Dual Aromatase−Steroid Sulfatase Inhibitors

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By introducting the steroid sulfatase inhibitory pharmacophore into aromatase inhibitor **1** (YM511), two series of single agent dual aromatase−sulfatase inhibitors (DASIs) were generated. The best DASIs in vitro (JEG-3 cells) are **5**, (IC_{50}(aromatase) = 0.82 nM; IC_{50}(sulfatase) = 39 nM), and **14**, (IC_{50}(aromatase) = 0.77 nM; IC_{50}(sulfatase) = 590 nM). X-ray crystallography of **5**, and docking studies of selected compounds into an aromatase homology model and the steroid sulfatase crystal structure are presented. Both **5** and **14** inhibit aromatase and sulfatase in PMSG pretreated adult female Wistar rats potently 3 h after a single oral 10 mg/kg dose. Almost complete dual inhibition is observed for **5** but the levels were reduced to 85% (aromatase) and 72% (sulfatase) after 24 h. DASI **5** did not inhibit aldosterone synthesis. The development of a potent and selective DASI should allow the therapeutic potential of dual aromatase-sulfatase inhibition in hormone-dependent breast cancer to be assessed.

Introduction

The aromatase enzyme catalyzes the conversion of androgens to estrogens, the last and rate-limiting step in the biosynthesis of estrogens. Aromatase inhibitors (AIs[a]) have been successfully developed, and one of the main therapeutic indications for AIs is hormone-dependent breast cancer (HDBC), which affects a substantial population of pre- and postmenopausal women in Western countries. Since the discovery of the aromatase inhibitory activity of aminoglutethimide in the late 1970s,[1] many AIs have been developed, of which the third-generation anastrozole, letrozole, and exemestane are highly potent and selective agents that suppress estradiol levels in plasma to virtually undetectable concentrations.[2] The use of these AIs in the clinic for treating patients with advanced stages of HDBC is well established. However, recent evidence has emerged to support a more front-line role for these third-generation AIs. Several randomized comparative clinical trials have demonstrated the advantages of using anastrozole, letrozole, and exemestane over tamoxifen as first-line agents in the treatment of patients with primary tumors.[3] From the results of the ATAC (arimidex, tamoxifen, alone, or in combination) trial analyzed after completion of 5 years of adjuvant treatment of breast cancer, it was proposed that anastrozole should be the preferred initial treatment for postmenopausal women with localized hormone receptor positive breast cancer.[4] The recent Breast International Group (BIG) 1-98 trial further confirmed the results of previous studies that showed the superiority of AIs over tamoxifen.[5] These findings, therefore, widen not only the indications for some leading third-generation AIs but also the population of patients who may benefit from receiving these agents.

While the clinical efficacy of third-generation AIs in the treatment of HDBC has clearly been demonstrated, there is now abundant and strong evidence to suggest that the deprivation of estrogen levels in patients treated with AIs can be augmented if steroid sulfatase (STS) is inhibited at the same time.[6,7] STS catalyzes the hydrolysis of steroid sulfates, such as estrone 3-sulfate (E1S) to estrone (E1), which is the main source of estrogens in tumors,[8] and modulates the production of 5-androstene-3β,17β-diol (Adiol), which contributes to the estrogenic stimulation of hormone-dependent breast tumors.[9] Hence, STS inhibitors, when used alone or in concert with an AI, may enhance the response of hormone-dependent breast tumors to this type of endocrine therapy by reducing not only the formation of E1 from E1S but also the synthesis of other steroids with estrogenic properties such as Adiol, from dehydroepiandrosterone (DHEA) sulfate via DHEA.

Since the discovery of estrone 3-*O*-sulfamate[10] (EMATE, Figure 1) as a highly potent time- and concentration-dependent inhibitor of STS, considerable progress has been made in the development of both steroidal and nonsteroidal STS irreversible inhibitors that are also highly potent but, in contrast to EMATE, devoid of estrogenicity.[7] STX64 (Figure 1), a benchmark nonsteroidal agent, is the first STS inhibitor that entered a phase I trial for treatment of HDBC.[11] Clinical data from this trial showed that an oral administration of STX64 at either a 5 or 20 mg daily dose inhibited STS activity potently in peripheral blood lymphocytes and biopsied tumor tissue without showing any serious drug-related adverse events. In addition, stable disease was observed in five out of eight evaluable patients. With these promising clinical results and the establishment of a "proof of principle" for STS inhibition in HDBC, it is anticipated that a great deal of interest in future clinical studies will be directed toward an investigation of the role of inhibiting aromatase and STS concomitantly for the treatment of HDBC.

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[a] Abbreviations: ACD, Advanced Chemistry Development; Adiol, 5-androstene-3β,17β-diol; AIs, aromatase inhibitors; ATAC, Arimidex, tamoxifen, alone, or in combination; BIG, Breast International Group; CAII, carbonic anhydrase II; DASI, dual aromatase−sulfatase inhibtior; DHEA, dehydroepiandrosterone; DMA, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; E1, estrone; E1S, estrone sulfate; HDBC, hormone-dependent breast cancer; PMSG, pregnant mares' serum gonadotropin; STS, steroid sulfatase.

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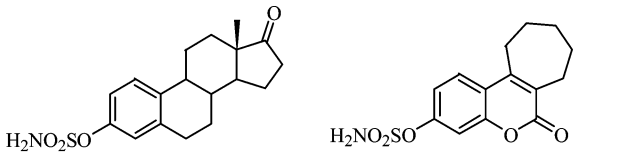


EMATE **STX64 (667COUMATE, BN83495)**

Figure 1. Structures of EMATE and STX64.

While coadministering an AI with an STS inhibitor as two individual agents or a fixed-dose bicomponent drug might be the obvious choice for attaining a combined endocrine therapy, an attractive alternative strategy is to design a dual aromatase−steroid sulfatase inhibitor (DASI) that will inhibit both enzymes as a single agent. Morphy amd Rankovic have recently appraised the design of a single chemical entity that has the ability to modulate multiple targets simultaneously and highlighted the pharmaceutical and development advantages of this drug design approach.[12]

In an earlier communication,[13] we explored the design strategy for a DASI by introducing the required pharmacophore for potent irreversible STS inhibition, i.e., a phenyl sulfamate ester, into **1** (YM511, Figure 2), which is a highly potent and selective nonsteroidal AI.[14] Two series of compounds were developed: *p*-sulfamates **2**−**5** and *m*-sulfamate **6** (Figure 2). In this work, we further expand the para-sulfamoylated series through introduction of substituent(s) that are considered to be electron-donating and/or electron-withdrawing at the position(s) ortho to the sulfamate group. Similarly, we also further exemplify the meta-sulfamoylated series with derivatives that bear a substituent at the para position of the phenyl ring. The in vitro dual inhibitory activities of these sulfamate-based compounds and the aromatase inhibitory activity of their corresponding parent phenolic compounds are studied in a human choriocarcinoma cell line (JEG-3) assay. Selected compounds are further investigated in vivo for their ability to reduce plasma estradiol levels and liver STS activity. In order to facilitate the SAR study, dockings of DASIs and their corresponding parent phenolic compounds into the homology model of aromatase published recently by Favia et al.[15] are carried out. Similarly, DASIs are also docked into the crystal structure of STS reported by Hernandez-Guzman et al.[16] in an attempt to understand how these compounds may interact with this enzyme.

Results and Discussion

Before the concept of designing a DASI was first validated in our earlier publication,[13] pioneering work in this field had already been carried by our group when the STS inhibitory pharmacophore was introduced to flavonoids,[17] some of which are known to exhibit aromatase inhibitory activity.[18] To this end, several flavone and flavanone sulfamates were found to show potent STS inhibition with moderate aromatase inhibitory activity in vitro.[17] The fact that these flavonoid sulfamates were not highly potent AIs can be attributed to the absence of an accessible heterocycle such as a triazole group that, through strong heme coordination, renders the high potency against aromatase observed in many established nonsteroidal AIs.

Recently, an attempt was made by Numazawa et al.[19] to apply the DASI concept to a number of moderately inhibitory steroidal AIs having an estrogenic steroid skeleton by preparing the 3-sulfamoylated derivatives of 2- and 4-halogeno (F, Cl, and Br) estrones and their estradiol analogues as well as 6β-methyl- and phenylestrones. As expected, these sulfamates showed good to potent STS inhibition. However, when these sulfamates were tested for aromatase inhibitory activity in a placental microsomes preparation, they were found to be very weak inhibitors with an IC_{50} of 41.8 ± 2.3 μM for the best compound, 6β-phenylestrone 3-*O*-sulfamate. It is likely that this strategy for achieving dual inhibition in a single molecule did not work particularly well because the template, a C18 steroid (estrogen) sulfamate, is more optimized for inhibiting STS than for aromatase. In contrast, most potent steroidal AIs, like 4-hydroxyandrostenedione and exemestane, are C19 steroids but they cannot be easily modified structurally to incorporate the pharmacophore for potent STS inhibition, i.e., a phenyl sulfamate ester, without disrupting the optimized functionalities for aromatase inhibition. In addition, an insertion of a sulfamate moiety into a C19 steroid is not expected to result in potent STS inhibition, as demonstrated by many alkyl *O*-sulfamates, like the C19 dehydroepiandrosterone 3β-*O*-sulfamate,[20] that were found to be significantly weaker STS inhibitors than aryl *O*-sulfamates such as EMATE.

Because of this foreseeable dilemma with designing a DASI using a steroidal template, we opted instead for the strategy of introducing the STS inhibitory pharmacophore into known, highly potent, and selective AIs of nonsteroidal nature. One such inhibitor is **1** (Figure 2). We reasoned that even though the incorporation of the aryl sulfamate motif into **1** might generate derivatives that are less optimized for aromatase inhibition, this strategy nonetheless may render the resulting compound a DASI because of the STS inhibitory potential introduced while retaining most of the aromatase inhibitory properties of **1**. There is an additional advantage for adopting the template of **1**. For symmetrical aromatase inhibitors like letrozole, which contains a tetrahedral carbon center, replacing one of its two *p*-cyanophenyl rings with a phenol sulfamate ester moiety will render the resulting DASI chiral. In contrast, there is no such complication with converting **1**, a tertiary amine, into a DASI because its central N atom can be substituted with three different substitutents and so one of which can be incorporated with the pharmacophore for STS inhibition.

The current study is a full development of the work carried out in our preliminary communication.[13] Two series of derivatives of **1**, namely, *p*- and *m*-sulfamates, are developed. The in vitro biological activities of these compounds and their parent phenols are tabulated in Tables 1−4. The in vivo activities of **5** and **14** are summarized in Table 5.

Chemistry

The synthetic routes to the compounds prepared in this study are outlined in Scheme 1 (for *p*-sulfamates **2**−**5** and **7**), Scheme

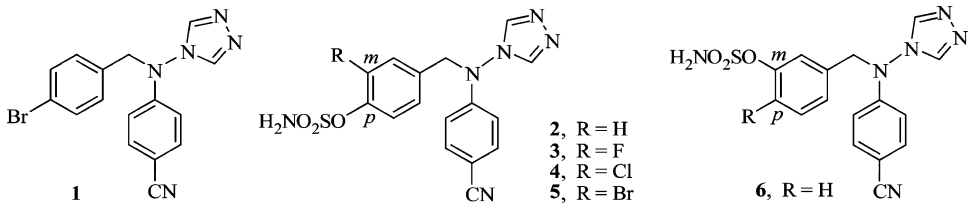
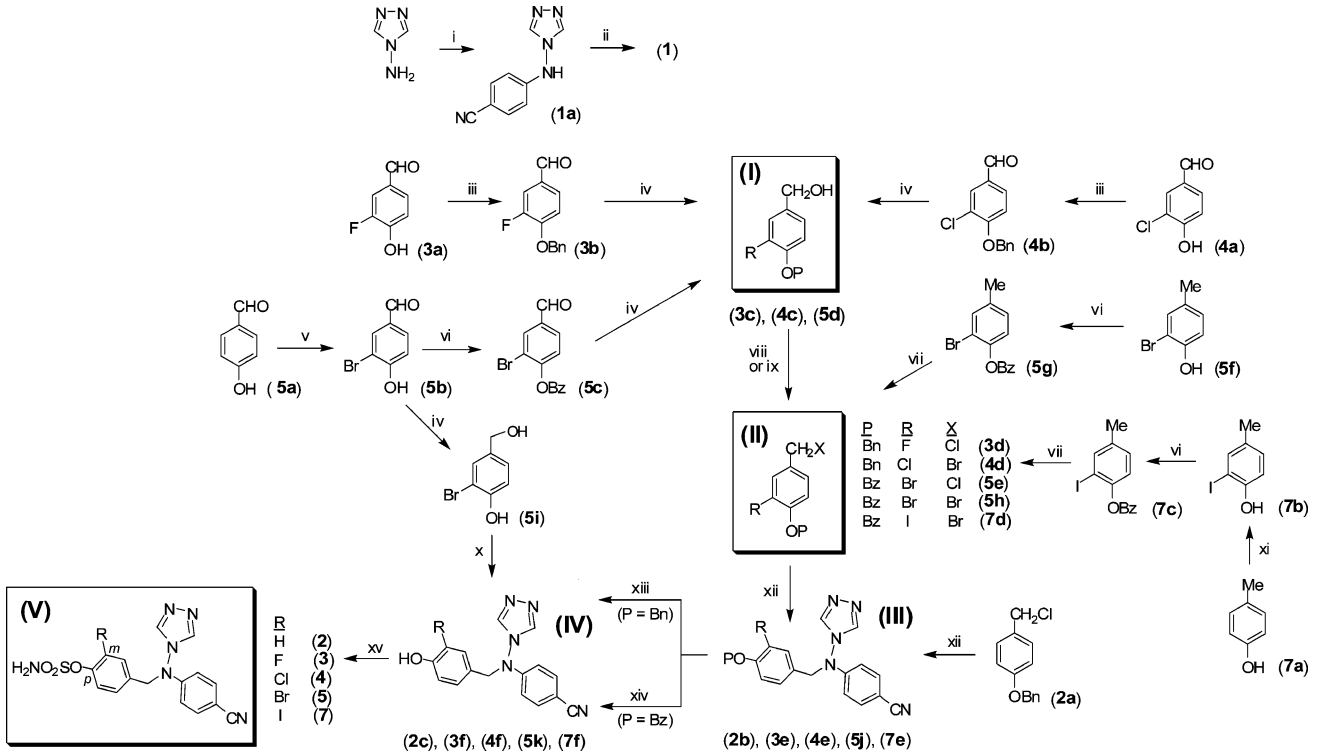


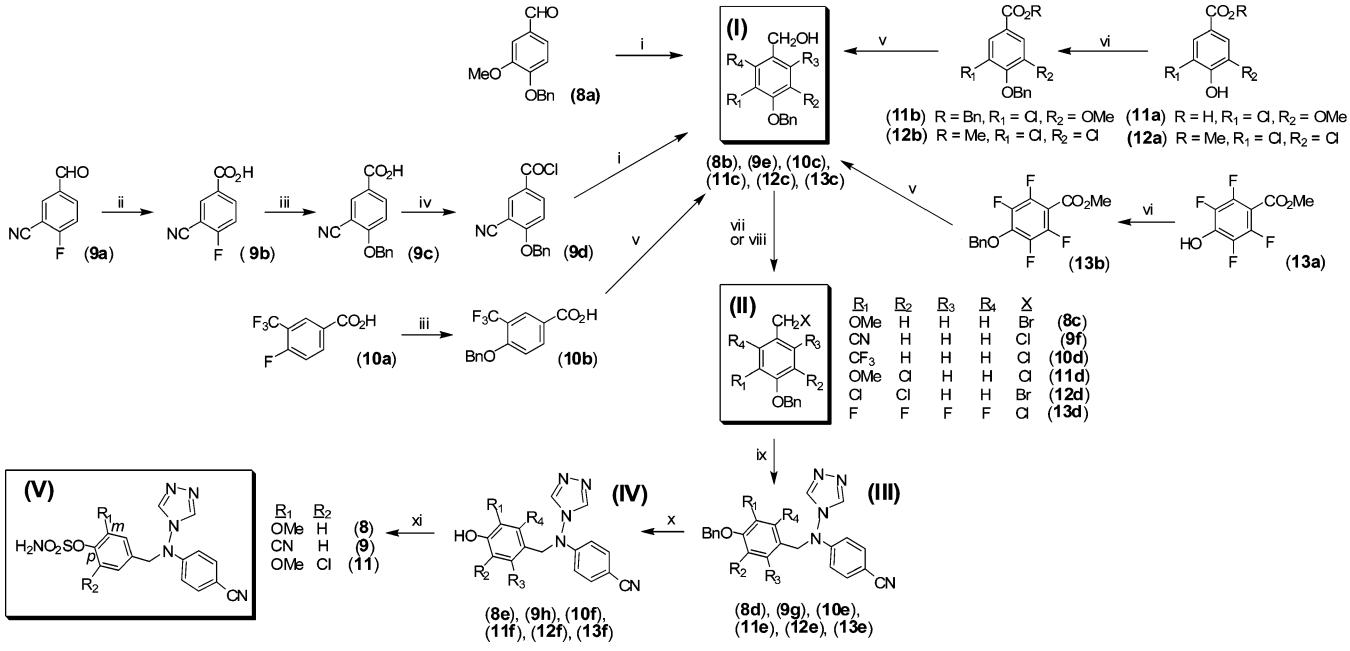
Figure 2. Structures of **1** (YM511), *p*-sulfamates **2**−**5**, and *m*-sulfamate **6**.

Scheme 1. Synthesis of *p*-Sulfamate Containing DASIs (**2**−**5** and **7**) and Their Precursors (**I**−**IV**)[a]



[a] Reagents and conditions: (i) KO^{t}Bu, 4-fluorobenzonitrile, DMSO; (ii) NaH, DMF, 4-bromobenzyl bromide; (iii) BnBr, K_2CO_3, DMF; (iv) $NaBH_4$, THF−EtOH, 0 °C to room temp; (v) Br_2, AcOH; (vi) Bz_2O, Et_3N, CH_2Cl_2, 0 °C; (vii) NBS, CCl_4, $(BzO)_2$, reflux; (viii) $SOCl_2$, CH_2Cl_2; (ix)) PBr_3, CH_2Cl_2, 0 °C; (x) (a) $SOCl_2$, (b) **1a**, K_2CO_3, DMF; (xi) KI, Oxone, MeOH; (xii) **1a**, NaH, DMF; (xiii) Pd−C (10% by weight), H_2, THF−MeOH; (xiv)) NaOH, MeOH, H_2O; (xv) $ClSO_2NH_2$, DMA, 0 °C.

