solid (1.97 g, 6.46 mmol, 64%), mp 71–72 °C. ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.68 (6H, s, $ArC(CH_3)_2CN$), 5.33 (2H, s, $ArCH_2N$), 7.40–7.41 (2H, t, $J =$ 1.7 Hz, ArH), 7.54–7.55 (1H, t, $J = 1.7$ Hz, ArH), 7.99 (1H, s, $C_2H_2N_3$) and 8.12 (1H, s, $C_2H_2N_3$); m/z (EI$^+$) 307 ((^{81}BrM + H)$^+$, 100%), 305 ((^{79}BrM + H)$^+$, 99).

2-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-(4-hydroxystyryl)phenyl}-2-methylpropanenitrile 25. Under inert conditions compound **24** (0.100 g, 0.328 mmol), 4-vinylphenol [10% wt in propylene glycol] (0.787 g, 0.655 mmol), NEt_3 (91.0 μL, 0.655 mmol) and PPh_3 (0.029 g, 0.112 mmol) were dissolved in anhydrous THF (1.5 mL) and the resulting clear solution was thoroughly degassed. $Pd(OAc)_2$ (0.012 g, 0.056 mmol) was added and the reaction mixture set to reflux for 22 h with constant stirring. After the reaction had been allowed to cool THF was removed *in vacuo* and the residues dissolved in CH_2Cl_2 (20 mL), washed with 2 M $KHSO_{4\,(aq)}$ (20 mL), distilled H_2O (20 mL × 3) and brine (20 mL). The organic portion was dried ($MgSO_4$) and solvent removed *in vacuo*. Column chromatography (EtOAc) eluted **25** as a colourless viscous oil (0.080 g, 71%) which was used without further purification. m/z (APCI$^+$) 345 ((M + H)$^+$, 100%).

Sulfamic acid 4-{2-[3-(cyanodimethylmethyl)-5-[1,2,4]triazol-1-ylmethylphenyl]vinyl}phenyl ester 26. Compound **26** was prepared from **25** using similar conditions to those described for the synthesis of compound **11**. Column chromatography (CH_2Cl_2–acetone 80 : 20) eluted **26** as a white solid (0.035 g, 36%), mp 151–152 °C. ^{1}H NMR δ_H (400 MHz, DMSO-d_6) 1.73 (6H, s, $ArC(CH_3)_2$)CN), 5.50 (2H, s, $ArCH_2N$), 7.30–7.33 (3H, m, ArH & vinyl), 7.35–7.38 (1H, d, $J = 16.6$ Hz, *trans* vinyl), 7.42 (1H, s, ArH), 7.51 (1H, s, ArH), 7.71 (1H, s, ArH), 7.72–7.74 (2H, d, $J =$ 8.4 Hz, ArH), 8.04 (1H, s, $C_2H_2N_3$), 8.06 (2H, br s, $ArOSO_2NH_2$) and 8.73 (1H, s, $C_2H_2N_3$); ^{13}C NMR δ_C (100.6 MHz, DMSO-d_6) 28.3 (CH_3), 36.6 (C), 51.9 (CH_2), 122.6 (CH), 123.0 (CH), 124.2 (CH), 124.5 (C), 125.4 (CH), 127.9 (CH), 128.2 (CH), 128.5 (CH), 135.2 (C), 137.5 (C), 138.0 (C), 142.5 (C), 144.4 (CH), 149.7 (C) and 151.9 (CH); m/z (APCI$^+$) 424 ((M + H)$^+$, 100%), 345 (27). HRMS (ES$^+$) 424.1435. $C_{21}H_{22}N_5O_3S$ requires 424.1438.

2-(3,5-Dimethylphenyl)-2-methylpropionitrile 27. To a solution of 3,3-dimethylphenylacetonitrile (2.50 g, 17.2 mmol) in anhydrous THF under inert conditions at −75 °C was added bis(trimethylsilyl)lithium amide (1.0 M in THF, 38.0 mL, 37.9 mmol). After stirring for 60 min at −75 °C, iodomethane (2.36 mL, 37.9 mmol) was introduced drop wise and the resulting light orange mixture was allowed to warm to room temperature and stir for an additional 4 h. Solvent was removed *in vacuo* to give a dark brown viscous oil This was diluted in EtOAc (100 mL), washed with 1 M $Na_2S_2O_{3\,(aq)}$ (100 mL × 2), brine (100 mL) and dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give a brown liquid. Column chromatography (EtOAc–hexane, 20 : 80 to 35 : 65) eluted **27** as a clear light brown liquid (2.30 g, 77%). ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.65 (6H, s, $ArC(CH_3)_2CN$), 2.29 (6H, s, $ArCH_3$), 6.97 (1H, s, ArH) and 7.11 (2H, s, ArH); m/z (FAB$^+$) 173 (M$^+$, 100%).