Scheme 2. Synthesis of *p*-Sulfamate Containing DASIs (**8**, **9**, and **11**) and Precursors (**I**−**IV**)[a]

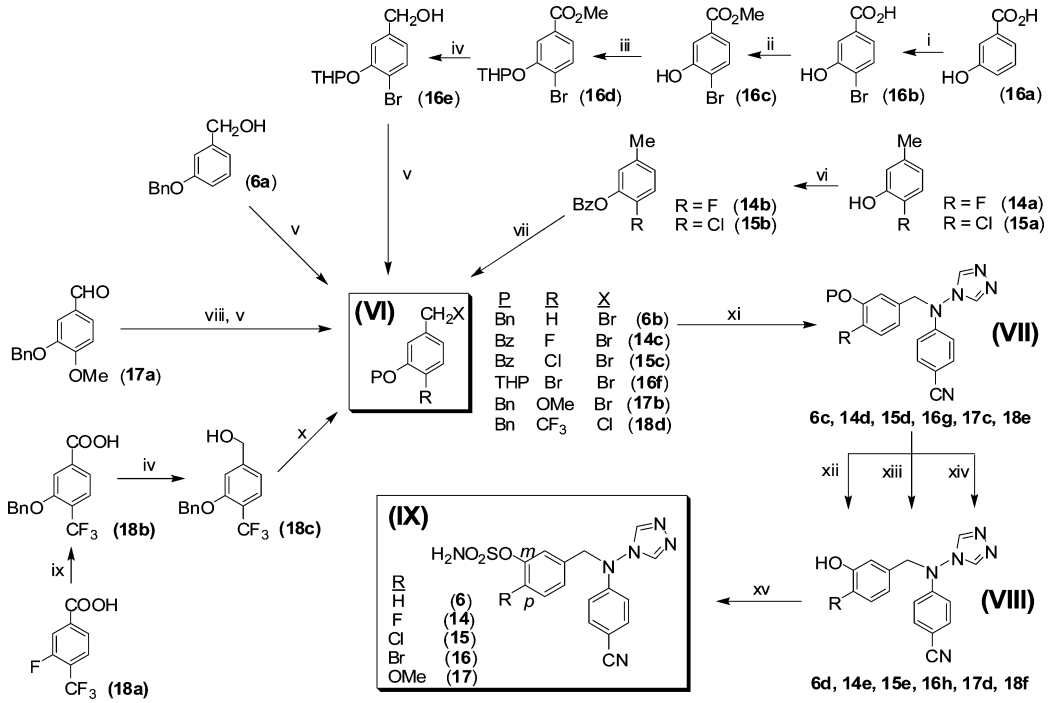


[a] Reagents and conditions: (i) $NaBH_4$, THF−EtOH, 0 °C to room temp; (ii) $NaClO_2$, H_2O_2, CH_3CN/H_2O; (iii) NaH, $PhCH_2OH$, DMSO; (iv) $SOCl_2$, reflux; (v) $LiAlH_4$, THF, 0 °C; (vi) BnBr, K_2CO_3, DMF; (vii) $SOCl_2$, CH_2Cl_2; (viii) PBr_3, CH_2Cl_2, 0 °C; (ix) **1a**, NaH, DMF; (x) Pd−C (10% by weight), H_2, THF−MeOH; (xi) $ClSO_2NH_2$, DMA, 0 °C.

2 (for *p*-sulfamates **8**, **9**, and **11**), and Scheme 3 (for *m*-sulfamates **6** and **14**−**17**). The structures of new compounds were characterized by standard analytical methods, elemental analysis (where stated), and HPLC and additionally by X-ray crystallography for **5**.

The synthesis of the *p*-sulfamate containing DASI candidates and their phenolic precursors was initiated using the commercially available benzaldehydes (**3a**, **4a**, and **5a** (Scheme 1), **8a** and **9a** (Scheme 2)), carboxylic esters or acids (**9b**, **10a**, **11a**, **12a**, and **13a** (Scheme 2)), or cresol derivatives (**5f** and **7a** (Scheme 1)). Though commercially available, carboxylic acid **9b** can easily be prepared in moderate yield by oxidation of the aldehyde **9a** using $NaClO_2/H_2O_2$. 3-Bromo-4-hydroxybenzaldehyde **5b**[21] and 2-iodo-4-methylphenol **7b**[22] were obtained

Scheme 3. Synthesis of *m*-Sulfamate Containing DASIs (**6** and **14**−**17**) and Their Precursors (**VI**−**VIII**)[a]



[a] Reagents and conditions: (i) Br_2, AcOH; (ii) MeOH, H_2SO_4; (iii) 3,4-dihydro-2*H*-pyran, *p*-TsOH, CH_2Cl_2, 0 °C; (iv) $LiAlH_4$, THF, 0 °C; (v) PBr_3, CH_2Cl_2, 0 °C; (vi) Bz_2O, Et_3N, CH_2Cl_2, 0 °C; (vii) NBS, CCl_4, $(BzO)_2$, reflux; (viii) $NaBH_4$, THF−EtOH, 0 °C to room temp; (ix) BnOH, KO^{t}Bu, DMSO; (x) $SOCl_2$, reflux; (xi) **1a**, NaH, DMF; (xii) Pd−C (10% by weight), H_2, THF−MeOH; (xiii) NaOH, MeOH, H_2O; (xiv) MeOH, *p*-TsOH, 0 °C to room temp; (xv) $ClSO_2NH_2$, DMA, 0 °C.

respectively from **5a** and *p*-cresol **7a** using established methods (Scheme 1). The 3-fluoro- **3a**, 3-chloro- **4a** (Scheme 1) and 3-chloro-5-methoxy **11a** (Scheme 2) derivatives were protected as their benzyl ethers and were converted to the corresponding benzyl alcohol derivatives **3c**, **4c**, and **11c** with either $NaBH_4$ or $LiAlH_4$. Subsequent halogenation with either thionyl chloride or phosphorus tribromide furnished the key halides **3d**, **4d**, and **11d** (an analogous strategy was applied by van Oeveren et al.[23] to prepare the 3-methoxy derivative **8c**, Scheme 2). Activated nitrile **9b** (Scheme 2) undergoes nucleophilic aromatic substitution with benzyl alcohol to give acid **9c**. The key 3-cyano-4-benzyloxybenzyl chloride **9f** was then obtained by mild reduction of the acid chloride **9d** (prepared by chlorination with thionyl chloride) with sodium borohydride and subsequent treatment of the benzyl alcohol derivative with thionyl chloride.

3-Bromo-4-benzoyloxybenzyl halides **5e** and **5h** (Scheme 1) were prepared in three steps from aldehyde **5b**, utilizing benzoate ester protection of the phenolic hydroxyl. The alternative protection strategy of the phenolic hydroxyl group as a benzyl ether was found to be unsatisfactory because of the variable degrees of debromination observed during the subsequent deprotection step by catalytic hydrogenation (data not shown). The benzoyl protective group, although being less robust than the benzyl group, was the preferred moiety for protecting the phenolic group in the preparation **5j**. A more convenient route to the benzyl bromide derivative **5e** was realized through radical bromination of protected cresol **5g**. This methodology was also successfully applied in the synthesis of 3-iodo compound **7d** (obtained from **7c** by iodination of **7a** with KI−Oxone and subsequent protection).

The synthesis of the "half-unit" 4-[(4-cyanophenyl)amino]-4*H*-[1,2,4]triazole, **1a**, was accomplished by nucleophilic aromatic substitution of 4-fluorobenzonitrile with 4-amino-4*H*-[1,2,4]triazole according to the previously described method by Okada et al.[14] Coupling of the anion of **1a** (obtained by deprotonation of **1a** with NaH in DMF) with either 4-bromobenzyl bromide or the required protected hydroxybenzyl halides (**II**, Schemes 1 and 2) (4-benzyloxybenzyl chloride, **2a**, was obtained from Sigma-Aldrich) gave the tertiary amines **1**[14] and **III** (Schemes 1 and 2). After deprotection by either (i) catalytic hydrogenation (in the case of benzyloxy derivatives **2b**, **3e**, **4e** (Scheme 1); **8d**, **9g**, **10e**, **11e**, **12e**, and **13e** (Scheme 2)) or (ii) base-catalyzed hydrolysis (in the case of **5j** and **7e** (Scheme 1)), the resulting phenols (**IV**, Schemes 1 and 2) were finally converted to their corresponding sulfamates (**V**, Schemes 1 and 2) according to conditions described by Okada et al. by reaction with an excess of sulfamoyl chloride[24] in *N*,*N*-dimethylacetamide (DMA).[25]

For the synthesis of **5k**, it was subsequently found that this brominated phenol could be made by coupling **1a** with freshly prepared unprotected phenol **5i** (Scheme 1). The overall yield of **5k** obtained by this shortened route (two steps) was 35%, which is significantly more efficient than the route using the benzoate **5c** as the starting reagent, which gave **5k** in a yield of 23% over five steps.

Various attempts were made to sulfamoylate the trifluoromethylated phenol **10f**, the dichlorinated phenol **12f**, and the tetrafluorinated phenol **13f** (Scheme 2). However, either no sulfamate derivative or a sulfamate heavily contaminated with the corresponding parent phenol was isolated. The failure of efficient sulfamoylation for these starting phenols can be attributed to the instability of the sulfamates formed either in situ or during workup, because of the strong leaving group ability of the phenols under the influence of their strong electron-withdrawing substituent(s).[26,27]

DASIs containing a *m*-sulfamate were prepared in an analogous manner to the *p*-sulfamate series from the commercially available 3-(benzyloxy)benzyl alcohol **6a**, *m*-cresols (**14a** and **15a**), or 3-benzyloxy-4-methoxybenzaldehyde **17a** (Scheme 3). 3-Benzyloxybenzyl bromide **6b**[28] and 3-benzyloxy-

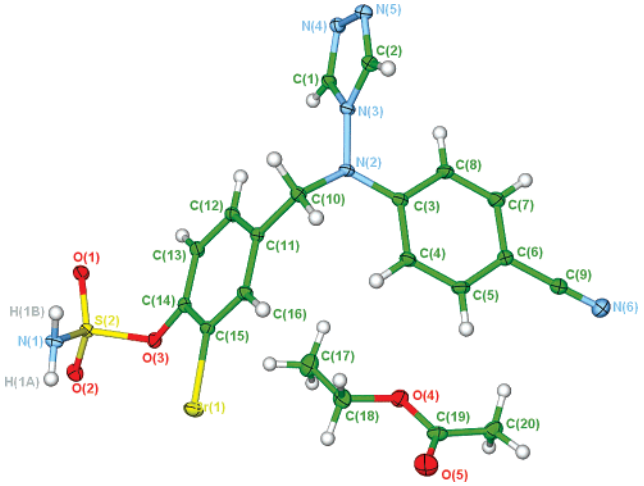


Figure 3. Ellipsoid plot (30% probability) of the asymmetric unit of **5** with a trapped molecule of ethyl acetate.

4-methoxybenzyl bromide **17b**[29] were prepared using established protocols from **6a** and **17a**, respectively. Protection of 4-fluoro-3-methylphenol **14a** and 4-chloro-3-methylphenol **15a** as benzoates followed by subsequent radical halogenation gave the required key bromides **14c** and **15c**. 4-Bromo-3-hydroxybenzoic acid **16b** was prepared from **16a** using the method reported by Buehler et al.[30] Subsequent reactions involving esterification to give **16c**, protection as the THP ether to give **16d**, and reduction with $LiAlH_4$ afforded the alcohol **16e**, which was then converted under mild conditions to the benzyl bromide **16f** over four steps. Coupling of the prerequisite bromides (**VI**, Scheme 3) with the anion of **1a** (Scheme 1) furnished the amines (**VII**). Standard deprotection afforded the phenols (**VIII**), which were finally converted to their corresponding *m*-sulfamates (**IX**) by reaction with an excess of sulfamoyl chloride in DMA. As experienced in the synthesis of the *p*-sulfamate congeners, the sulfamoylation of the trifluorinated phenol **18f** by standard methods was not successful in yielding the corresponding sulfamate product with an acceptable level of purity after workup. The main contaminant was found to be the starting phenol **18f**.

An X-ray crystal structure of **5** was obtained. Crystals suitable for X-ray analysis were grown by slow diffusion of *n*-hexane into a solution of **5** in ethyl acetate. A crystal (approximate dimensions of 0.20 mm × 0.13 mm × 0.05 mm) was used for data collection. The asymmetric unit of **5** is shown in Figure 3 with the labeling scheme used. In Figure 4, the stacking of the molecules within the crystal is arranged in such a manner that the NH_2 of the sulfamate groups interacts with the N atoms of neighboring triazolyl groups through intermolecular hydrogen bonding. The display of an asymmetric unit of four molecules of **5** using DSViewerPro software is shown in Figure 5. In addition to intermolecular hydrogen bonding already described

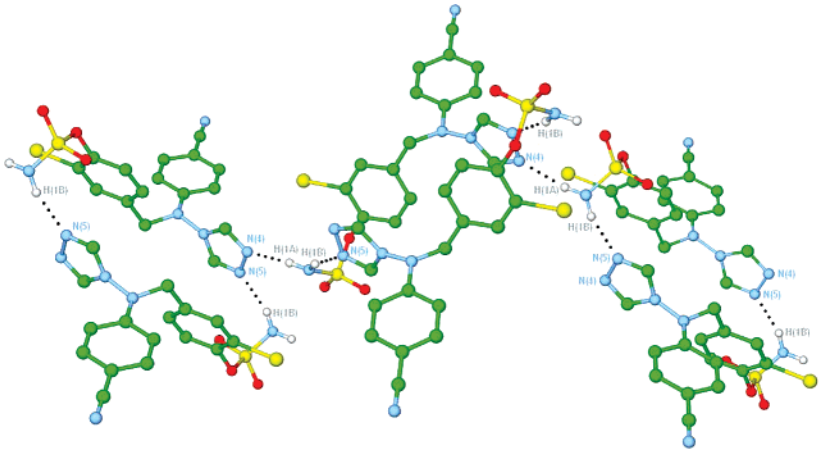


Figure 4. Packing plot of **5** in the X-ray crystal structure without trapped ethyl acetate showing intermolecular hydrogen bondings between N−H of sulfamate groups and the N atoms of the triazolyl groups.

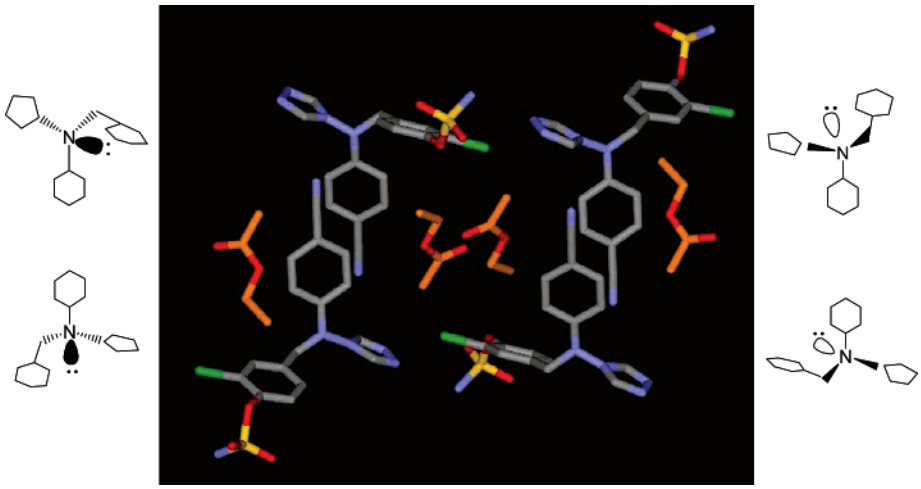
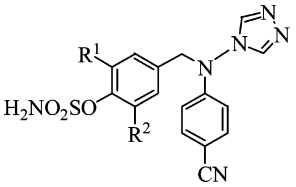


Figure 5. Display of four proximate asymmetric units in the structure of **5** by DSViewerPro software (Accelrys Inc., San Diego, CA) showing invertamers: bromine atom (green), carbon (gray except orange for ethyl acetate), nitrogen (blue), oxygen (red), and sulfur (yellow). The enantiomers on the left are in the *R*-configuration, whereas the enantiomers on the right are in the *S*-configuration.

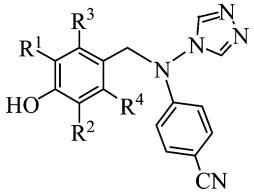
Table 1. In Vitro Inhibition of Aromatase and STS Activity Produced by *p*-sulfamates Assessed Using Intact Monolayers of JEG-3 Cells[a]



compd	R^1	R^2	aromatase IC_{50} (nM)	STS IC_{50} (nM)
1			0.5 ± 0.03	ND
STX64			ND	1.5 ± 0.3
2[b]	H	H	100 ± 7.8	227 ± 29
3[b]	F	H	12 ± 1.8	40 ± 3.8
4[b]	Cl	H	2.3 ± 0.3	20 ± 2.1
5[b]	Br	H	0.82 ± 0.3	39 ± 4.2
7	I	H	1.5 ± 0.1	190 ± 19
8	OMe	H	42 ± 1	380 ± 31
9	CN	H	27 ± 10	>10000
11	OMe	Cl	2.9 ± 0.2	536 ± 38

[a] Each value represents the mean ± SE of triplicate measurements. When JEG-3 cells were pretreated with **2** at 1 μM and cells were washed to remove unbound inhibitor, the inhibition of aromatase was reduced by 63.4%, whereas that of STS remained essentially unaffected (from 91.1 ± 0.4% to 89.9 ± 0.1%). ND: not determined. [b] Data from Woo et al.[13]

Table 2. In Vitro Inhibition of Aromatase Activity in a JEG-3 Cells Preparation by *p*-Phenols

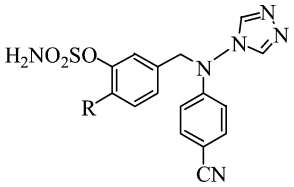


compd	R^1	R^2	R^3	R^4	aromatase IC_{50} (nM)
2c	H	H	H	H	23 ± 1
3f	F	H	H	H	2.9 ± 0.4
4f	Cl	H	H	H	2.5 ± 0.3
5k	Br	H	H	H	1.1 ± 0.1
7f	I	H	H	H	0.33 ± 0.05
8e	OMe	H	H	H	2.8 ± 0.5
9h	CN	H	H	H	24 ± 5.5
10f	CF_3	H	H	H	0.88 ± 0.1
11f	OMe	Cl	H	H	0.51 ± 0.06
12f	Cl	Cl	H	H	7.6 ± 2
13f	F	F	F	F	159 ± 2.1

above, likely $\pi-\pi$ stacking between *p*-cyanophenyl rings is also observed in this view. Interestingly, on examination of the conformation of each molecule, the groups about the central tertiary amine show a different orientation. The geometry of the amine is flattened but remains trigonal pyramidal with the pair of molecules on the right-hand side of Figure 5, as illustrated, exhibiting *S*-stereochemistry and those on the left *R*-stereochemistry as invertamers. As expected, the unit contains a 50:50 mixture of either stereoisomer. However, in our recently published crystal structure of human carbonic anhydrase (CA) II complexed with **5**, the stereochemistry around the central tertiary amine appeared to be in the *S*-configuration, although the molecule too had a flattened geometry.[31] It is most likely that this configurational bias observed for the invertible nitrogen of **5** is due to the conformation adopted by its bromosulfamate-bearing aryl ring interacting with the amino acid residues within the chiral CAII active site.

(A) In Vitro Activities, SAR, and Modeling. (i) *p*-Sulfamates (Table 1). As anticipated, replacing the *p*-bromo atom of **1** with a much more polar sulfamate group to give **2**

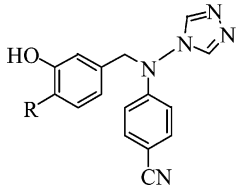
Table 3. In Vitro Inhibition of Aromatase and STS Activity in a JEG-3 Cells Preparation by *m*-Sulfamates



compd	R^1	aromatase IC_{50} (nM)	STS IC_{50} (nM)
6[a]	H	39 ± 4	5133 ± 65.4
14	F	0.77 ± 0.03	590 ± 19
15	Cl	0.92 ± 0.03	>10000
16	Br	3.9 ± 0.9	>10000
17	OMe	12 ± 1.9	>10000

[a] Data from Woo et al.[13]

Table 4. In Vitro Inhibition of Aromatase Activity in a JEG-3 Cells Preparation by *m*-Phenols



compd	R	aromatase IC_{50} (nM)
6d	H	2.8 ± 0.8
14e	F	0.6 ± 0.1
15e	Cl	0.18 ± 0.04
16h	Br	0.5 ± 0.1
17d	OMe	1.2 ± 0.08
18f	CF_3	0.4 ± 0.02

significantly reduces its potency as an AI. The IC_{50} (100 nM) for **2** against aromatase is some 200-fold higher than that for **1** (0.5 nM). It is clear that the interactions between the enzyme active site and the bromo atom of **1** are more favorable than those provided by the sulfamate group of **2**. Although **2** is 150-fold (IC_{50} = 227 nM) less potent than STS inhibitor STX64 tested in clinical trial (IC_{50} = 1.5 nM), this compound nonetheless demonstrates a significant degree of STS inhibitory activity, rendering **2** as the first DASI of this structural class. When a halogen is introduced ortho to the sulfamate group to give **3**−**5** and **7**, the ability of these derivatives to inhibit aromatase and STS improves significantly, with the bromo compound **5** showing a similar potency against aromatase (IC_{50} = 0.82 nM) compared to **1** (IC_{50} = 0.5 nM). It is possible that the increase in lipophilicity enhances the binding of these halogenated derivatives to the aromatase enzyme active site through hydrophobic interactions, compensating some of the disrupting effects provided by the sulfamate moiety for aromatase inhibition as observed for **2** vs **1**. However, despite its highest lipophilicity, the finding that the iodo compound **7** is not the most potent AI of this series suggests that the 2-fold reduction in aromatase inhibitory activity observed for **7** (IC_{50} = 1.5 nM), relative to its bromo congener **5**, is likely the result of steric hindrance.

The docking of **5** into the active site of the aromatase homology model together with **1** and letrozole is shown in Figure 6. (Because of the failure of the docking program GOLD in recognizing the heme ligating property of the triazole group of DASIs, the distance between the coordinating nitrogen atom of the triazole group and the iron atom of the heme was constrained, using the constraint distance functionality within

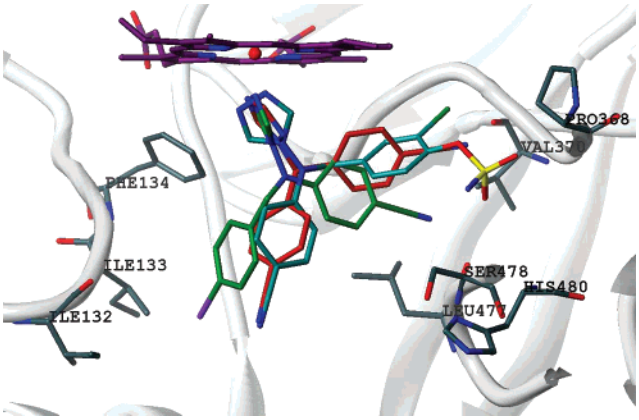


Figure 6. Docking of **1** (green), letrozole (red), and compound **5** (blue) into the aromatase homology model. The heme group is in purple.

GOLD, to be between 2.00 and 2.30 Å. For further details and discussion, refer to the Experimental Section.) It is interesting to note that the *p*-cyano group of **1** and one of the two of letrozole, which potentially can act as a hydrogen-bond acceptor, are docked closely to Ser478, a putative hydrogen-bond-donating amino acid residue that is postulated to be involved in the interaction with some nonsteroidal AIs.[15] The distances between the cyano group and Ser478 are 3.29 and 5.39 Å for **1** and letrozole, respectively. In contrast, it is the sulfamate group of **5** that resides more closely to Ser478, rendering its *p*-cyano group some 6.22 Å away from this amino acid residue.

In comparison with the unsubstituted compound **2** (IC_{50} = 227 nM), a halogen substituted ortho to the sulfamate group clearly lowers the IC_{50} against STS by between 1.2-fold (**7**, IC_{50} = 190 nM) and 11-fold (**4**, IC_{50} = 20 nM). Previous work carried out by our group showed that the lowering of the pK_a of a phenol, and hence its leaving group ability, by electronic effects will enhance the STS inhibitory activity of the corresponding sulfamate.[26,32−34] The higher potency observed for **3**−**5** compared with **2** against STS is consistent with these previous observations (as predicted by ACD/Labs software, version 8.14, the pK_a values of their corresponding 2-substituted 4-methylphenols are 9.01 for 2-fluoro, 8.79 for 2-chloro, and 8.73 for 2-bromo; cf. 10.21 for unsubstituted 4-methylphenol). However, it is interesting to note that despite the variation in the pK_a values of their corresponding phenols, the STS inhibitory activities of **3**−**5** are of the same order of magnitude, suggesting that the leaving group ability of phenols is not the only contributory factor toward the biological activities observed for **3**−**5**. Like its effect on aromatase inhibitory activity, the size of the iodo group of **7** (IC_{50} = 190 nM) could contribute to the nearly 10-fold reduction in potency against STS compared with its chloro congener **4** (IC_{50} = 20 nM). According to pK_a prediction alone, the potency of **7** is anticipated to be much closer to that of **4**, since the pK_a for 2-iodo-4-methylphenol (8.81) is very similar to that for 2-chloromethylphenol (vide supra). Hence, it is likely that through steric hindrance the iodo atom of **7** interferes with the binding of its sulfamate group to the catalytic site, preventing the inhibitor from inactivating the enzyme effectively in the same manner as its congeners **3**−**5**.

The methoxy derivative **8** inhibits STS with an IC_{50} of 380 nM, about 1.6-fold higher than that achieved by the unsubstituted compound **2** (IC_{50} = 227 nM). This reduction in potency exerted by the introduction of a methoxy group here is comparatively moderate considering the 13-fold decrease in potency when the steroidal EMATE (Figure 1) is methoxylated at the 2-position to give 2-methoxyestrone 3-*O*-sulfamate.[26] The finding that **8** is less active than **2** is likely the result of steric hindrance exerted on the sulfamate group by the neighboring methoxy group. However, it is also reasonable to expect that electronic factors may also play a role here. The methoxy group, which has an overall electron-donating effect, should stabilize the S−O bond of the sulfamate group. This stabilization effect weakens the ability of the sulfamate group to be transferred to an essential amino acid residue within the enzyme active site, a mechanism that has been postulated to be crucial for irreversible inactivation of STS.[7,13]

The introduction of an electron-withdrawing chloro atom at the other position ortho to the sulfamate group of **8** does not improve the STS inhibitory activity of the resulting compound **11**. The even slightly higher IC_{50} against STS observed for **11** (IC_{50} = 536 nM) compared with **8** (IC_{50} = 380 nM) suggests that despite the anticipated beneficial electron-withdrawing effect of the chloro atom, the sulfamate group of **11** may not be activated effectively for the inactivation of the enzyme probably because it is shielded by the two flanking substituents. A similar finding was observed for several 2,4-dialkylated derivatives of EMATE, which were much weaker inhibitors of STS than EMATE.[26]

There is nearly a 2.5-fold increase in aromatase inhibitory activity observed for **8** (IC_{50} = 42 nM) in comparison with the unsubstituted compound **2** (IC_{50} = 100 nM). Despite its small negative effect on STS inhibition, a methoxy group substituted ortho to the sulfamate group apparently renders compound **8** a stronger AI. This effect is further enhanced by the introduction of a chloro atom at the position ortho to the sulfamate of **8** to give compound **11** (IC_{50} = 2.9 nM), which is over 30-fold more potent than compound **2**.

The cyano group is a common feature found in potent nonsteroidal AIs like **1**, letrozole, and anastrozole. It has been reasoned that the cyano group functions as a hydrogen-bond acceptor and secures a tighter binding of the inhibitor to the aromatase active site, inter alia, through interaction with the putative hydrogen bond donor Ser478 in the enzyme active site.[15] However, the finding that **9** is only a modest AI of the series (IC_{50} = 27 nM) suggests that the cyano group does not act effectively as a hydrogen-bond acceptor at this position or in the presence of an adjacent sulfamate group. In order to investigate the potential reason for this result, we synthesized two derivatives that have the *p*-bromo atom of **1** removed and the resulting aryl ring substituted with either a *p*-cyano or *m*-cyano group, i.e., 4-[(4-cyanobenzyl)(4-cyanophenyl)amino]-4*H*-[1,2,4]triazole and 4-[(3-cyanobenzyl)(4-cyanophenyl)-amino]-4*H*-[1,2,4]triazole, respectively. When these agents were assayed in JEG-3 cells, their respective aromatase IC_{50} values were found to be 1.2 and 6.3 nM. These findings support the notion that a cyano group substituted at the meta position of the aryl ring, as in the case with **9**, does not interact with the aromatase active site amino acid residue(s) as well as a cyano group substituted at the para position. It is also reasonable to expect that the *p*-sulfamate group of **9** further attenuates the aromatase inhibitory potency of the molecule.

For STS inhibition, **9** is the weakest ($IC_{50} \geq 10\,000$ nM) STS inhibitor in the series. Although **9** was synthesized and characterized successfully, it appears that this compound has a significant problem with its chemical stability once in solution. Indeed, as monitored by HPLC, a solution of **9** in dimethyl sulfoxide stored at room temperature was found to degrade to its parent phenol **9h** at a rate much faster than those observed for **4** (chloro) and **5** (bromo) stored under similar conditions. While complete degradation of **9** to **9h** was observed in less than 2 days, the half-lives of **4** and **5** were found to be between

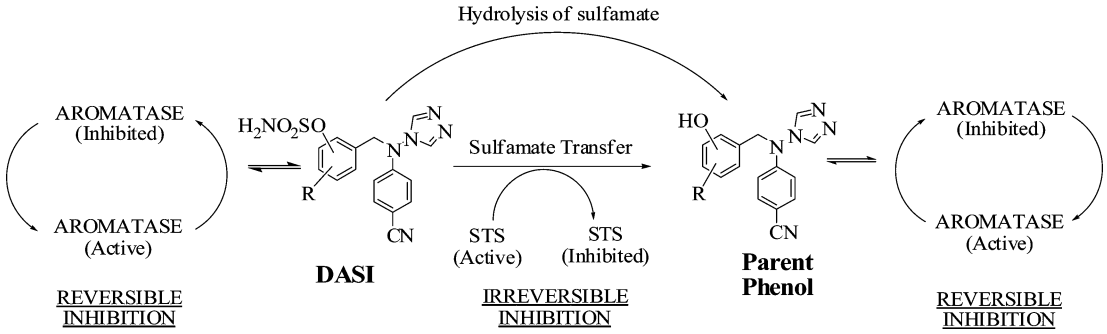


Figure 7. Proposed mechanisms of action for DASI compounds.

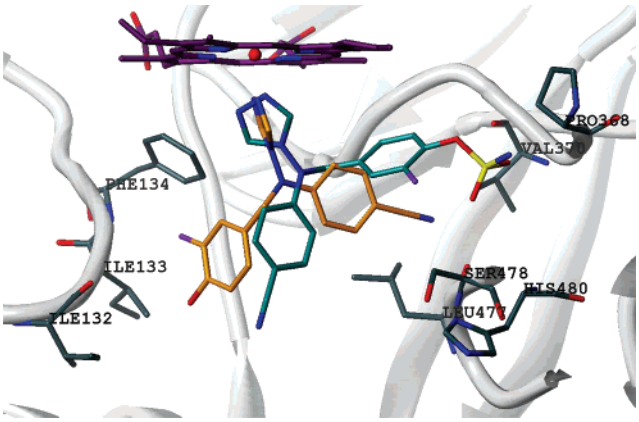


Figure 8. Docking of the *p*-sulfamate **3** (blue) and *p*-hydroxyl compound **3f** (orange) into the aromatase homology model. The heme group is in purple.

7 and 8 days (unpublished observation). Though only qualitative, this analytical study often gives a good indication of the relative resistance of sulfamate esters to hydrolysis (desulfamoylation) in protic polar solvents. Hence, the sulfamate group of **9** is considered to be more susceptible to hydrolysis than other compounds tested in this series. As discussed in several of our previous publications,[26,27] the chemical instability of an aryl sulfamate ester is related to the pK_a value of its parent phenol. Indeed, using ACD/Labs software, version 8.14, the pK_a value of 2-cyano-4-methylphenol is predicted to be 7.47, which is at least 1 log unit lower than those predicted for 2-halogen-4-methylphenols (vide supra). It is therefore highly likely that the surprising low STS inhibitory activity exhibited by **9** is the result of its poor stability once in solution. Incidentally, the finding that the IC_{50} for the corresponding phenol **9h** (24 nM, Table 2, vide infra) is very similar to that observed for **9** (27 nM) lends support to this explanation. Hence, there might have been a significant level of desulfamoylation of **9** taking place during biological testing, rendering a reduction in the concentration of **9** available for inactivating STS and resulting in the weak potency of **9** observed.

(ii) *p*-Phenols (Table 2). The recent STX64 phase I trial has shown evidence that 667COUMARIN, the parent phenolic compound, is the main metabolite detected in plasma, although levels are not high.[11a] According to the various mechanisms of action proposed by our group,[32−34] the immediate product released after the irreversible inactivation of STS by a sulfamate-based STS inhibitor is expected to be its corresponding phenol, although the quantity released by this route in vivo is limited once all the STS activity has been inactivated.[35] However, given the limited chemical stability observed for some aryl sulfamates, it is reasonable to expect that the formation of phenol continues as a result of the hydrolytic cleavage of the sulfamate group in the plasma over a period of time. Therefore, the corresponding phenols of DASIs **2**−**5**, **7**−**9**, and **11**, once released through STS inactivation or chemical hydrolysis of their sulfamate group, are anticipated to act also as AIs in their own right because of the retention of the aromatase inhibitory pharmacophore (i.e., a heme ligating moiety like a triazole) in these phenols. This concept is illustrusted in Figure 7.

As shown in Table 2, all *p*-phenolic compounds tested show moderate to highly potent inhibition of aromatase. The best AI is the iodo derivative **7f** whose inhibitory activity (IC_{50} = 0.33 nM) is even stronger than that exhibited by **1** (0.5 nM, Table 1) obtained from the same assay. With the exception of **4f**, **5g**, and **9h**, all hydroxyl compounds tested are more potent AIs than their corresponding sulfamates (**2** vs **2c**, **3** vs **3f**, **7** vs **7f**, **8** vs **8e**, **11** vs **11f**). These findings could be attributed to the fact that the sulfamate moiety may be too large or polar relative to the hydroxyl group for effective binding of these inhibitors to the aromatase active site.

When **3f** is docked into the active site of the aromatase homology model (Figure 8), the orientation of the molecule resulting is clearly different from that observed for the *p*-sulfamate **3**, with the hydroxyl group of **3f** seen pointing to a direction opposite that of the sulfamate group of **3**. The reason for this difference in docking modes observed between this hydroxyl and sulfamate pair of compounds is not entirely clear, but it is possible that it may involve a steric factor caused by the sulfamate group. On closer examination of the docking mode of **3f**, it is apparent that the resulting orientation is similar to that observed for **1** (cf. Figure 6) with its *p*-cyanophenyl ring docking closely to Ser478. It is therefore possible that upon induced fitting of **3f** to the enzyme, the closer proximity of its cyano group to Ser478 than that of **3** may lead to a significantly tighter binding of the inhibitor to the enzyme active site through hydrogen bonding, rendering the stronger aromatase inhibition observed for **3f** compared to **3**.

The reason for the aromatase inhibitory activity observed for **9h** has already been discussed (vide supra). For the chloro- (**4f** vs **4**) and bromo- (**5g** vs **5**) phenols and sulfamates, it is not clear why their inhibitory activities are similar. It could be argued that the similar aromatase inhibitory activities observed are due to the degradation of sulfamates to phenols, in the same manner as discussed for the cyano pair, **9** vs **9f**. However, the fact that sulfamates **4** and **5** are the two most potent STS inhibitors in the *p*-sulfamate series does not fully support this argument. In other words, if the degradation of sulfamates **4** and **5** to their respective phenols were a significant contributory factor to their aromatase inhibitory activities observed in Table 1, the STS inhibition produced by these sulfamates should appear weak, which is not the case observed.

For phenols **10f**, **12f**, and **13f**, because their corresponding sulfamates are too unstable to be isolated and/or purified

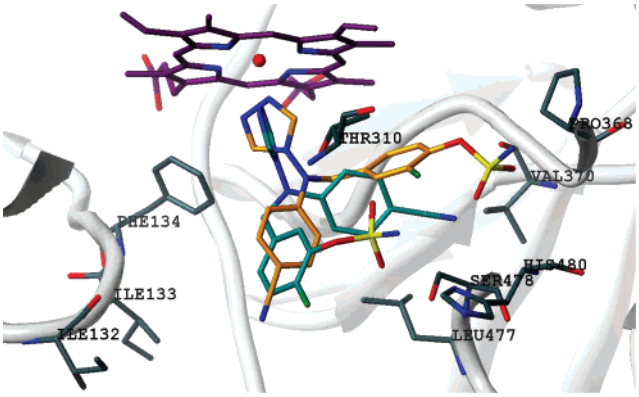


Figure 9. Docking of the *p*-sulfamate **3** (orange) and *m*-sulfamate **14** (blue) into the aromatase homology model. The heme group is in purple.

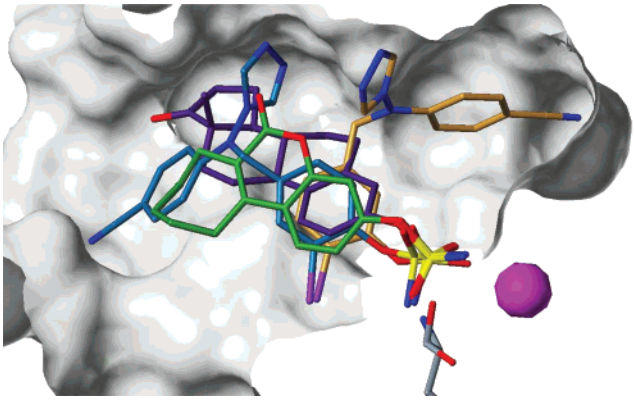


Figure 10. Docking of the *p*-sulfamate **5** (blue), the *m*-sulfamate **16** (brown), STX64 (green), and E1S (dark purple) into the crystal structure of human STS as reported by Hernandez-Guzman et al.[16] The coordinating Ca^{2+} atom (light purple) and formylglycine in its *gem*-diol form are shown.

chemically, no similar comparison can be made but it is anticipated that these phenols are stronger aromatase inhibitors than their corresponding sulfamates. The best inhibitor here is **10f** (IC_{50} = 0.88 nM), and the most likely explanation for this is the increase in hydrophobicity imparted by its CF_3 group. Di- and tetrasubstitutions on the hydroxyl-bearing aryl ring with halogens do not improve the resulting derivatives toward aromatase inhibition compared with their corresponding monohalogenated counterparts (**3f** vs **13f**; **4f** vs **12f**). Despite the apparent increase in hydrophobicity expected for **12f** and **13f**, which should benefit aromatase inhibition, steric hindrance, inter alia, could render a less favorable binding of these phenols to the aromatase active site.

(iii) *m*-Sulfamates (Table 3). When the positions of the meta substituent and the *p*-sulfamate on the aryl ring are interchanged, this results in a series of highly potent AIs (Table 3). Apart from the bromo derivative **16**, all other compounds in the series studied give significantly more potent inhibition of aromatase than their corresponding *p*-sulfamate shown in Table 1. In general, these results suggest that a sulfamate group substituted at the meta position, either with or without a substituent at the para position, is better tolerated by the enzyme than having the sulfamate group placed at the para position. The best AI of this series is the fluoro derivative **14** (IC_{50} = 0.77 nM), which is some 16-fold more potent than its *m*-fluoro counterpart **3** (IC_{50} = 12 nM, Table 1).

On examination of the docking orientations of **3** and **14** in the active site of the aromatase homology model (Figure 9), it is clear that the docking orientations of the two molecules are very different. For *m*-sulfamate **14**, its *p*-cyanophenyl group resides close to Ser478, like that of **1**, while its benzyl moiety folds itself toward its own *p*-cyanophenyl moiety rendering the sulfonyl oxygens of the sulfamate group also within hydrogen-bonding distances (1.63 Å) with Ser478. These docking results suggest that **14** may bind to the aromatase active site in a manner different from that of its *p*-sulfamate counterpart and may interact more favorably with amino acid residues lining the active site, including Ser478, resulting in the higher potency observed.

Despite the promising STS inhibition observed for the *p*-sulfamate series, all meta-sulfamoylated compounds are significantly weaker STS inhibitors with the best inhibition shown by the fluoro derivative **14** (IC_{50} = 590 nM).