2-(3-Bromomethyl-5-methylphenyl)-2-methylpropionitrile 28. To a solution of **27** (2.25 g, 12.98 mmol) in CCl_4 (50 mL) was added *N*-bromosuccinimide (2.33 g, 13.0 mmol) and benzoyl peroxide (0.126 g, 0.520 mmol). The light yellow suspension was then refluxed under inert conditions for 18 h. After cooling to room temperature, the suspension was filtered and the filter cake collected was washed with $CHCl_3$ (30 mL × 5). The combined filtrates were evaporated *in vacuo* to give a yellow liquid. Column chromatography (EtOAc–hexane, 10 : 90 to 20 : 80) eluted **28** as a light yellow oil (2.15 g, 66%). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.67 (6H, s, $ArC(CH_3)_2CN$), 2.33 (3H, s, $ArCH_3$), 4.70 (2H, s, $ArCH_2Br$), 7.24 (1H, s, ArH), 7.28 (1H, s, ArH) and 7.39 (1H, d, $J = 1.9$ Hz, ArH); m/z (FAB$^+$) 252 ((^{79}BrM + H)$^+$, 33%), 225 (50), 172 (100); HRMS (FAB$^+$) 254.0381. $C_{12}H_{15}{}^{81}BrN$ requires 254.0367.

2-[3-(4-Hydroxyphenylsulfanylmethyl)-5-methylphenyl]-2-methylpropionitrile 29. A solution of **28** (1.5 g, 5.95 mmol) in anhydrous DMF (30 mL) at room temperature and under inert conditions was treated with K_2CO_3 (8.25 g, 59.5 mmol) followed by a solution of 4-hydroxythiophenol (0.940 g, 7.14 mmol) in anhydrous DMF (0.5 mL). The resulting dark yellow suspension was stirred at room temperature for 30 min and then acidified with 1 M $HCl_{(aq)}$. After diluting with EtOAc (100 mL), the organic layer was washed with distilled H_2O (50 mL × 3), brine (50 mL), dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give yellow residues. Column chromatography (EtOAc–hexane 20 : 80 to 50 : 50) eluted **29** as a white solid (1.50 g, 85%), mp 77–78 °C (Found: C, 72.5; H, 6.4; N, 4.5. $C_{18}H_{19}NOS$ requires C, 72.7; H, 6.4; N, 4.7%). ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.63 (6H, s, $ArC(CH_3)_2CN$), 2.33 (3H, s, $ArCH_3$), 3.91 (2H, s, $ArCH_2SAr$), 5.49 (1H, br s, ArOH), 6.73 (2H, m, ArH), 6.85 (1H, s, ArH), 6.99 (1H, s, ArH), 7.12 (1H, s, ArH) and 7.17 (2H, m, ArH).

Sulfamic acid 4-[3-(cyanodimethylmethyl)-5-methylbenzylsulfanyl]phenyl ester 30. Compound **30** was prepared from **29** using similar conditions to those described for the synthesis of compound **11**. Column chromatography (EtOAc) eluted **30** as a pale yellow viscous oil that on standing crystallised to a pale yellow solid (0.282 g, 74%), mp 107–110 °C. ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.61 (6H, s, $ArC(CH_3)_2CN$), 2.35 (3H, s, $ArCH_3$), 4.00 (2H, s, $ArCH_2SAr$), 5.27 (2H, br s, OSO_2NH_2), 6.80 (1H, s, ArH), 7.01 (1H, s, ArH), 7.08 (1H, s, ArH), 7.22 (2H, m, ArH) and 7.32 (2H, m, ArH); ^{13}C NMR δ_C (100.6 MHz, DMSO-d_6) 21.0 (CH_3), 28.3 (CH_3), 36.5 (C), 36.9 (CH_2), 122.8

(CH), 122.9 (CH), 124.6 (C), 124.7 (CH), 128.9 (CH), 130.0 (CH), 134.1 (C), 138.0 (C), 138.4 (C), 141.5 (C) and 148.4 (C); *m/z* (ES^+) 377 ($(M + H)^+$, 100%); HRMS (ES^+) 377.0972. $C_{18}H_{21}N_2O_3S_2$ requires 377.0988.

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