In an attempt to understand these unexpected results, the *p*-sulfamates (**2**−**5** and **7**, Table 1) and *m*-sulfamates (**6**, **14**−**16**, Table 3) were docked, with the sulfamate group in its monoanionic form (i.e., $-OSO_2NH^-$), into the crystal structure of STS as reported by Hernandez-Guzman et al.[16] Compounds from individual series dock in a similar manner showing a highly conserved binding mode (graphic not shown). STX64, the phase I trial STS inhibitor, and E1S, the natural substrate for STS, are also docked for comparison. As shown in Figure 10, the *p*-sulfamate **5** docks in the same region of the active site as that occupied by either STX64 or E1S, with the sulfamate group directed toward the catalytic cavity where the coordinating Ca^{2+} ion and formylglycine in its *gem*-diol form reside. Like the skeleton of STX64, the *p*-cyanophenyl moiety and partly the triazolyl group of **5** docked occupy the predominantly hydrophobic tunnel which leads to the entry to the active site. It is anticipated that the interactions between these two functionalities of DASI with the amino acid residues lining the hydrophobic tunnel will not be as optimal as those achieved by a tricyclic coumarin sulfamate or a steroidal inhibitor like EMATE, rendering the weaker STS inhibition observed for DASIs studied in Table 1.

For *m*-sulfamate **16**, it is apparent that it docks in a manner very different from that observed for its *p*-sulfamate counterpart **5** (Figure 10). Although its sulfamate group is also directed toward the catalytic cavity, most parts of the molecule clearly occupy a different pocket within the active site. With the much weaker STS inhibition shown by the *m*-sulfamates in Table 3, one can reason that the docking orientation as predicted for the *p*-sulfamate **5** in Figure 10 is indeed more favorable for enzyme inactivation. The positioning of the sulfamate group at the para position of the aryl ring appears to allow both the sulfamate group and the rest of the molecule to interact productively with the catalytic cavity and binding site of the enzyme. According to the docking studies, these favorable interactions will be significantly disrupted when the sulfamate group is positioned at the meta position of the aryl ring.

(iv) *m*-Phenols (Table 4). As shown in Table 4, all meta phenolic compounds tested show highly potent aromatase inhibition. The chloro derivative **15e** is the best AI whose inhibitory activity (IC_{50} = 0.18 nM) is some 3-fold more potent than that exhibited by **1** (0.5 nM, Table 1) obtained from the same assay. Apart from **18f**, whose sulfamate derivative cannot be isolated in high purity possibly because of its chemical instability in solution, all hydroxyl compounds tested are more potent AIs than their corresponding sulfamates (**6** vs **6d**, **14** vs **14e**, **15** vs **15e**, **16** vs **16h**, **17** vs **17d**). Similar to what has been discussed for the *p*-hydroxy and meta-substituent series, these findings reinforce the conclusion that the sulfamate moiety may be too large or polar relative to the hydroxyl group for effective binding of these inhibitors to the aromatase active site.

(B) In Vivo Inhibition. We reported in our previous publication that DASIs **2** and **5** showed promising dual inhibition in adult female Wistar rats pretreated with 200 IU/0.1 mL sc of PMSG (pregnant mares' serum gonadotropin).[13] In this work, we have retested compound **5** alongside compound **14**, the best DASI in the *m*-sulfamate series, and assessed their ability to inhibit aromatase and STS 3 and 24 h after oral administration of drug at a 10 mg/kg dose. The reference inhibitors used in the study are letrozole (instead of **1**) and STX64.

As shown in Table 5, the reduction of plasma estradiol levels attained is complete and indistinguishable 3 h after letrozole and **5** are administered. Compound **5** performs better in the current study than in the previous experiment when the reduction of plasma estradiol levels achieved 3 h after drug administration was lower at 68%.[13] When the level of inhibition is assessed 21 h later, letrozole remains fully inhibitory, while compound **5** only achieves 85% inhibition. These results suggest that the duration of action of letrozole is longer than **5** possibly as a result of more favorable pharmacokinetic properties. Despite their similar potencies in vitro, the *m*-sulfamate **14** is less potent than its *p*-sulfamate counterpart **5**, achieving a lower 82% inhibition 3 h after dosing. After 24 h, the level of aromatase inhibition achieved is even lower at 20% for **14**. Although the reason for the difference in the in vivo aromatase inhibitory activities observed between **5** and **14** remains to be elucidated, they are nonetheless highly effective AIs, albeit not as potent as the established AI letrozole.

Table 5. Percentage Inhibition of Aromatase and STS Activities in Female Wistar Rats Produced by Letrozole, DASIs **5** and **14**, and STX64[a]

	aromatase inhibition (%)		STS inhibition (%)	
treatment	3 h	24 h	3 h	24 h
control	0 ± 1	8 ± 13	0 ± 10	8 ± 6
PMSG	0 ± 22	0 ± 13	4 ± 4	0 ± 6
letrozole	100 ± 6	100 ± 13	3 ± 0.6	6 ± 6
5	100 ± 7	85 ± 7	98 ± 0.2	72 ± 2
14	82 ± 8	20 ± 44	98 ± 0.1	33 ± 5
STX64	ND	ND	100 ± 1.8	97 ± 1.4

[a] Results are expressed as the percentage inhibition of PMSG stimulated estradiol levels for aromatase activity or percentage of activity in untreated animals for STS activity (mean ± SE, $n = 3$). Where no error bars are shown, SE is less than 1%. The statistical significance for aromatase and STS Activities in control and treated groups was assessed using Student's *t* test: (a) $p < 0.05$; (b) $p < 0.001$. ND: not determined.

When the selectivity of **5** toward the inhibition of aromatase is assessed in vivo, it shows no significant inhibition on the plasma aldosterone levels 3 and 24 h after treatment, suggesting that **5**, like **1**, is a selective AI for aromatase (unpublished data).

When the liver STS activity is assayed in animals treated with a single oral dose of each inhibitor at 10 mg/kg, both **5** and **14** almost give 100% inhibition 3 h later despite **14** (Table 3) being a weaker STS inhibitor than **5** (Table 1) in vitro by some 15-fold. Although the mechanism for this phenomenon is not clear, this is not the first time when we have observed a compound that is an apparently weak STS inhibitor in vitro but shows potent inhibition in vivo. Thus, the unsubstituted congener **2** (Table 1), like **5**, was found to inhibit rat liver STS activity by nearly 100% at an oral dose of 10 mg/kg despite **2** being 6-fold weaker than **5** as an STS inhibitor in vitro.[13] In addition, while a letrozole-based DASI gave an IC_{50} of $>10\ \mu M$ in JEG-3 cells, it inhibited rat liver STS activity by 88% 24 h after a single oral dose of 10 mg/kg was administered to the animals.[36] Similarly, the congener of STX64, 6615COUMATE, despite its IC_{50} in a placental microsomal preparation being 370 times higher than that of 6610COUMATE, inhibited rat liver STS activity significantly more strongly than 6610COUMATE.[37] These results clearly suggest that the STS inhibitory activity of a sulfamate-based compound in JEG-3 cells, particularly for those DASIs studied here, may not reflect the full STS inhibitory potential of such a compound in vivo where other factors could be involved in transforming the inhibitor and/or delivering the active species to the target enzyme.

Unlike STX64 where almost complete inhibition is observed, the levels of inhibition attained by compounds **5** and **14** are reduced to 72% and 33%, respectively, 24 h after dosing. This is surprising because compound **5**, like its congener **2**,[13] was found to be an irreversible inhibitor of STS (data not shown). Although compound **14** has not been studied in the same manner, it is anticipated that it will share the same mechanism of action as its congeners. One possible explanation for this observation is that compounds **5** and **14** are metabolized more efficiently than STX64 in vivo. It has been shown that in vivo STX64 is sequestered into the RBCs through reversible binding to CAII, whose IC_{50} values were determined to be in the range 17−25 nM.[31,38,39] This interaction of STX64 with CAII has been postulated to be an essential mechanism for transporting STX64 in vivo, protecting it from first-pass degradation, and hence contributing to the excellent bioavailability of 95% observed in rats.[40] Like STX64, the sulfamate-containing **5** also interacts with CAII albeit less effectively as shown by its higher IC_{50} of 118 nM.[31] It is therefore possible, inter alia, that **5**, being a weaker CAII inhibitor, complexes to a lower extent with CAII than STX64. As a result, **5** may be less protected from first-pass metabolism, rendering the shorter duration of action observed in vivo. The ability of **14** to inhibit CAII has not been studied, although the reduction in the level of inhibition observed for this compound 24 h after dosing can also be attributed to the less favorable pharmacokinetics properties of **14**. Further investigation into the biological activities of **5** and **14** is warranted, but these studies are beyond the scope of the work reported here.

Conclusions

Compound **1** is a highly potent and selective nonsteroidal AI. In this work, replacing its *p*-bromo atom with a sulfamate ester introduces the pharmacophore for irreversible STS inhibition. SAR studies have produced a series of *p*- and *m*-sulfamates that are either unsubstituted or substituted with a halogen, OMe, or CN group at the position(s) ortho to the sulfamate. Upon screening in JEG-3 cells, the most promising DASIs are the *m*-bromo-*p*-sulfamate **5** (IC_{50}(aromatase) = 0.82 nM and IC_{50}(STS) = 39 nM) and the *p*-fluoro-*m*-sulfamate **14** (IC_{50}(aromatase) = 0.77 nM and IC_{50}(STS) = 590 nM). Relocation of the sulfamate group from the para to the meta position of the aryl ring generally improves the aromatase, but weakens the sulfatase, inhibitory activity of the derivatives compared to their *p*-sulfamate counterparts. Most phenolic precursors inhibit aromatase more strongly than their corresponding sulfamates. Hence, it is reasoned that these phenols may further sustain aromatase inhibition in vivo when they are released as a result of chemical degradation and/or by sulfatase-mediated cleavage of their corresponding sulfamates upon STS inactivation. The SAR of this class of DASIs was explored by molecular modeling studies. Different docking orientations within the active site of an aromatase homology model are observed for different series of sulfamates and phenols. However, it appears that the higher potency observed for some molecules may be attributed partly to the proximity of their *p*-cyanophenyl ring to the putative

hydrogen bond donating residue Ser478. For STS, *p*-sulfamates dock into a region similar to that occupied by either STX64 or E1S in the active site, while *m*-sulfamates, which are weaker STS inhibitors, dock differently with their *p*-cyanophenyl rings residing in a different binding pocket.

In vivo, both **5** and **14** show potent dual inhibition, with **5** achieving almost complete inhibition 3 h after a single oral 10 mg/kg dose. This level of efficacy is comparable to that produced by STX64 (STS inhibitor) and letrozole (AI) individually at the same time point. However, the dual inhibition of **5** is reduced to 85% (aromatase) and 72% (STS) after 24 h when STX64 and letrozole still inhibit potently (>97%). Compound **5** inhibits aldosterone synthesis insignificantly *in vivo* 3 and 24 h after dosing, suggesting that this DASI, like **1**, is also selective toward aromatase inhibition. While **5** thus represents a promising YM511-based DASI, the challenge is to develop analogues that not only exhibit potent dual inhibition in vitro but also possess an optimized and favorable inhibitory profile in vivo. This is underway. The development of a highly effective DASI should allow the therapeutic potential of estrogen ablation effected by dual aromatase−STS inhibiton in HDBC to be evaluated.

Experimental Section

Chemistry. All reagents were of commercial quality obtained from either Sigma-Aldrich (Gillingham, Dorset, U.K.) or Lancaster Synthesis (Morecambe, Lancashire, U.K.). Solvents were dried, where necessary, using standard procedures. Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets, silica gel 60 F_{254}) with detection by UV light or with phosphomolybdic acid in EtOH followed by heating. Flash chromatography was performed on silica gel (particle size 40-63 μm). NMR spectra were recorded with a JEOL EX-270 or a Varian Mercury VX 400 spectrometer. ^{1}H NMR and ^{13}C NMR chemical shifts are measured in ppm (δ) relative to internal tetramethylsilane (TMS). Signals, where declared, are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Coupling constants (J) are given in Hz. LRMS and HRMS mass spectra were recorded at the University of Bath on a Finnigan MAT 8340 instrument or at the EPSRC National Mass Spectrometry Service Centre, University of Wales Swansea, Chemistry Department. High-performance liquid chromatography (HPLC) analysis was performed on a Waters 717 with an autosampler and PDA detector. For chromatograhic conditions, refer to Supporting Information. Unless stated otherwise, all biologically tested compounds attained a purity level of >95% by HPLC. Liquid chromatography−mass spectrometry (LCMS) analysis was performed using a Waters "Symmetry" C18 (packing: 3.5 μm, 4.6 mm × 100 mm) column on a Waters 2790 Alliance, ZQ MicroMass spectrometer with PDA detector. Gradient was 5:95 MeCN/H_2O (flow rate of 0.5 mL/min) to 95:5 MeCN/H_2O (flow rate of 1 mL/min) over 10 min. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points (mp) were determined using a Reichert-Jung hot stage microscope apparatus, a Gallenkamp MPD-350 apparatus, or Stanford Research Systems OptiMelt MPA100 and are uncorrected.

Biology. The extent of in vitro inhibition of STS and aromatase activity by compounds was assessed using JEG-3 cells. Cells were seeded into 24-well culture plates and maintained in MEM (Flow Laboratories, Irvine, U.K.) 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 (0.001−10000 nmol/L) inhibitor. The product E1 was separated from E1S by 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 (2−3 nM, 30 Ci/mmol; Perkin-Elmer Life Sciences, MA) was incubated with JEG-3 cells for 1 h in the presence or absence of inhibitor. The product, E1, was separated using dextran-coated charcoal at 4 °C for 2 h, and remaining radioactivity was measured by scintillation spectrometry. Each IC_{50} represents the mean ± SE of triplicate measurements.

The in vivo inhibition of aromatase and STS activity by letrozole, DASIs **5** and **14**, and STX64 was assessed in female Wistar rats. Animals received a single subcutaneous injection of pregnant mares' serum gonadotropin (PMSG, 200 IU, Sigma). Three days later drugs (10 mg/kg) were administered orally in THF/propylene glycol (10:90) as a single dose. Blood and liver samples were obtained 3 and 24 h after drug administration. Plasma concentrations of estradiol were measured using a radioimmunoassay 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 inhibition of PMSG stimulated estradiol levels for aromatase activity or percentage of activity in untreated animals for STS activity (means ± SE, $n = 3$).

The effects of **5** on cytochrome P450 dependent biosynthesis of steroids and hence the selectivity of this DASI toward inhibiting aromatase were assessed by monitoring the plasma aldosterone level (after 3 and 24 h) in treated animals using a radioimmunoassay kit (Diagnostic Systems Laboratories Inc., TX).

Molecular Modeling. (i) Homology Model of the Human Aromatase Enzyme. A homology model of the human aromatase enzyme, which is based on the recently published crystal structure of the human CYP2C9 metabolic enzyme,[41] was processed as described by Favia et al.[15] using the Sybyl 7.1[42] molecular modeling software suite. The process involved the addition of hydrogen to all atoms and the fixing of side chain amides, followed by the energy minimization of hydrogen atom positions with fixed heavy atoms. The AMBER FF02 force field was used with Gasteiger−Hückel charges applied, as implemented within Sybyl 7.1.

The ligands to be docked into the aromatase homology model were generated using the Sybyl 7.1 molecular modeling package after they had been minimized using the MMFF94s force field with MMFF94 charges applied, as implemented in Sybyl 7.1. All docking calculations were performed using the GOLD[43] molecular docking package with each ligand docked a total of 35 times.

Not all the compounds are found to dock in a fashion as anticipated for this class of nonsteroidal AIs with the nitrogen-containing heterocycle, the triazole group in our case, coordinating with the heme iron via one of its N atoms. It is found that the docking of DASIs into the homology model shows their sulfamate moiety interacting with the heme iron instead of their triazole group. The GOLD docking program recognizes the interactions of the sulfamate moiety with the heme iron being a more dominant feature than the coordinate triazole−iron bond. This failure of the docking program to recognize the heme ligating property of nitrogen-containing heterocycles was also reported by Verras et al.[44] where the DOCK program failed to recognize the coordinate imidazole−iron bond and produced unreasonable inhibitor orientations. In order to rectify this undesirable orientation of DASIs experienced during the docking calculation, the ligand−protein distance was defined using the constraint distance functionality within GOLD. So the distance between the ligand nitrogen atom and the heme iron atom was constrained to be between 2.00 and 2.30 Å as estimated by examination of crystal structures from the CSD.[45]

(ii) Crystal Structure of Steroid Sulfatase. The 1P49 crystal structure of human placental estrone/DHEA sulfatase was used for the building of the *gem*-diol form of STS.[16] This involved a point mutation of ALS75 residue in the crystal structure to the *gem*-diol form using the structure editing tools within Sybyl 7.1. The resulting structure was then minimized with the backbone atoms fixed to allow the *gem*-diol and surrounding side chain atoms to adopt low-energy conformations. Minimizations were undertaken using Sybyl 7.1, applying the AMBER7 99 force field with Gasteiger−Hückel charges as implemented within Sybyl 7.1. In order to mimic the sulfate group of E1S, all sulfamate-based compounds are docked

into the active site with their sulfamate group in its monoanionic form (i.e., $-OSO_2NH^-$).[34]

4-[(4-Cyanophenyl)amino]-4*H*-[1,2,4]triazole (1a). The title compound was prepared according to the method reported by Okada et al.[14] using potassium *tert*-butoxide (6.7 g, 59.5 mmol), 4-amino-4*H*-[1,2,4]triazole (5.0 g, 59.5 mmol), and 4-fluorobenzonitrile (3.60 g, 29.7 mmol) in anhydrous DMSO (40 mL). Upon workup, **1a** crystallized out of the neutralized aqueous solution as small yellow crystals upon standing at room temperature overnight. Recrystallization from H_2O gave **1a** as colorless crystals (2.08 g, 38%): mp 200-204 °C [lit.[14] 206−208 °C (acetone)].

4-[(4-Bromobenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (1). The title compound was prepared according to the method reported by Okada et al.[14]

4-[(4-Benzyloxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (2b). To a stirred suspension of NaH (60% dispersion in oil, 0.151 g, 3.78 mmol) in anhydrous DMF (5 mL) was added a solution of 4-[(4-cyanophenyl)amino]-4*H*-[1,2,4]triazole, **1a** (0.7 g, 3.78 mmol), in anhydrous DMF (3 mL), and the mixture stirred at room temperature for 0.5 h. A solution of 4-benzyloxybenzyl chloride, **2a** (0.968 g, 4.16 mmol) in anhydrous DMF (2 mL), was then added and the mixture heated at 80−90 °C overnight. The mixture was cooled, diluted with EtOAc (50 mL), washed with water (4 × 100 mL) and brine (100 mL), and dried (Na_2SO_4). Concentration in vacuo gave an orange residue, which was crystallized from *i*-PrOH to give **2b** as a pale-cream powder (1.35 g, 72%): mp 206-211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 4.98 (s, 2H), 5.06 (s, 2H), 6.77 (AA′BB′, 2H), 6.95 (AA′BB′, 2H), 7.21 (AA′BB′, 2H), 7.30−7.46 (m, 5H), 7.76 (AA′BB′, 2H), and 8.75 (s, 2H). Anal. ($C_{23}H_{19}N_5O$) C, H, N.

4-[(4-Hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (2c). To a solution of **2b** (1.0 g, 2.62 mmol) in anhydrous THF/MeOH (180 mL) was added Pd−C (10% by weight, 0.1 g). The black suspension was then stirred under an atmosphere of hydrogen (balloon) for 72 h. The catalyst was removed by filteration through Celite and exhaustively washed with THF. The filtrate was concentrated in vacuo to give a beige residue. Recrystallization from EtOH gave **2c** as a white solid (0.74 g, 97%): mp 229-233 °C; ^{1}H NMR δ_H (400 MHz, DMSO-d_6) 4.91 (s, 2H), 6.67 (AA′BB′, 2H), 6.77 (AA′BB′, 2H), 7.06 (AA′BB′, 2H), 7.76 (AA′BB′, 2H), 8.71 (s, 2H), and 9.49 (s, 1H, exchanges with D_2O). Anal. ($C_{16}H_{13}N_5O$) C, H, N.

Sulfamic Acid 4-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]-methyl}phenyl Ester (2). To an ice-cooled solution of **2c** (0.20 g, 0.69 mmol) in anhydrous DMA (2 mL) was added sulfamoyl chloride (0.69 M solution in toluene, 2.2 mL; the toluene was removed in vacuo (not allowing the temperature of the water bath to exceed 30 °C prior to addition, 1.51 mmol), and the mixture was stirred (under a positive flow of dry nitrogen) overnight. The mixture was diluted with EtOAc (25 mL), washed with water (3 × 50 mL) and brine (50 mL), and concentrated in vacuo (not allowing the temperature of the water bath to exceed 30 °C). The residue was purified by gradient elution flash column chromatography [SiO_2, $CHCl_3$/MeOH (7:1) to $CHCl_3$/MeOH (3.5:1)] to give **2** as a white powder (0.244 g, 95%): mp 153-158 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 5.10 (s, 2H), 6.74 (AA′BB′, 2H), 7.23 (AA′BB′, 2H), 7.42 (AA′BB′, 2H), 7.77 (AA′BB′, 2H), 8.03 (br s, 2H, exchanges with D_2O), and 8.85 (s, 2H). Anal. ($C_{16}H_{14}N_6O_3S$) C, H.

4-Benzyloxy-3-fluorobenzaldehyde (3b). A mixture of 3-fluoro-4-hydroxybenzaldehyde, **3a** (4.90 g, 35.0 mmol), benzyl bromide (6.84 g, 40.0 mmol, 4.80 mL), and K_2CO_3 (9.66 g, 70.0 mmol) in DMF (50 mL) was stirrred for 18 h at room temperature and then diluted with EtOAc (100 mL) and water (50 mL). The organic layer was separated, washed with water (2 × 50 mL) and brine (30 mL) and dried (Na_2SO_4). Concentration in vacuo gave a white solid residue. Recrystallization from CH_2Cl_2/*n*-hexane gave **3b** as colorless needles (7.65 g, 95%): mp 94−95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 5.24 (s, 2H), 7.12 (dd, J = 8.2 Hz, 8.2 Hz, 1H), 7.34−7.48 (m, 5H), 7.59−7.66 (m, 2H), 9.85 (d, J = 2.0 Hz, 1H). Anal. ($C_{14}H_{11}FO_2$) C, H.

4-Benzyloxy-3-fluorobenzyl Alcohol (3c). To a solution of **3b** (7.32 g, 31.8 mmol) in EtOH (40 mL) and THF (40 mL) was added $NaBH_4$ (0.5 g, 13.2 mmol) at 0 °C. The clear solution was allowed to warm to room temperature and stirred for 12 h at this temperature. The reaction was quenched by addition of EtOAc (150 mL) and water (50 mL). The organic layer was separated, washed with water (2 × 50 mL) and brine (50 mL), and dried (Na_2SO_4). Concentration in vacuo gave a white solid residue, which dissolved in the minimum volume of CH_2Cl_2 and precipitated by addition of *n*-hexane. The precipitate was filtered and dried under high vacuum to give **3c** as a white powder (7.16 g, 97%): mp 56-57 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 1.71 (s, 1H, exchanges with D_2O), 4.61 (s, 2H), 5.15 (s, 2H), 6.97 (dd, J = 8.6 Hz, 8.6 Hz, 1H), 7.02 (dd, J = 8.6 Hz, 1.9 Hz, 1H), 7.13 (dd, J = 11.7 Hz, 1.9 Hz, 1H), 7.30−7.46 (m, 5H). Anal. ($C_{14}H_{13}FO_2$) C, H.

4-Benzyloxy-3-fluorobenzyl Chloride (3d). Thionyl chloride (5 mL) was added to solution of **3c** (6.80 g, 29.28 mmol) in anhydrous CH_2Cl_2 (50 mL). The solution was stirred for 1 h at room temperature and concentrated under reduced pressure. Et_2O (100 mL) and water (20 mL) were added, and the organic layer was separated, washed with saturated $NaHCO_3$ solution (10 mL), and dried (Na_2SO_4). Concentration in vacuo and subsequent trituration from CH_2Cl_2 solution by addition of *n*-hexane afforded a white precipitate. The product was filtered off and dried under high vacuum to give **3d** as a white powder (7.01 g, 95%): mp 67−69 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.52 (s, 2H), 5.15 (s, 2H), 6.96 (dd, J = 8.2 Hz, 8.2 Hz, 1H), 7.04-7.06 (m, 1H), 7.15 (dd, J = 11.7 Hz, 2.4 Hz, 1H), 7.31−7.45 (m, 5H). Anal. ($C_{14}H_{12}ClFO$) C, H.

4-[(4-Benzyloxy-3-fluorobenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (3e). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.4 g, 10 mmol), **1a** (1.852 g, 10 mmol), and **3d** (2.51 g, 10 mmol) in anhydrous DMF (50 mL) to give **3e** as a white solid (3.12 g, 78%) after recrystallization from *i*-PrOH: mp >220 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 4.97 (s, 2H), 5.12 (s, 2H), 6.73 (AA′BB′, 2H), 7.01 (dd, J = 8.2 Hz, 1.2 Hz, 1H), 7.16 (dd, J = 8.6 Hz, 8.6 Hz, 1H), 7.21 (dd, J = 8.6 Hz, 2.4 Hz, 1H), 7.30−7.44 (m, 5H), 7.75 (AA′BB′, 2H), 8.80 (s, 2H).

4-[(3-Fluoro-4-hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (3f). The title compound was hydrogenated (over 18 h) in a manner similar to that for **2c**, using **3e** (2.83 g, 7.09 mmol) and Pd−C (5% by weight, 0.15 g) in THF/EtOH/EtOAc (1:1:1) (150 mL) to give **3f** as a colorless solid (2.13 g, 97%) after recrystallization from *i*-PrOH: mp >200 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 4.93 (s, 2H), 6.75 (AA′BB′, 2H), 6.82-6.89 (m, 2H), 7.07−7.12 (m, 1H), 7.76 (AA′BB′, 2H), 8.77 (s, 2H), 9.95 (s, 1H, exchanges with D_2O). Anal. ($C_{16}H_{12}FN_5O$) C, H, N.

Sulfamic Acid 4-{[(4-Cyanophenyl)[1,2,4]triazol-4-yl-amino]-methyl}-2-fluorophenyl Ester (3). The title compound was sulfamoylated in a manner similar to that for **2**, using **3f** (0.22 g, 0.71 mmol) and sulfamoyl chloride (2.1 mmol) in anhydrous DMA (5 mL) to give **3** as a white powder (0.228 g, 83%) after precipitation from acetone/Et_2O: mp 164−165 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 5.11 (s, 2H), 6.71 (AA′BB′, 2H), 7.23 (dd, J = 8.2 Hz, 1.2 Hz, 1H), 7.39 (dd, J = 8.2 Hz, 8.2 Hz, 1H), 7.43 (dd, J = 11.1 Hz, 2.1 Hz, 1H), 7.77 (AA′BB′, 2H), 8.28 (s, 2H, exchanges with D_2O), 8.92 (s, 2H). Anal. ($C_{16}H_{13}FN_6O_3S$) C, H.

4-Benzyloxy-3-chlorobenzaldehyde (4b). The title compound was prepared in a manner similar to that for **3b**, using 3-chloro-4-hydroxybenzaldehyde **4a** (7.83 g, 50.0 mmol) and benzyl bromide (9.41 g, 55.0 mmol) and K_2CO_3 (13.8 g, 0.1 mol) in DMF (100 mL) to give **4b** as a white solid (11.35 g, 92%): mp 92-93 °C (MeOH) [lit.[46] 88−89 °C (EtOH)]; ^{1}H NMR (270 MHz, $CDCl_3$) 5.25 (s, 2H), 7.09 (d, J = 8.4 Hz, 1H), 7.30-7.52 (m, 5H), 7.72 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 7.93 (d, J = 2.2 Hz, 1H), 9.87 (s, 1H). Anal. ($C_{14}H_{11}ClO_2$) C, H.

(4-Benzyloxy-3-chlorophenyl)methanol (4c). The title compound was prepared in a manner similar to that for **3c**, using **4b** (4.93 g, 20.0 mmol) and $NaBH_4$ (1.0 g, 26.4 mmol) in EtOH (100 mL) to give **4c** as a white solid (4.43 g, 89%): mp 53-56 °C; ^{1}H

NMR δ_H (400 MHz, $CDCl_3$) 2.14 (s, 1H, exchanges with D_2O), 4.56 (s, 2H), 5.16 (s, 2H), 6.93 (d, J = 8.6 Hz, 1H), 7.14 (dd, J = 8.6 Hz, 2.3 Hz, 1H), 7.31−7.48 (m, 6H). Anal. ($C_{14}H_{13}ClO_2$) C, H.

1-Benzyloxy-4-bromomethyl-2-chlorobenzene (4d). To a solution of **4c** (3.40 g, 13.7 mmol) in CH_2Cl_2 (50 mL) was added PBr_3 (2.0 mL, 5.76 g, 21.3 mmol) at 0 °C. The mixture was stirred for 1 h at this temperature. Then water (30 mL) was added, and the organic layer was separated, washed with water (2 × 30 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated in vacuo to give a white solid (4.27 g, 100%): mp 73−76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.44 (s, 2H), 5.17 (s, 2H), 6.92 (d, J = 8.2 Hz, 1H), 7.21 (dd, J = 8.2 Hz, 2.3 Hz, 1H), 7.31−7.48 (m, 6H). Anal. ($C_{14}H_{12}BrClO$) C, H.

4-[(4-Benzyloxy-3-chlorobenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (4e). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.2 g, 5.0 mmol), **1a** (0.926 g, 5.0 mmol), and **4d** (1.56 g, 5.0 mmol) in anhydrous DMF (20 mL) to give **4e** as a white solid (1.38 g, 66%) after recrystallization from *i*-PrOH: mp 212−214 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 4.99 (s, 2H), 5.17 (s, 2H), 6.76 (AA′BB′, 2H), 7.16 (d, J = 8.6 Hz, 1H), 7.20 (dd, J = 8.6 Hz, 2.2 Hz, 1H), 7.32−7.47 (m, 6H), 7.76 (AA′BB′, 2H), 8.81 (s, 2H). Anal. ($C_{23}H_{18}ClN_5O$) C, H, N.

4-[(3-Chloro-4-hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (4f). The title compound was hydrogenated (over 18 h) and prepared in a manner similar to that for **2c**, using **4e** (1.04 g, 2.50 mmol), Pd−C (10% by weight, 0.05 g) in THF/MeOH/EtOAc (1:1:1) (75 mL) to give **4f** as a colorless solid (0.484 g, 59%) after recrystallization from *i*-PrOH: mp 221−222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 4.93 (s, 2H), 6.76 (AA′BB′, 2H), 6.86 (d, J = 8.6 Hz, 1H), 7.02 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.27 (d, J = 2.0 Hz, 1H), 7.76 (AA′BB′, 2H), 8.77 (s, 2H), 10.29 (s, 1H, exchanges with D_2O). Anal. ($C_{16}H_{12}ClN_5O$) C, H, N.

Sulfamic Acid 2-Chloro-4-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}phenyl Ester (4). The title compound was prepared in a manner similar to that for **2**, using **4f** (0.163 g, 0.50 mmol) and sulfamoyl chloride (2.1 mmol) in anhydrous DMA (5 mL) to give **4** as a white powder (0.059 g, 29%) after precipitation from acetone/Et_2O: mp 103−108 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 5.11 (s, 2H), 6.72 (AA′BB′, 2H), 7.38 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.59 (d, J = 2.0 Hz, 1H), 7.77 (AA′BB′, 2H), 8.31 (s, 2H, exchanges with D_2O), 8.91 (s, 2H). Anal. ($C_{16}H_{13}ClN_6SO_3$) C, H, N.

3-Bromo-4-hydroxybenzaldehyde (5b). The title compound is commercially available but was prepared according to the method reported by Kelly et al.[47] using 4-hydroxybenzaldehyde, **5a**, and Br_2 in glacial AcOH.

3-Bromo-4-benzoyloxybenzaldehyde (5c). To a solution of **5b** (8.0 g, 40.0 mmol) in EtOAc (100 mL) was added NEt_3 (5.58 mL, 40.0 mmol), and the mixture was stirred at room temperature for 0.5 h. Benzoyl chloride (4.64 mL, 40.0 mmol) was then added, and the mixture was stirred at room temperature for 5 h. The precipitated NEt_3·HCl was filtered off and the organic solution dried (Na_2SO_4). Concentration in vacuo gave a grayish residue. Recrystallization from EtOAc/*n*-hexane gave **5c** as a slightly yellow solid (10.9 g, 89%): mp 89−91 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 7.48 (d, J = 8.4 Hz, 1H), 7.54 (t, J = 8.2 Hz, 2H), 7.68 (t, J = 7.2 Hz, 1H), 7.90 (dd, J = 8.2 Hz, 1.5 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H), 8.24 (d, J = 7.2 Hz, 1H), 9.97 (s, 1H). Anal. ($C_{14}H_9O_3Br$) C, H.

3-Bromo-4-benzoyloxybenzyl Alcohol (5d). The title compound was prepared in a manner similar to that for **3c**, using **5c** (4.93 g, 20.0 mmol) and $NaBH_4$ (1.13 g, 30.0 mmol) in EtOH (100 mL) to give **4d** as a white solid. Recrystallization from EtOAc/*n*-hexane gave **5d** as colorless needles (5.89 g, 96%): mp 101−103 °C; ^{1}H NMR δ_H (270 MHz, $CDCl_3$) 1.82 (t, J = 5.9 Hz, 1H, exchanges with D_2O), 4.70 (d, J = 6.2 Hz, 2H), 7.26 (d, J = 8.2 Hz, 1H), 7.36 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 7.53 (t, J = 7.9 Hz, 1H), 7.66 (t, J = 7.4 Hz, 1H), 7.68 (d, J = 2.0 Hz, 1H), 8.24 (d, J = 7.2 Hz, 2H); LRMS (FAB+) m/z (rel intensity) 308 (10, [M + H]), 105 (100). Anal. ($C_{14}H_{11}O_3Br$) C, H.

4-Benzoyloxy-3-bromobenzyl Chloride (5e). Thionyl chloride (3.22 mL, 44.20 mmol) was added to a solution of **5d** (9.05 g, 29.46 mmol) in anhydrous CH_2Cl_2 (100 mL). The mixture was stirred at room temperature for 2 h, and the volatiles were removed in vacuo. The residue was redissolved and coevaporated three times with CH_2Cl_2 (3 × 20 mL) and recrystallized from EtOAc/*n*-hexane to give **5e** as a white needles (8.01 g, 84%): mp 63−65 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 4.55 (s, 2H), 7.26 (d, J = 8.4 Hz, 1H), 7.38 (dd, J = 8.2 Hz, 1.7 Hz, 1H), 7.51 (t, J = 7.9 Hz, 2H), 7.65 (t, J = 7.2 Hz, 1H), 7.68 (d, J = 1.5 Hz, 1H), 8.22 (d, J = 7.7 Hz, 2H). Anal. ($C_{14}H_{10}O_2BrCl$) C, H.

4-Benzoyloxy-3-bromotoluene (5g). The title compound was prepared by adapting the method for **5c**, using 2-bromo-4-methylphenol, **5f** (9.35 g, 50.0 mmol), NEt_3 (6.97 mL, 50.0 mmol), and benzoyl chloride (5.80 mL, 50.0 mmol) in EtOAc (125 mL) to give **5g** as colorless needles (9.27 g, 64%) after recrystallization from *n*-hexane: mp 71−73 °C [lit.[48] 71−72 °C]; ^{1}H NMR (270 MHz, $CDCl_3$) 2.35 (s, 3H), 7.17 (d, J = 8.2 Hz, 1H), 7.17 (dd, J = 8.2 Hz, 1.6 Hz, 1H), 7.47 (d, J = 1.6 Hz, 1H), 7.52 (m, 2H), 7.65 (m, 1H), 8.24 (m, 2H).

4-Benzoyloxy-3-bromobenzyl Bromide (5h). Method A. To a solution of **5d** (3.07 g, 10.0 mmol) in anhydrous CH_2Cl_2 (45 mL) at 0 °C was added PBr_3 (0.98 mL, 10.3 mmol). The mixture was stirred at 0 °C for 2 h and then at room temperature for 1 h. The mixture was poured onto ice−water (400 mL) and allowed to warm to room temperature. The aqueous solution was extracted with Et_2O (5 × 100 mL) and the combined ethereal solution dried ($MgSO_4$). Concentration in vacuo afforded a slightly yellow oil, which upon purification by column chromatography [SiO_2, EtOAc/*n*-hexane (1:7)] gave **5h** as colorless needles (3.22 g, 87%): mp 59-61 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 4.56 (s, 2H), 7.25 (d, J = 8.4 Hz, 1H), 7.40 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.53 (t, J = 7.9 Hz, 2H), 7.66 (t, J = 7.7 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H), 8.23 (d, J = 8.4 Hz, 2H). Anal. ($C_{14}H_{10}O_2Br_2$) C, H.

Method B (Radical Bromination). Commercial *N*-bromosuccinimide (NBS) was recrystallized from water and thoroughly dried in vacuo (24 h) prior to use (mp 180−182 °C, lit. 180−183 °C). To a solution of **5g** (2.61 g, 8.96 mmol) in anhydrous CCl_4 (15 mL) was added finely powdered NBS (1.59 g, 8.96 mmol) and benzoyl peroxide (0.05 g, 0.19 mmol). The mixture was heated at reflux for 2 h and then cooled, and the precipitated succinimide was filtered off and washed with anhydrous carbon tetrachloride (20 mL). The combined filtrate was concentrated in vacuo and the pale-yellow residue purified by chromatography [SiO_2, EtOAc/*n*-hexane (1:7)] to give **5h** as white needles (2.05 g, 62%). The analytical data for this sample of **5h** were identical to the data obtained for the sample prepared by method A.

2-Bromo-4-hydroxymethylphenol (5i). The title compound was prepared in a manner similar to that for **3c**, using 3-bromo-4-hydroxybenzaldehyde **5b** (4.02 g, 20.0 mmol) and $NaBH_4$ (1.0 g, 26.4 mmol) in EtOH (50 mL) to give **5i** as a light-yellow solid (2.40 g, 59%): mp 127−129 °C (EtOAc/hexane) [lit.[49] 128 °C (CCl_4)]; ^{1}H NMR (400 MHz, DMSO-d_6) 4.37 (s, 2H), 5.11 (s, 1H), 6.89 (d, J = 8.2 Hz, 1H), 7.10 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 10.08 (s, 1H). Anal. ($C_7H_7BrO_2$) C, H.

Benzoic Acid 2-Bromo-4-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}phenyl Ester (5j). The title compound was prepared by adapting the method for **2b**, using NaH (60% dispersion in oil, 0.22 g, 5.4 mmol), **1a** (1.0 g, 5.68 mmol), and **5e** (1.85 g, 5.68 mmol) or **5h** (2.00 g, 5.4 mmol) in anhydrous DMF (5 mL) to give **5j** as a colorless solid (1.90 g, 74% from **5e** and 1.99 g, 78% from **5h**) after recrystallization: mp 196−206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 5.14 (s, 2H), 6.77 (AA′BB′, 2H), 7.44 (d, J = 8.2 Hz, 1H), 7.61−7.66 (m, 2H), 7.76−7.80 (m, 5H), 8.15 (AA′BB′, 2H), 8.92 (s, 2H).

4-[(3-Bromo-4-hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (5k). Method 1 (from 5j, with the Phenolic Group Protected). To a suspension of **5j** (2.0 g, 4.22 mmol) in MeOH (10 mL) was added KOH (1.42 g, 25.3 mmol), and the mixture was stirred at room temperature for 2 h. The solvents were reduced to ∼2 mL in vacuo, and the slurry was treated with saturated

$NaHCO_3$ solution. The white precipitate was filtered off, washed with the minimum of cold water, and boiled in *i*-PrOH to give **5k** as a white powder (1.20 g, 77%): mp 229−233 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 4.93 (s, 2H), 6.77 (AA′BB′, 2H), 6.85 (d, J = 8.2 Hz, 1H), 7.06 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.76 (AA′BB′, 1H), 8.76 (s, 1H), 10.35 (br s, 1H, exchanges with D_2O). Anal. ($C_{16}H_{12}BrN_5O$) C, H, N.

Method 2 (from 5i, without Protection of the Phenolic Hydroxy Group). A solution of **5i** (2.03 g, 10.0 mmol) in $SOCl_2$ (5 mL) was stirred at room temperature for 2 h. The excess $SOCl_2$ was removed under reduced pressure, and the residue was dissolved in DMF (20 mL). Then **1a** (1.85 g, 10.0 mmol) and K_2CO_3 (2.76 g, 20.0 mmol) were added and stirring was continued for 18 h. The mixture was diluted with EtOAc (60 mL), washed with water (3 × 50 mL) and brine (30 mL), and dried (Na_2SO_4). Concentration in vacuo gave a solid residue, which was purified by flash column chromatography [SiO_2, $CHCl_3$/acetone (1:1)] to give **5k** (2.15 g, 58%) as a white solid. The analytical data for this sample of **5k** were identical to the data obtained for the sample prepared by method A.

Sulfamic Acid 2-Bromo-4-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}phenyl Ester (5). The title compound was sulfamoylated by adapting the method for **2**, using **5k** (0.50 g, 1.35 mmol) and sulfamoyl chloride (8.1 mmol) in anhydrous DMA (2 mL) to give **5** as a colorless amorphous solid (0.107 g, 44%) after gradient elution gravity column chromatography [SiO_2, EtOAc/*n*-hexane (1:4) to EtOAc (100%)]: mp 133-138 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 5.17 (s, 2H), 6.80 (AA′BB′, 2H), 7.50 (s, 2H), 7.78 (s, 1H), 7.84 (AA′BB′, 2H), 8.38 (s, 2H), 8.96 (s, 2H). Anal. ($C_{16}H_{13}BrN_6O_3S\cdot{}^1/_4EtOAc$) C, H, N. Crystals suitable for X-ray analysis were grown by slow diffusion of *n*-hexane into a solution of **5** in EtOAc.

4-[(3-Benzyloxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (6c). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.22 g, 5.4 mmol), **1a** (1.0 g, 5.68 mmol), and 3-benzyloxybenzyl bromide,[50] **6b** (1.57 g, 5.66 mmol), in anhydrous DMF (5 mL) to give **6c** as a colorless solid (0.58 g, 28%) after chromatography [SiO_2, EtOAc/*n*-hexane (1:1)] and recrystallization from EtOH: mp 168−170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.85 (s, 2H), 5.03 (s, 2H), 6.62 (AA′BB′, 2H), 6.77 (d, J = 7.8 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.96 (dd, J = 7.8 Hz, 2.4 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 7.34−7.37 (m, 5H), 7.57 (AA′BB′, 2H), 8.04 (s, 2H).

4-[(3-Hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (6d). The title compound was hydrogenated (over 16 h) in a manner similar to that for **2c**, using **6c** (0.4 g, 1.05 mmol), Pd−C (10% by weight, 0.04 g) in THF/MeOH (1:1) (20 mL) to give **6d** as a colorless solid (0.27 g, 88%) after recrystallization from EtOH: mp 228−231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 4.97 (s, 2H), 6.68 (AA′BB′, 2H), 6.72-6.75 (m, 3H), 7.11 (m, 1H), 7.76 (AA′BB′, 2H), 8.77 (s, 2H), 9.49 (br s, 1H, exchanges with D_2O).

Sulfamic Acid 3-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}phenyl Ester (6). The title compound was sulfamoylated in a manner similar to that for **2**, using **6d** (0.10 g, 0.34 mmol) and sulfamoyl chloride (0.69 mmol) in anhydrous DMA (2 mL) to give **6** as a colorless solid (0.06 g, 47%) after recrystallization from acetone/hexane: mp 161−164 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 5.11 (s, 2H), 6.74 (AA′BB′, 2H), 7.20−7.27 (m, 3H), 7.40 (t, J = 7.8 Hz, 1H), 7.77 (AA′BB′, 2H), 7.98 (br s, 2H, exchanges with D_2O), 8.81 (s, 2H).

2-Iodo-4-methylphenol (7b). The title compound was prepared using the method reported by Narender et al.[51] To a well-stirred solution of *p*-cresol and **7a** (6.49 g, 60 mmol) in MeOH (300 mL) was added potassium iodide (10.96 g, 66 mmol) and Oxone (40.58 g, 66 mmol), and the mixture was stirred at room temperature overnight. The resulting mixture was filtered. The solids were washed with MeOH, and the combined organic filtrates were concentrated in vacuo. The purple residue was purified by flash column chromatography [SiO_2, EtOAc/*n*-hexane (1:20)] to give **7b** as a light-brown oil (7.61 g, 54%): ^{1}H NMR (270 MHz, $CDCl_3$) 2.24 (s, 3H), 5.20 (br s, 1H, exchanges with D_2O), 6.86 (d, J = 8.2 Hz, 1H), 7.02 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H).

4-Benzoyloxy-3-iodotoluene (7c). The title compound was prepared in a manner similar to that for **5c**, using **7b** (7.54 g, 32.22 mmol), NEt_3 (4.49 mL, 32.22 mmol), and benzoyl chloride (3.74 mL, 32.22 mmol) in EtOAc (275 mL) to give **7c** as white needles (5.13 g, 47%) after recrystallization from EtOAc/*n*-hexane: mp 75−77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 2.36 (s, 3H), 7.12 (d, J = 8.2 Hz, 1H), 7.20 (dd, J = 8.2 Hz, 1.6 Hz, 1H), 7.22 (m, 2H), 7.53 (m, 2H), 7.66 (m, 1H), 7.69 (d, J = 1.6 Hz, 1H), 8.26 (m, 1H). Anal. ($C_{14}H_{11}IO_2$) C, H.

4-Benzoyloxy-3-iodobenzyl Bromide (7d). The title compound was prepared in a manner similar to that for **5h** (method B), using **7c** (3.03 g, 8.96 mmol), NBS (1.75 g, 9.86 mmol), and benzoyl peroxide (0.05 g, 0.19 mmol) in anhydrous CCl_4 (15 mL) to give **7d** as colorless needles (2.80 g, 75%) after chromatography [SiO_2, EtOAc/*n*-hexane (1:7)]: mp 74−75 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 4.44 (s, 2H), 7.22 (d, J = 8.2 Hz, 1H), 7.43 (dd, J = 8.2 Hz, 2.2 Hz, 1H), 7.53 (m, 2H), 7.66 (m, 1H), 7.90 (d, J = 2.2 Hz, 1H), 8.26 (m, 2H).

Benzoic Acid 4-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}-2-iodophenyl Ester (7e). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.20 g, 5.03 mmol), **1a** (0.89 g, 4.80 mmol), and **7d** (2.20 g, 5.28 mmol) in anhydrous DMF (10 mL) to give **7e** as a colorless solid (1.47 g, 59%) after chromatography [SiO_2, EtOAc (100%)] and recrystallization from EtOAc: mp 192−195 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 5.12 (s, 2H), 6.77 (AA′BB′, 2H), 7.34 (d, J = 8.4 Hz, 1H), 7.44 (dd, J = 8.4 Hz, 1.7 Hz, 1H), 7.65 (t, J = 7.7 Hz, 2H), 7.78 (m, 1H), 7.79 (AA′BB′, 2H), 7.91 (d, J = 1.7 Hz, 1H), 8.17 (d, J = 7.7 Hz, 2H), 8.90 (s, 2H). Anal. ($C_{23}H_{16}IN_5O_2$) C, H, N.

4-[(4-Hydroxy-3-iodobenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (7f). The title compound was prepared in a manner similar to that for **5j**, using **7e** (1.41 g, 2.70 mmol) and KOH (0.30 g, 5.41 mmol) in MeOH (10 mL) to give **7f** (0.71 g, 63%) after recrystallization from *i*-PrOH: mp 186−197 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 4.87 (s, 2H), 6.69 (d, J = 8.2 Hz, 1H), 6.75 (AA′BB′, 2H), 7.00 (dd, J = 8.2 Hz, 1.6 Hz, 1H), 7.51 (d, J = 1.6 Hz, 1H), 7.73 (AA′BB′, 1H), 8.71 (s, 1H). Ar*OH* is too broad to be observed. Anal. ($C_{16}H_{12}IN_5O$) C, H, N.

Sulfamic Acid 4-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}-2-iodophenyl Ester (7). The title compound was sulfamoylated in a manner similar to that for **2**, using **7f** (0.5 g, 1.35 mmol) and sulfamoyl chloride (8.1 mmol) in anhydrous DMA (2 mL) to give **7** as a colorless amorphous solid (0.107 g, 44%) after gradient elution gravity column chromatography [SiO_2, EtOAc/*n*-hexane (1:4) to EtOAc (100%)]: mp >140 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 5.07 (s, 2H), 6.72 (AA′BB′, 2H), 7.36 (d, J = 8.2 Hz, 1H), 7.41 (dd, J = 8.2 Hz, 1.6 Hz, 1H), 7.75 (AA′BB′, 2H), 7.85 (d, J = 1.6 Hz 1H), 8.27 (s, 2H), 8.86 (s, 2H).

4-[(4-Benzyloxy-3-methoxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (8d). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.22 g, 5.4 mmol), **1a** (1.0 g, 5.68 mmol), and 3-methoxy-4-benzyloxybenzyl bromide[23] (**8c**, 1.66 g, 5.4 mmol) in anhydrous DMF (5 mL) to give **8d** as an off-white powder (0.769 g, 35%) after recrystallization from *i*-PrOH: mp 210−214 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 3.80 (s, 3H), 4.79 (s, 2H), 5.12 (s, 2H), 6.64 (d, J = 2.0 Hz, 1H), 6.66 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 6.69 (AA′BB′, 2H), 6.81 (d, J = 8.2 Hz, 1H), 7.29−7.42 (m, 5H), 7.59 (AA′BB′, 2H), 8.06 (s, 2H).

4-[(4-Hydroxy-3-methoxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (8e). The title compound was hydrogenated (over 16 h) in a manner similar to that for **2c**, using **8d** (0.411 g, 0.999 mmol) and Pd−C (10% by weight, 0.042 g) in THF/MeOH (1:1) (20 mL) to give **8e** as a colorless powder (0.232 g, 72%) after recrystallization from *i*-PrOH: mp >220 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 3.71 (s, 3H), 4.90 (s, 2H), 6.61 (dd, J = 7.8 Hz, 2.0 Hz, 1H), 6.65 (d, J = 7.8 Hz, 1H), 6.79 (AA′BB′, 2H), 6.80 (d, J

= 2.0 Hz, 1H), 7.76 (AA′BB′, 2H), 8.72 (s, 2H), 9.06 (br s, 1H, exchanges with D_2O).

Sulfamic Acid 4-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]-methyl}-2-methoxyphenyl Ester (8). The title compound was sulfamoylated in a manner similar to that for **2**, using **8e** (0.10 g, 0.31 mmol) and sulfamoyl chloride (1.87 mmol) in anhydrous DMA (2 mL) to give **8** as a colorless amorphous solid (0.04 g, 32%) after precipitation from a EtOAc solution by addition of *n*-hexane: mp >150 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 3.77 (s, 3H), 5.12 (s, 2H), 6.76 (AA′BB′, 2H), 6.90 (dd, J = 8.2 Hz, 2.3 Hz, 1H), 7.08 (d, J = 2.3 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.22 (AA′BB′, 2H), 7.95 (s, 2H), 8.87 (s, 2H).

3-Cyano-4-fluorobenzoic Acid (9b). A solution of KH_2PO_4 (2.4 g in 25 mL of water) and H_2O_2 (3 mL, 30% in water) was added to a solution of 3-cyano-4-fluorobenzaldehyde **9a** in acetonitrile (50 mL). The mixture was cooled to 0 °C, and a solution of $NaClO_2$ (6.0 g in 50 mL of water, 80% technical grade) was slowly added with vigorous stirring. Stirring was continued until the production of O_2 ceased (~2 h), at which time a solution of Na_2SO_3 (3.0 g in 10 mL of water) was added. The mixture was stirred for another hour. Then 2 N HCl (50 mL) was added and the resulting mixture was extracted with EtOAc (5 × 50 mL). The combined organic fractions were washed with water (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo to give **9b** (4.63 g, 93%) as a white solid: mp 187−188 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 7.68 (t, J = 8.9 Hz, 1H), 8.23-8.38 (1H, m), 8.43 (dd, J = 6.2 Hz, 2.2 Hz, 1H), 13.55 (br s, 1H). Anal. ($C_8H_4FNO_2$) C, H, N.

4-Benzyloxy-3-cyanobenzoic Acid (9c). To a solution of **9b** (4.13 g, 25.0 mmol) and benzyl alcohol (4.33 g, 40.0 mmol) in DMSO (50 mL) was added NaH (2.40 g, 60% in oil, 60.0 mmol) in small portions. The mixture was stirred for 4 h at room temperature, poured into water (100 mL), and acidified with concentrated HCl. The white precipitate was filtered off, dissolved in EtOAc (100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The resulting solid was recrystallized from $CHCl_3$/hexane to give **9c** (4.76 g, 75%) as a light-yellow solid: mp 209−211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.38 (s, 2H), 7.44-7.50 (m, 6H), 8.21−8.23 (m, 2H), 13.22 (br s, 1H).

4-Benzyloxy-3-cyanobenzoyl Chloride (9d). A solution of **9c** (3.80 g, 15.0 mmol) in $SOCl_2$ (10 mL) was heated to reflux until the production of gas ceased. The excess $SOCl_2$ was destilled off under reduced pressure, the residue was dissolved in dichloromethane, and hexane was added to precipitate the product. The resulting white solid was filtered off and dried under high vacuum to give **9d** (4.07 g, 100%): mp 100−102 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 5.29 (s, 2H), 7.11 (d, J = 9.2 Hz,1H), 7.38−7.42 (5H, m), 8.23 (dd, J = 9.2 Hz, 2.5 Hz, 1H), 8.36 (d, J = 2.5 Hz, 1H). Anal. ($C_{15}H_{10}ClNO_2$) C, H, N.

2-Benzyloxy-5-hydroxymethylbenzonitrile (9e). To a cooled solution of **9d** (3.80 g, 14.0 mmol) in THF (25 mL) and EtOH (25 mL) was added $NaBH_4$ (0.50 g, 13.2 mmol) in small portions. The mixture was stirred for 1 h at 0 °C and for another hour at room temperature. Then water (50 mL) was then added and the product was extracted with EtOAc (3 × 40 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried (Na_4SO_4), and concentrated in vacuo. The resulting solid was recrystallized from $CHCl_3$/hexane to give **9e** (3.12 g, 93%) as a white solid: mp 109−110 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 2.03 (s, 1H), 4.60 (s, 2H), 5.20 (s, 2H), 6.96 (d, J = 8.7 Hz, 1H), 7.28−7.50 (m, 6H), 7.55 (d, J = 2.2 Hz, 1H). Anal. ($C_{15}H_{13}NO_2$) C, H, N.

2-Benzyloxy-5-chloromethylbenzontrile (9f). A mixture of **9e** (2.87 g, 12.0 mmol) and $SOCl_2$ (5 mL) was heated to reflux until the production of gas ceased. The excess $SOCl_2$ was distilled off under reduced pressure. The resulting solid was recrystallized from CH_2Cl_2/hexane to give **9f** (2.97 g, 96%) as fine light-yellow needles: mp 106−108 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 4.51 (s, 2H), 5.22 (s, 2H), 6.98 (d, J = 8.9 Hz, 1H), 7.28−7.54 (m, 6H), 7.59 (d, J = 2.5 Hz, 1H). Anal. ($C_{15}H_{12}ClNO$) C, H, N.

2-Benzyloxy-5-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]-methyl}benzonitrile (9g). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.20 g, 5.0 mmol), **1a** (0.93 g, 5.0 mmol), and **9f** (1.29 g, 5.0 mmol) in anhydrous DMF (20 mL) to give **9g** as a white powder (1.67 g, 82%) after recrystallization from *i*-PrOH: mp 230−233 °C; ^{1}H NMR (270 MHz, DMSO-d_6) 5.03 (s, 2H), 5.26 (s, 2H), 6.76 (AA′BB′, 2H), 7.29 (d, J = 8.7 Hz, 1H), 7.34−7.50 (m, 5H), 7.57 (dd, J = 8.7 Hz, 2.2 Hz, 1H), 7.74 (d, J = 2.2 Hz, 1H), 7.77 (AA′BB′, 2H), 8.85 (s, 2H).

5-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}-2-hydroxybenzonitrile (9h). The title compound was hydrogenated (over 18 h) in a manner similar to that for **2c**, using **9g** (1.32 g, 3.25 mmol) and Pd−C (10% by weight, 0.10 g) in THF/MeOH (1:1) (100 mL) to give **9h** as a white solid (0.95 g, 92%) after recrystallization from *i*-PrOH: mp >230 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 4.96 (s, 2H), 6.76 (AA′BB′, 2H), 6.93 (d, J = 8.7 Hz, 1H), 7.38 (dd, J = 8.7 Hz, 2.2 Hz, 1H), 7.55 (d, J = 2.2 Hz, 1H), 7.77 (AA′BB′, 2H), 8.80 (s, 2H), 11.27 (br s, 1H).

Sulfamic Acid 2-Cyano-4-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}phenyl Ester (9). The title compound was sulfamoylated in a manner similar to that for **2**, using **9h** (0.158 g, 0.71 mmol) and sulfamoyl chloride (3.5 mmol) in anhydrous DMA (5 mL) to give **9** as a white solid (0.038 g, 19%) after crystallization from acetone/Et_2O: mp >155 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 5.16 (s, 2H), 6.72 (AA′BB′, 2H), 7.55 (d, J = 8.7 Hz, 1H), 7.76−7.80 (m, 3H), 7.97 (d, J = 2.2 Hz, 1H), 8.55 (s, 2H), 8.95 (s, 2H).

4-Benzyloxy-3-trifluoromethylbenzoic Acid (10b). The title compound was prepared in a manner similar to that for **9c**, using NaH (60% dispersion in oil, 1.80 g, 45.0 mmol), 4-fluoro-3-trifluoromethylbenzoic acid **10a** (4.16 g, 20.0 mmol), and benzyl alcohol (3.25 g, 30.0 mmol) in anhydrous DMSO (50 mL) to give **10b** as a white solid (4.25 g, 72%) after recrystallization from EtOAc/hexane: mp 183−185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 5.37 (s, 2H), 7.32−7.48 (m, 6H), 8.12 (d, J = 2.0 Hz, 1H), 8.18 (dd, J = 8.6 Hz, 2.0 Hz, 1H), 13.16 (br s, 1H). Anal. ($C_{15}H_{11}F_3O_3$) C, H.

(4-Benzyloxy-3-trifluoromethylphenyl)methanol (10c). A solution of **10b** (3.56 g, 12.0 mmol) in THF (20 mL) was added dropwise to a suspension of lithium aluminium hydride (1.0 g, 26.3 mmol) in THF (20 mL). The mixture was stirred for 0.5 h at room temperature and then quenched by addition of 2 N NaOH (5 mL). The resulting white precipitate was filtered off and washed with dichloromethane (100 mL). The filtrate was dried (Na_2SO_4) and concentrated in vacuo to give **10c** (3.31 g, 98%) as a white solid after recrystallization from Et_2O/hexane: mp 78−80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 1.72 (t, J = 5.9 Hz, 1H), 4.66 (d, J = 5.9 Hz, 2H), 5.21 (s, 2H), 7.02 (d, J = 8.2 Hz, 1H), 7.30−7.48 (m, 6H), 7.61 (d, J = 2.3 Hz, 1H). Anal. ($C_{15}H_{13}F_3O_2$) C, H.

1-Benzyloxy-4-chloromethyl-2-trifluoromethylbenzene (10d). The title compound was prepared in a manner similar to that for **3d**, using **10c** (3.10 g, 11.0 mmol) and $SOCl_2$ (2.0 mL) in anhydrous dichloromethane (20 mL) to give **10d** (3.25 g, 98%) as a light-yellow solid: mp 58−60 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.57 (s, 2H), 5.22 (s, 2H), 7.02 (d, J = 8.5 Hz, 1H), 7.31−7.52 (m, 6H), 7.63 (d, J = 2.0 Hz, 1H). Anal. ($C_{15}H_{12}ClF_3O$) C, H.

4-[(4-Benzyloxy-3-trifluoromethylbenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (10e). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.20 g, 5.0 mmol), **1a** (0.926 g, 5.0 mmol), and **10d** (1.504 g, 5.0 mmol) in anhydrous DMF (50 mL) to give **10e** as a white solid (1.865 g, 83%) after recrystallization from *i*-PrOH: mp 240−242 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 5.05 (s, 2H), 5.23 (s, 2H), 6.78 (AA′BB′, 2H), 7.26 (d, J = 8.6 Hz, 1H), 7.30−7.44 (m, 5H), 7.51 (dd, J = 8.6 Hz, 2.0 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.77 (AA′BB′, 2H), 8.79 (s, 2H). Anal. ($C_{24}H_{18}F_3N_5O$) C, H, N.

4-[(4-Hydroxy-3-trifluoromethylbenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (10f). The title compound was hydrogenated (over 18 h) in a manner similar to that for **2c**, using **10e** (1.75 g, 3.89 mmol) and Pd−C (10% by weight, 0.10 g) in THF/EtOH/MeCN (1:1:1) (120 mL) to give **10f** as a white solid (1.31 g, 94%) after recrystallization from *i*-PrOH: mp 219−221 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 4.98 (s, 2H), 6.78 (AABB′, 2H), 6.91 (d, J =

8.6 Hz, 1H), 7.31 (dd, J = 8.6 Hz, 2.0 Hz, 1H), 7.39 (d, J = 2.0 Hz, 1H), 7.76 (AA′BB′, 2H), 8.74 (s, 2H), 10.66 (s, 1H). Anal. ($C_{17}H_{12}F_3N_5O$) C, H, N.

4-Benzyloxy-3-chloro-5-methoxybenzoic Acid Benzyl Ester (11b). The title compound was prepared in a manner similar to that for **3b**, using 5-chlorovanillic acid **11a** (4.05 g, 20.0 mmol), benzyl bromide (8.55 g, 50.0 mmol), and K_2CO_3 (6.90 g, 0.05 mol) in DMF (60 mL) to give **11b** as fine colorless needles (7.34 g, 96%) after recrystallization from CH_2Cl_2/hexane: mp 55−56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.92 (s, 3H), 5.13 (s, 2H), 5.36 (s, 2H), 7.31−7.51 (m, 10H), 7.53 (d, J = 2.0 Hz, 1H), 7.73 (d, J = 2.0 Hz, 1H). Anal. ($C_{22}H_{19}ClO_4$) C, H.

(4-Benzyloxy-3-chloro-5-methoxyphenyl)methanol (11c). The title compound was prepared in a manner similar to that for **10c**, using **11b** (3.83 g, 10.0 mmol) and $LiAlH_4$ (0.50 g, 13.15 mmol) in anhydrous THF (50 mL) to give **11c** (2.70 g, 97%) as a light-yellow oil after removal of most of the benzyl alcohol on a Kugelrohr distillation apparatus in vacuo at 120 °C. The crude product was used for the synthesis of **11d** without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) 2.09 (s, 1H), 3.86 (s, 3H), 4.56 (s, 2H), 5.02 (s, 2H), 6.83 (d, J = 2.0 Hz, 1H), 6.95 (d, J = 2.0 Hz, 1H), 7.31−7.41 (m, 3H), 7.52−7.55 (m, 2H).

2-Benzyloxy-1-chloro-5-chloromethyl-3-methoxybenzene (11d). The title compound was prepared in a manner similar to that for **3d**, using **11c** (2.70 g, 9.7 mmol) and $SOCl_2$ (2.0 mL) in anhydrous dichloromethane (10 mL) to give **11d** (2.73 g, 95%) as a light-yellow solid: mp 39−41 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.89 (s, 3H), 4.52 (s, 2H), 5.07 (s, 2H), 6.87 (d, J = 2.0 Hz, 1H), 7.03 (d, J = 2.0 Hz, 1H), 7.33−7.43 (m, 3H), 7.53−7.58 (m, 2H). Anal. ($C_{15}H_{14}Cl_2O_2$) C, H.

4-[(4-Benzyloxy-3-chloro-5-methoxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (11e). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.20 g, 5.0 mmol), **1a** (0.926 g, 5.0 mmol), and **11d** (1.486 g, 5.0 mmol) in anhydrous DMF (50 mL) to give **11e** as a white solid (1.761 g, 79%) after recrystallization from *i*-PrOH: mp 177−178 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 3.78 (s, 3H), 4.80 (s, 2H), 5.05 (s, 2H), 6.52 (d, J = 2.0 Hz, 1H), 6.67 (AA′BB′, 2H), 6.89 (d, J = 2.0 Hz, 1H), 7.32−7.40 (m, 3H), 7.46−7.50 (m, 2H), 7.61 (AA′BB′0, 2H), 8.75 (s, 2H). Anal. ($C_{24}H_{20}ClN_5O_2$) C, H, N.

4-[(3-Chloro-4-hydroxy-5-methoxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (11f). The title compound was hydrogenated (over 18 h) in a manner similar to that for **2c**, using **10f** (1.34 g, 3.0 mmol) and Pd−C (5% by weight, 0.10 g) in THF/EtOH (1:1) (100 mL) to give **11f** as a white solid (1.06 g, 99%) after recrystallization from *i*-PrOH: mp >230 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 3.77 (s, 3H), 4.92 (s, 2H), 6.79 (AA′BB, 2H), 6.81 (d, J = 1.8 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H), 7.77 (AA′BB′, 2H), 8.79 (s, 2H), 9.49 (s, 1H). Anal. ($C_{17}H_{14}ClN_5O_2$) C, H, N.

Sulfamic Acid 2-Chloro-4-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}-6-methoxyphenyl Ester (11). The title compound was sulfamoylated in a manner similar to that for **2**, using **11f** (0.212 g, 0.60 mmol) and sulfamoyl chloride (2.1 mmol) in anhydrous DMA (5 mL) to give **11** as a white solid (0.219 g, 84%) after crystallization from acetone/hexane: mp >210 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 3.78 (s, 3H), 6.74 (AA′BB′, 2H), 7.04 (d, J = 2.0 Hz, 1H), 7.07 (d, J = 2.0 Hz, 1H), 7.77 (AA′BB′, 2H), 7.98 (s, 2H), 8.91 (s, 2H). Anal. ($C_{17}H_{15}ClN_6O_4S\cdot H_2O$) C, H, N.

4-Benzyloxy-3,5-dichlorobenzoic Acid Methyl Ester (12b). The title compound was prepared in a manner similar to that for **3b**, using 5-chlorovanillic acid **12a** (5.25 g, 25.0 mmol), benzyl bromide (5.13 g, 30.0 mmol), and K_2CO_3 (6.91 g, 0.05 mol) in DMF (100 mL) to give **12b** as fine colorless needles (7.47 g, 96%) after recrystallization from EtOAc/hexane: mp 105−106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.93 (s, 3H), 5.12 (s, 2H), 7.35−7.44 (m, 3H), 7.54−7.58 (m, 2H), 8.01 (s, 2H). Anal. ($C_{15}H_{12}Cl_2O_3$) C, H.

(4-Benzyloxy-3,5-dichlorophenyl)methanol (12c). The title compound was prepared in a manner similar to that for **10c**, using **12b** (7.20 g, 23.14 mmol) and $LiAlH_4$ (1.50 g, 39.5 mmol) in anhydrous THF (60 mL) to give **12c** (5.83 g, 89%) as a white solid: mp 81−82 °C (EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) 1.85 (t, J = 5.9 Hz, 1H), 4.63 (d, J = 5.9 Hz, 2H), 5.04 (s, 2H), 7.33 (s, 2H), 7.35−7.44 (m, 3H), 7.55−7.59 (m, 2H).

2-Benzyloxy-5-bromomethyl-1,3-dichlorobenzene (12d). The title compound was prepared in a manner similar to that for **4d**, using **12c** (5.50 g, 19.4 mmol) and PBr_3 (2 mL) in anhydrous dichloromethane (40 mL) to give **12d** (5.24 g, 78%) as a white solid after recrystallization from dichloromethane/hexane: mp 79−80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.39 (s, 2H), 5.05 (s, 2H), 7.37 (s, 2H), 7.38−7.44 (m, 3H), 7.54−7.58 (m, 2H). Anal. ($C_{14}H_{11}BrCl_2O$) C, H.

4-[(4-Benzyloxy-3,5-dichlorobenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (12e). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.20 g, 5.0 mmol), **1a** (0.926 g, 5.0 mmol), and **12d** (1.73 g, 5.0 mmol) in anhydrous DMF (20 mL) to give **12e** as a white solid (1.76 g, 78%) after recrystallization from *i*-PrOH: mp >200 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 5.00 (s, 2H), 5.07 (s, 2H), 6.74 (AA′BB′, 2H), 7.36−7.44 (m, 3H), 7.47−7.50 (m, 2H), 7.50 (s, 2H), 7.77 (AA′BB′, 2H), 8.92 (s, 2H).

4-[(3,5-Dichloro-4-hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (12f). The title compound was hydrogenated (over 18 h) in a manner similar to that for **2c**, using **12f** (1.13 g, 2.5 mmol) and Pd−C (10% by weight, 0.10 g) in THF/MeOH (1:1) (100 mL) to give a white solid, which was suspended in *i*-PrOH and refluxed for 5 min. The solid was collected by filtration and dried in vacuo to give **12f** (0.31 g, 34%) as a white powder: mp 233−236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 4.97 (s, 2H), 6.76 (AA′BB′, 2H), 7.31 (s, 2H), 7.77 (AA′BB′, 2H), 8.85 (s, 2H), 10.29 (s, 1H); LRMS (FAB+) m/z (rel intensity) 360.0 (100, $[M + H]^+$). Anal. ($C_{16}H_{11}Cl_2N_5O$) C, H, N.

4-Benzyloxy-2,3,5,6-tetrafluorobenzoic Acid Methyl Ester (13b). The title compound was prepared in a manner similar to that for **3b**, using 5-chlorovanillic acid **13a** (4.48 g, 20.0 mmol), benzyl bromide (3.59 g, 21.0 mmol), and K_2CO_3 (6.91 g, 0.05 mol) in DMF (40 mL) to give **13b** as fine colorless needles (5.85 g, 85%) after recrystallization from EtOAc/hexane: mp 57−60 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.95 (s, 3H), 5.35 (s, 2H), 7.35−7.44 (m, 5H). Anal. ($C_{15}H_{10}F_4O_3$) C, H.

(4-Benzyloxy-2,3,5,6-tetrafluorophenyl)methanol (13c).[52] The title compound was prepared in a manner similar to that for **3c**, using **13b** (1.89 g, 6.0 mmol) and $NaBH_4$ (0.30 g, 7.9 mmol) in EtOH/THF (1:1) (40 mL) to give **13c** (1.60 g, 93%) as a white solid: mp 65−66 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 1.88 (t, J = 6.0 Hz, 1H), 4.74 (d, J = 6.0 Hz, 2H), 5.19 (s, 2H), 7.35-7.44 (m, 5H). Anal. ($C_{14}H_{10}F_4O_2$) C, H.

1-Benzyloxy-4-chloromethyl-2,3,5,6-tetrafluorobenzene (13d). The title compound was prepared in a manner similar to that for **10d**, using **13c** (1.49 g, 5.20 mmol) and $SOCl_2$ (10 mL) to give **13d** (1.47 g, 93%) as a white solid: mp 108−109 °C (dichloromethane/hexane); ^{1}H NMR (270 MHz, $CDCl_3$) 5.05 (s, 2H), 5.28 (s, 2H), 7.35−7.42 (m, 5H). Anal. ($C_{14}H_9ClF_4O_2$) C, H.

4-[(4-Benzyloxy-2,3,5,6-tetrafluorobenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (13e). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.17 g, 4.30 mmol), **1a** (0.796 g, 4.3 mmol), and **13d** (1.31 g, 4.3 mmol) in anhydrous DMF (20 mL) to give **13e** as a white solid (1.33 g, 68%) after recrystallization from *i*-PrOH: mp >210 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 5.20 (s, 2H), 5.28 (s, 2H), 6.81 (AA′BB′, 2H), 7.34−7.42 (m, 5H), 7.78 (AA′BB′, 2H), 8.80 (s, 2H). Anal. ($C_{23}H_{15}F_4N_5O$) C, H, N.

4-[(4-Benzyloxy-2,3,5,6-tetrafluorobenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (13f). The title compound was hydrogenated (over 18 h) in a manner similar to that for **2c**, using **13e** (0.227 g, 0.50 mmol) and Pd−C (10% by weight, 0.10 g) in THF/MeOH/EtOAc (1:1) (90 mL) to give **13f** (0.151 g, 83%) as a white solid: mp >180 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 5.15 (s, 2H), 6.82 (AA′BB′, 2H), 7.80 (AA′BB′, 2H), 8.83 (s, 2H), 11.78 (br s, 1H); LRMS (FAB+) m/z (rel intensity) 240.1 (70, $[M + H]^+$), 91.1

(100); HRMS (FAB+) found 364.0818, $C_{16}H_{10}F_4N_5O$ $[M + H]^+$ requires 364.0821. Anal. ($C_{16}H_9F_4N_5O$) C, H, N.

3-Benzoyloxy-4-fluorotoluene (14b). The title compound was prepared in a manner similar to that for **5c**, using 4-fluoro-3-hydroxytoluene, **14a** (3.78 g, 30.0 mmol), benzoyl chloride (4.22 g, 30.0 mmol), and NEt_3 (5 mL) in CH_2Cl_2 (50 mL). After being stirred for 18 h, the mixture was concentrated in vacuo. The residue was redissolved in Et_2O (200 mL) and washed with water (100 mL), 2 N NaOH (2 × 30 mL), and brine (20 mL). The ethereal layer was dried (Na_2SO_4) and concentrated in vacuo to give **14b** as a white solid (6.60 g, 96%): mp 81−83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 2.36 (s, 3H), 7.01−7.12 (m, 3H), 7.49−7.55 (m, 2H), 7.63−7.68 (m, 1H), 8.19−8.23 (m, 2H). Anal. ($C_{14}H_{11}FO_2$) C, H.

3-Benzoyloxy-4-fluorobenzyl Bromide (14c). The title compound was prepared in a manner similar to that for **5h** (method B), using **14b** (2.47 g, 10.0 mmol), NBS (1.96 g, 11.0 mmol), and benzoyl peroxide (0.01 g) in anhydrous CCl_4 (25 mL). After 2 h, the mixture was cooled and diluted with Et_2O (100 mL) and water (50 mL). The ethereal layer was washed with brine (20 mL) and dried (Na_2SO_4). Concentration in vacuo and subsequent purification by flash column chromatography [SiO_2, EtOAc/hexane (1:25)] gave **14c** as a white solid (1.80 g, 58%): mp 104−105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.48 (s, 2H), 7.18 (dd, J = 9.8 Hz, 8.6 Hz, 1H), 7.28 (ddd, J = 8.6 Hz, 4.3 Hz, 2.3 Hz, 1H), 7.34 (dd, J = 7.0 Hz, 2.3 Hz, 1H), 7.50−7.55 (m, 2H), 7.64−7.69 (m, 1H), 8.18−8.23 (m, 2H). Anal. ($C_{14}H_{10}BrFO_2$) C, H.

Benzoic Acid 5-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]-methyl}-2-fluorophenyl Ester (14d). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.20 g, 5.0 mmol), **1a** (0.926 g, 5.0 mmol), and **14c** (1.55 g, 5.0 mmol) in anhydrous DMF (20 mL) to give **14d** as a white solid (1.16 g, 56%) after chromatography [SiO_2, EtOAc (100%)]. This product was used for the next reaction without further purification: mp 165−168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.91 (s, 2H), 6.67 (AA′BB′, 2H), 7.07 (ddd, J = 8.2 Hz, 4.3 Hz, 2.0 Hz, 1H), 7.18 (dd, J = 9.4 Hz, 8.6 Hz, 1H), 7.24 (dd, J = 7.0 Hz, 2.3 Hz, 1H), 7.49−7.56 (m, 2H), 7.58 (AA′BB′, 2H), 7.62−7.70 (m, 1H), 8.15−8.20 (m, 2H), 8.21 (s, 2H).

4-[(4-Fluoro-3-hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (14e). The title compound was prepared in a manner similar to that for **5k**, using **14d** (0.958 g, 2.32 mmol) and NaOH (0.25 g, 6.25 mmol) in MeOH (10 mL). The solution was refluxed for 5 min and concentrated in vacuo. Water (10 mL) was added, and the milky suspension was neutralized (pH 7−8) with 2 N HCl. The white precipitate was filtered off, washed with a small amount of water (5 mL), and dried under high vacuum to give **14e** as a white solid (0.476 g, 66%): mp >210 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 4.95 (s, 2H), 6.70 (ddd, J = 11.4 Hz, 8.4 Hz, 2.4 Hz, 1H), 6.75 (AA′BB′, 2H), 6.84 (dd, J = 8.4 Hz, 2.4 Hz, 1H), 7.06 (dd, J = 11.3 Hz, 8.4 Hz, 1H), 7.76 (AA′BB′, 2H), 8.75 (s, 2H), 9.90 (s, 1H, exchanges with D_2O). Anal. ($C_{16}H_{12}FN_5O$) C, H, N.

Sulfamic Acid 5-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]-methyl}-2-fluorophenyl Ester (14). The title compound was sulfamoylated in a manner similar to that for **2**, using **14e** (0.075 g, 0.22 mmol) and sulfamoyl chloride (1.05 mmol) in anhydrous DMA (5 mL) to give **15** as a white powder (0.136 g, 55%) after precipitation from acetone/Et_2O: mp 148−150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 3.23 (t, J = 7.0 Hz, 2H), 4.07 (t, J = 7.0 Hz, 2H), 6.54 (AA′BB′, 2H), 7.08−7.12 (m, 1H), 7.20−7.26 (m, 1H), 7.39 (dd, J = 9.2 Hz, 9.2 Hz, 1H), 7.69 (AA′BB′, 2H), 8.03 (s, 2H), 8.94 (s, 2H). Anal. ($C_{16}H_{13}FN_6SO_3$) C, H, N.

3-Benzoyloxy-4-chlorotoluene (15b). The title compound was prepared in a manner similar to that for **5c**, using 4-chloro-3-hydroxytoluene, **15a** (3.92 g, 27.5 mmol), benzoyl chloride (3.51 g, 25 mmol), and NEt_3 (5 mL) in CH_2Cl_2 (100 mL) to give **15b** as a white solid (5.82 g, 94%): mp 39−40 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 2.38 (s, 3H), 7.05 (d, J = 8.2 Hz, 1H), 7.11 (s, 1H), 7.36 (d, J = 8.2 Hz, 1H), 7.51−7.56 (m, 2H), 7.64−7.70 (m, 1H), 8.22−8.28 (m, 2H). Anal. ($C_{14}H_{11}ClO_2$) C, H.

3-Benzoyloxy-4-chlorobenzyl Bromide (15c). The title compound was prepared in a manner similar to that for **14c**, using **15b** (2.47 g, 10.0 mmol), NBS (1.96 g, 11.0 mmol), and benzoyl peroxide (10 mg) in carbon tetrachloride (25 mL). The residue was purified by flash column chromatography [SiO_2, EtOAc/*n*-hexane (1:40)] to give **15c** as a colorless oil (2.01 g, 62%) that solidified on standing: mp 71−74 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.48 (s, 2H), 7.27 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.50−7.58 (m, 2H), 7.64−7.71 (m, 1H), 8.22−8.28 (m, 2H). Anal. ($C_{14}H_{10}BrClO_2$) C, H.

Benzoic Acid 2-Chloro-5-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}phenyl Ester (15d). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.20 g, 5.0 mmol), **1a** (0.926 g, 5.0 mmol), and **15c** (1.63 g, 5.0 mmol) in anhydrous DMF (20 mL) to give **30c** as a white solid (1.773 g, 82%) after chromatography [SiO_2, EtOAc (100%)]. This product was used for the next reaction without further purification: mp >150 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) 4.93 (s, 2H), 6.66 (AA′BB′, 2H), 7.06 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.27 (d, J = 2.0 Hz, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.47−7.58 (m, 4H), 7.60−7.69 (m, 1H), 8.16−8.21 (m, 2H), 8.24 (s, 2H). Anal. ($C_{23}H_{16}ClN_5O_2$) C, H, N.

4-[(4-Chloro-3-hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (15e). A mixture of **15d** (1.13 g, 2.63 mmol), NaOMe (500 mg) in MeOH (20 mL), and water (5 mL) was refluxed for 30 min. After the mixture was cooled to room temperature, most of the solvent was removed in vacuo and a concentrated $NaHCO_3$ solution (20 mL) and EtOAc (50 mL) were added. The organic layer was separated and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The resulting white powder was refluxed in EtOAc (10 mL; product did not dissolve completely). The product was filtered off and dried in vacuo to give **15e** as a white powder (0.412 g, 48%): mp >130 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 4.97 (s, 2H), 6.71−6.75 (m, 3H, AA′BB′ and Ar*H*), 6.84 (d, J = 2.0 Hz, 1H), 7.25 (d, J = 8.2 Hz, 1H), 7.75 (AA′BB′, 2H), 8.76 (s, 2H), 10.21 (s, 1H, exchanges with D_2O). Anal. ($C_{16}H_{12}ClN_5O$) C, H, N.

Sulfamic Acid 2-Chloro-5-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}phenyl Ester (15). The title compound was sulfamoylated in a manner similar to that for **2**, using **15e** (0.20 g, 0.614 mmol) and sulfamoyl chloride (2.1 mmol) in anhydrous DMA (5 mL) to give **15** as a white powder (0.136 g, 55%) after precipitation from acetone/Et_2O: mp >210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 5.11 (s, 2H), 6.75 (AA′BB′, 2H), 7.25 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.54 (d, J = 8.2 Hz, 1H), 7.77 (AA′BB′, 2H), 8.32 (s, 2H, exchanges with D_2O), 8.82 (s, 2H).

4-Bromo-3-hydroxybenzoic Acid (16b). The title compound was prepared according to the method reported by Buehler et al.[53] using 3-hydroxybenzoic acid, **16a** (18.2 g, 132.0 mmol), and Br_2 (6.76 mL, 132.0 mmol) in glacial AcOH (250 mL) to give **16b** as a colorless crystalline solid (3.43 g, 12%) after recrystallization from Et_2O/*n*-hexane: mp 225−226 °C [lit.[53] 225-226 °C (H_2O)]; ^{1}H NMR (270 MHz, DMSO-d_6) 7.28 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 7.51 (d, J = 1.7 Hz, 1H), 7.60 (d, J = 8.3 Hz, 1H), 10.66 (br s, 1H, exchanges with D_2O), and 13.04 (br s, 1H, exchanges with D_2O).

Methyl 4-Bromo-3-hydroxybenzoate (16c). The title compound was prepared according to the method reported by Faltis et al.[54] using 4-bromo-3-hydroxybenzoic acid, **16c** (5.7 g, 26.26 mmol), and concentrated sulfuric acid (98% solution, 1.4 mL, 26.26 mmol) in anhydrous MeOH (150 mL) to give **16c** as a colorless crystalline powder (5.4 g, 89%) after recrystallization from MeOH; mp 121−123 °C [lit.[54] 124−125 °C (petroleum ether)]; ^{1}H NMR (270 MHz, $CDCl_3$) 3.90 (s, 3H), 5.75 (br s, 1H, exchanges with D_2O), 7.46 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.67 (d, J = 1.7 Hz, 1H).

Methyl 4-Bromo-3-(tetrahydropyran-2-yloxy)benzoate (16d). To a solution of the methyl 4-bromo-3-hydroxybenzoate, **16c** (4.0 g, 17.31 mmol), in anhydrous CH_2Cl_2 (30 mL) was added 3,4-dihydro-2*H*-pyran (3.95 mL, 43.28 mmol) and *p*-toluenesulfonic

acid (0.01 g, 0.17 mmol) at 0 °C. The mixture was stirred at this temperature for 90 min, warmed to room temperature, and quenched with saturated $NaHCO_3$ solution (50 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated in vacuo. The pale-yellow residue was purified by flash column chromatography [SiO_2, Et_2O/*n*-hexane (4:6)] to give a colorless oil. The oil solidified on standing at room temperature to give **16d** as a colorless solid (4.12 g, 76%): mp 50−53 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 1.59−1.77 (m, 3H), 1.86−2.13 (m, 3H), 3.60−3.64 (m, 1H), 3.83−3.85 (m, 1H), 3.88 (s, 3H), 5.60−5.62 (m, 1H), 7.52 (dd, J = 8.2 Hz, 1.7, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 2.0 Hz, 1H).

4-Bromo-3-(tetrahydropyran-2-yloxy)benzyl Alcohol (16e). To a suspension of $LiAlH_4$ (0.72 g, 19.04 mmol) in anhydrous Et_2O (90 mL) was slowly added a solution of **16d** (4.0 g, 12.69 mmol) in anhydrous Et_2O (10 mL). The mixture was stirred at room temperature for 2 h and then cautiously quenched with $Na_2SO_4 \cdot 10H_2O$ (until gas evolution ceased). The solids were filtered off and washed with Et_2O (100 mL). The combined organic fractions were then dried (Na_2SO_4) and concentrated in vacuo. The pale-yellow residue was purified by flash column chromatography [SiO_2, Et_2O/*n*-hexane (7:3)] to give **16e** as a colorless oil (3.21 g, 88%): ^{1}H NMR (270 MHz, $CDCl_3$) 1.54−1.76 (m, 3H), 1.82−2.12 (m, 3H), 3.54−3.64 (m, 1H), 3.80−3.94 (m, 1H), 4.61 (d, J = 4.9 Hz, 2H), 5.52 (m, 1H), 6.85 (dd, J = 7.9 Hz, 1.5 Hz, 1H), 7.13 (d, J = 2.0 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H). ArCH_2O*H* is too broad to be observed.

4-Bromo-3-(tetrahydropyran-2-yloxy)benzyl Bromide (16f). The title compound was prepared by adapting the method reported by Ishida et al.[56] with the following modifications. To a solution of **16e** (3.0 g, 10.45 mmol), CBr_4 (6.93 g, 20.90 mmol), and anhydrous pyridine (0.85 mL, 10.45 mmol) in anhydrous Et_2O (50 mL) at 0 °C was added dropwise a solution of triphenylphosphine (5.48 g, 20.90 mmol) in anhydrous Et_2O (10 mL). The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The solvent was removed in vacuo, and the residue was suspended in *n*-hexane and filtered. The filtered solid was washed with *n*-hexane, and the combined filtrates were concentrated in vacuo to ∼10 mL. The residue was purified by flash column chromatography [SiO_2, EtOAc/hexane (5:95)] to give **16f** as a pale-yellow solid (2.82 g, 77%,): mp 61−63 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 1.58−2.12 (m, 6H), 3.58−3.65 (m, 1H), 3.83−3.92 (m, 1H), 4.41 (d, J = 12.6 Hz, 2H), 5.53 (m, 1H), 6.89 (dd, J = 7.9 Hz, 2.0 Hz, 1H), 7.16 (d, J = 2.2 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H).

4-{[4-Bromo-3-(tetrahydropyran-2-yloxy)benzyl][1,2,4]triazol-4-ylamino}benzonitrile (16g). The title compound was prepared by adapting the method for the synthesis of **2b**. To a stirred suspension of NaH (60% dispersion in oil, 0.27 g, 6.78 mmol) in anhydrous DMF (10 mL) was added a solution of 4-[(4-cyanophenyl)amino]-4*H*-[1,2,4]triazole, **1a** (1.26 g, 6.78 mmol), in anhydrous DMF (5 mL), and the mixture was stirred at room temperature for 0.5 h. A solution of **16f** (2.61 g, 7.46 mmol) in anhydrous DMF (5 mL) was then added and the mixture heated at 80−90 °C for 3 h. The mixture was cooled, diluted with EtOAc (50 mL), washed with water (4 × 100 mL) and brine (100 mL), and dried (Na_2SO_4). Concentration in vacuo and subsequent purification by flash column chromatography [SiO_2, EtOAc (100%)] gave **16g** as a yellow oil (3.05 g, 99%): ^{1}H NMR (270 MHz, $CDCl_3$) 1.61−1.99 (m, 6H), 3.55−3.59 (m, 1H), 3.73 (m, 1H), 4.75 (d, J = 14.6 Hz, 1H), 4.90 (d, J = 14.6 Hz, 1H), 5.41 (m, 1H), 6.64 (AA′BB′, 2H), 6.69 (dd, J = 8.2 Hz, 2.2 Hz, 1H), 7.00 (d, J = 2.2 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.57 (AA′BB′, 2H), 8.15 (s, 2H).

4-[(4-Bromo-3-hydroxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (16h). The title compound was prepared by adapting the method reported by Tafi et al.[56] To a solution of **16g** (3.05 g, 6.71 mmol) in MeOH (60 mL) was added a catalytic amount of *p*-toluenesulfonic acid at 0 °C, and the mixture was stirred (allowing to slowly warm to room temperature) overnight. The solvent was removed in vacuo and the residue dissolved in EtOAc (100 mL). The organic fraction was washed with Na_2CO_3 solution (1 M aqueous solution, 3 × 50 mL) and brine (3 × 50 mL) and dried (Na_2SO_4). The solvent was removed in vacuo and the pale-yellow residue recrystallized from EtOH to give **16h** as an off-white powder (1.48 g, 60%): mp >210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 5.00 (s, 2H), 6.72 (dd, J = 8.3 Hz, 2.1, 1H), 6.76 (AA′BB′, 2H), 6.86 (d, J = 1.8 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.78 (AA′BB′, 2H), 8.81 (s, 2H), 10.32 (s, 1H, exchanges with D_2O). Anal. ($C_{16}H_{12}N_5OBr$) C, H, N.

Sulfamic Acid 2-Bromo-5-{[(4-cyanophenyl)[1,2,4]triazol-4-ylamino]methyl}phenyl Ester (16). The title compound was sulfamoylated in a manner similar to that for **2**, using **16h** (0.5 g, 1.35 mmol) and sulfamoyl chloride (13.5 mmol) in anhydrous DMA (5 mL) to give **16** as an off-white solid (0.39 g, 64%) after chromatography [SiO_2, EtOAc (100%)]; mp >140 °C (dec); ^{1}H NMR δ_H (400 MHz, DMSO-d_6) 5.11 (s, 2H), 6.77 (AA′BB′, 2H), 7.21 (dd, J = 8.1 Hz, 1.8 Hz, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.80 (AA′BB′, 2H), 8.34 (br s, 2H, exchanges with D_2O), 8.85 (s, 2H). Anal. ($C_{16}H_{13}N_6SO_3Br$) C, H, N.

4-[(3-Benzyloxy-4-methoxybenzyl)[1,2,4]triazol-4-ylamino]-benzonitrile (17c). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.22 g, 5.4 mmol), **1a** (1.0 g, 5.68 mmol), and 4-methoxy-3-benzyloxybenzyl bromide[57] (**17b**, 1.66 g, 5.4 mmol) in anhydrous DMF (5 mL) to give **17c** as fine colorless needles (0.97 g, 44%) after recrystallization from EtOH: mp 147−149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.89 (s, 3H), 4.72 (s, 2H), 5.12 (s, 2H), 6.60 (AA′BB′), 6.62 (d, J = 2.0 Hz, 1H), 6.64 (dd, J = 7.8 Hz, 2.0 Hz, 1H), 6.79 (d, J = 7.8 Hz, 1H), 7.28−7.34 (m, 5H), 7.57 (AA′BB′, 2H), 7.74 (s, 2H). Anal. ($C_{24}H_{21}N_5O_2$) C, H, N.

4-[(3-Hydroxy-4-methoxybenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (17d). The title compound was hydrogenated (over 16 h) in a manner similar to that for **2c**, using **17c** (0.411 g, 1.00 mmol) and Pd−C (10% by weight, 0.042 g) in THF/MeOH (1:1) (20 mL) to give **17d** as a white powder (0.164 g, 51%) after recrystallization from acetone: mp >230 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 3.71 (s, 3H), 4.88 (s, 2H), 6.52 (dd, J = 8.2 Hz, 2. Hz, 1H), 6.68 (d, J = 2.0 Hz, 1H), 6.75 (AA′BB′, 2H), 6.80 (d, J = 8.2 Hz, 1H), 7.74 (AA′BB′, 2H), 8.70 (s, 2H), 9.00 (s, 1H). Anal. ($C_{17}H_{15}N_5O_2$) C, H, N.

Sulfamic Acid 5-{[(4-Cyanophenyl)[1,2,4]triazol-4-ylamino]-methyl}-2-methoxyphenyl Ester (17). The title compound was sulfamoylated in a manner similar to that for **2**, using **31d** (0.10 g, 0.31 mmol) and sulfamoyl chloride (1.87 mmol) in anhydrous DMA (2 mL) to give **17** as a white solid (0.04 g, 32%) after precipitation from EtOAc solution by addition of *n*-hexane: mp 169−171 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) 3.77 (s, 3H), 5.00 (s, 2H), 6.77 (AA′BB′, 2H), 7.06 (d, J = 8.6 Hz, 1H), 7.13 (dd, J = 8.6 Hz, 2.3 Hz, 1H), 7.27 (d, J = 2.3 Hz, 1H), 7.77 (AA′BB′, 2H), 7.94 (s, 2H), 8.75 (s, 2H).

3-Benzyloxy-4-trifluoromethylbenzoic Acid (18b). The title compound was prepared in a manner similar to that for **9c**, using ^{t}BuOK (5.60 g, 50.0 mmol) instead of NaH, 3-fluoro-4-trifluoromethyl benzoic acid **18a** (4.70 g, 22.6 mmol), and benzyl alcohol (3.25 g, 30.0 mmol) in anhydrous DMSO (50 mL) to give **18b** (4.13 g, 62%) as colorless plates after recrystallization from EtOAc/hexane: mp 190−191 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 5.26 (s, 2H), 7.28−7.48 (m, 5H), 7.66−7.80 (m, 3H), 12.00 (br s, 1H). Anal. ($C_{15}H_{11}F_3O_3$) C, H.

(3-Benzyloxy-4-trifluoromethylphenyl)methanol (18c). The title compound was prepared in a manner similar to that for **10c**, using **18b** (3.92 g, 13.2 mmol) and $LiAlH_4$ (1.0 g, 26.3 mmol) in anhydrous THF (40 mL) to give **18c** (3.68 g, 99%) as a light-yellow solid: mp 45−47 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 1.90 (br s, 1H), 4,70 (s, 2H), 5.19 (s, 2H), 6.96 (d, J = 8.0 Hz, 1H), 7.08 (s, 1H), 7.28−7.48 (m, 5H), 7.56 (d, J = 8.0 Hz, 1H).

1-Benzyloxy-3-chloromethyl-2-trifluoromethylbenzene (18d). A mixture of $SOCl_2$ (15 mL) and **18c** (3.59 g, 12.7 mmol) was refluxed for 3 h. The excess $SOCl_2$ was removed under reduced pressure, and the residue was dissolved in $CHCl_3$ (50 mL), washed with water (20 mL), dried ($MgSO_4$), and concentrated in vacuo to give **18d** (3.81 g, 99%) as a light-yellow solid: mp 49−51 °C; ^{1}H

NMR δ_H (270 MHz, $CDCl_3$) 4.55 (s, 2H), 5.20 (s, 2H), 7.01 (d, J = 7.6 Hz, 1H), 7.08 (1s, 1H), 7.28−7.48 (m, 5H), 7.57 (d, J = 7.6 Hz, 1H).

4-[(3-Benzyloxy-4-trifluoromethylbenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (18e). The title compound was prepared in a manner similar to that for **2b**, using NaH (60% dispersion in oil, 0.392 g, 9.8 mmol), **1a** (1.815 g, 9.8 mmol), and **18d** (2.945 g, 9.8 mmol) in anhydrous DMF (50 mL) to give **18e** as light-yellow plates (3.011 g, 68%) after recrystallization from *i*-PrOH: mp 184−185 °C; ^{1}H NMR (270 MHz, DMSO-d_6) 5.11 (s, 2H), 5.25 (s, 2H), 6.71 (AA′BB′, 2H), 7.01 (d, J = 7.6 Hz, 1H), 7.26−7.42 (m, 6H), 7.56 (d, J = 7.6 Hz, 1H), 7.75 (AA′BB′, 2H), 8.81 (s, 2H). Anal. ($C_{24}H_{18}F_3N_5O$) C, H, N.

4-[(3-Hydroxy-4-trifluoromethylbenzyl)[1,2,4]triazol-4-ylamino]benzonitrile (18f). The title compound was hydrogenated (over 18 h) in a manner similar to that for **2c**, using **18e** (2.25 g, 5.0 mmol) and Pd−C (5% by weight, 0.10 g) in THF/MeOH (1:1) (100 mL) to give **18f** as a white solid (1.65 g, 92%) after precipitation from acetone witth hexane: mp >190 °C (dec); ^{1}H NMR (270 MHz, DMSO-d_6) 5.08 (s, 2H), 6.73 (AA′BB′, 2H), 6.89−6.95 (m, 2H), 7.46 (d, J = 7.9 Hz, 1H), 7.77 (A′BB′, 2H), 8.84 (s, 2H), 10.64 (br s, 1H). Anal. ($C_{17}H_{12}F_3N_5O$) C, H, N.

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Supporting Information Available: (A) Combustion/elemental analysis results for **2b**, **2**, **3b**−**d**,**f**, **3**, **4b**,**d**,**f**, **4**, **5b**−**e**,**h**,**k**, **5**, **7c**,**e**−**f**, **9b**,**d**−**f**, **10b**−**e**, **11b**,**d**−**f**, **11**, **12b**,**d**,**f**, **13b**−**f**, **14b**−**c**,**e**, **14**, **15b**−**d**, **16h**, **16**, **17d**, and **18b**,**e**; (B) HPLC data for biologically tested compounds; (C) LRMS/HRMS data for compounds except **1a**, **1**, **11c**, **15b**, and **16f**; (D) ^{13}C data for **4d**,**f**, **5i**, **5**, **7b**,**f**, **7**, **9c**,**e**−**h**, **9**, **12b**−**f**, **15b**, **16h**, and **16**; (E) X-ray crystallographic data for **5**. This material is available free of charge via the Internet at http://pubs.acs.org. In addition, the crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as CCDC 628906 and are available free of charge on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, U.K. (fax, (+44) 1223 336033; e-mail, deposit@ccdc.cam.ac.uk).

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