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LETTER

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Hybrid Dual Aromatase-Steroid Sulfatase Inhibitors with Exquisite Picomolar Inhibitory Activity

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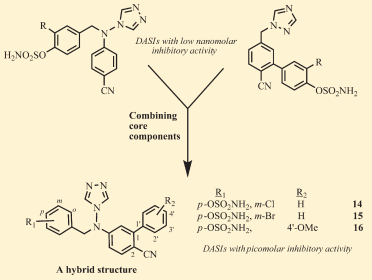
Supporting Information

ABSTRACT: Single agents against multiple drug targets are highly topical. Hormone-dependent breast cancer (HDBC) may be more effectively treated by dual inhibition of aromatase and steroid sulfatase (STS), and several dual aromatase-sulfatase inhibitors (DASIs) have been recently reported. The best compounds from two leading classes of DASI, **3** and **9**, are low nanomolar inhibitors. In search of a novel class of DASI, core motifs of two leading classes were combined to give a series of hybrid structures, with several compounds showing markedly improved dual inhibitory activities in the picomolar range in JEG-3 cells. Thus, DASIs **14** (IC_{50}: aromatase, 15 pM; STS, 830 pM) and **15** (IC_{50}: aromatase, 18 pM; STS, 130 pM) are the first examples of an exceptional new class of highly potent dual inhibitor that should encourage further development toward multitargeted therapeutic intervention in HDBC.



KEYWORDS: Hybrid, dual inhibitors, aromatase, sulfatase, cancer

Estrogen deprivation has been an effective therapeutic intervention for hormone-dependent breast cancer (HDBC). One established and clinically proven approach involves the inhibition of the aromatase enzyme,[1−3] although the inhibition of steroid sulfatase (STS) is an emerging new strategy, as demonstrated by promising results for STX64 (Irosustat, BN83495),[4−6] the first STS inhibitor that entered clinical trials. Since both aromatase and STS are targets for treating HDBC, it has been reasoned that estrogen deprivation may be more comprehensively achieved by dual inhibition of both enzymes.

Designing single agents that act against multiple biological targets is of increasing interest and prominence. In recent years, an increasing volume of work has been published exemplifying the successful use of this strategy.[7−17] We have pioneered this approach to augment our strategy directed at the first-in-class clinical target of STS inhibition and successfully developed three series of single agent dual aromatase and sulfatase inhibitors (DASIs) that are sulfamate derivatives of the nonsteroidal aromatase inhibitor (AI) 4-((4-bromobenzyl)-[1,2,4]-triazol-4-ylamino)benzonitrile (**1**) (e.g., **2** and **3**),[18−20] letrozole **4** (e.g., **5**),[21,22] and anastrozole **6** (e.g., **7**) (Figure 1).[23] The design of these DASIs shares a common principle of engendering the irreversible STS inhibitory pharmacophore (ie. an aryl sulfamate ester, $ArOSO_2NH_2$) into a clinical or experimental AI with minimal structural change incurred on the original scaffold in order to retain and maximize aromatase inhibition. More recently, a different design approach was employed. A series of biphenyl-based DASIs was developed as a result of incorporating the reversible aromatase inhibitory pharmacophore, which is principally a heme-ligating nitrogen-containing heterocycle, into a known sulfamate-based STS inhibitor (e.g., **8** and **9**, Figure 1).[24] On evaluating the lead candidates from these four structural classes of DASI, all of them showed *in vitro* and *in vivo* dual inhibitory activities with potency ranging from good to very high.

Given this success in the design of DASIs to date, we explored further the feasibility of expanding structural diversity but, at the same time, set a challenge of maintaining or increasing still further the high potency. One obvious route to take would be to repeat the two aforementioned strategies for designing a DASI and apply them to other reported aromatase and STS inhibitors. However, repetitive application of the same strategies lacks some novelty and so pursuing a new design approach seemed more desirable and challenging .

On reviewing computational docking studies of leading DASIs and other related molecules performed with a homology model of aromatase,[19,23,25] we noticed that the molecules docked in different orientations with unoccupied space available within the enzyme site. This observation suggested that further interactions with available amino acid residues might be exploited in principle if additional functionality could be built into the docked molecule in a complementary manner. For STS,[19,23] a similar observation was realized on reviewing docking studies of lead DASIs carried

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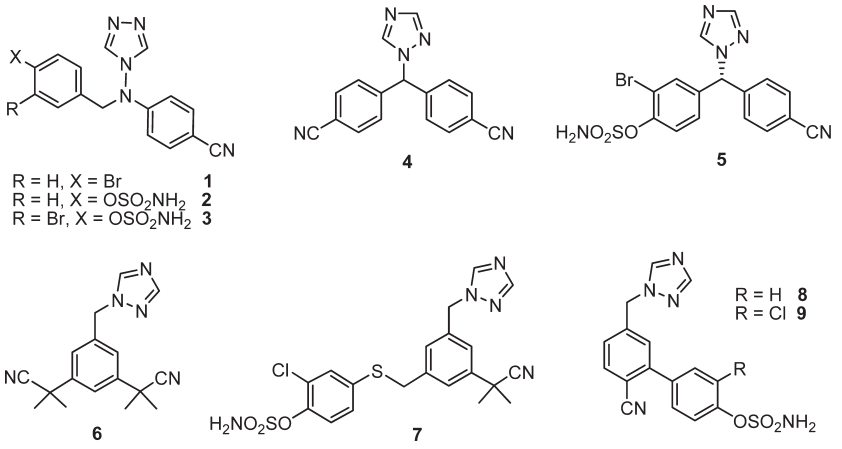


Figure 1. Structures of nonsteroidal AIs **1**, **4**, and **6**, and DASIs **2**, **3**, **5**, and **7** − **9**.

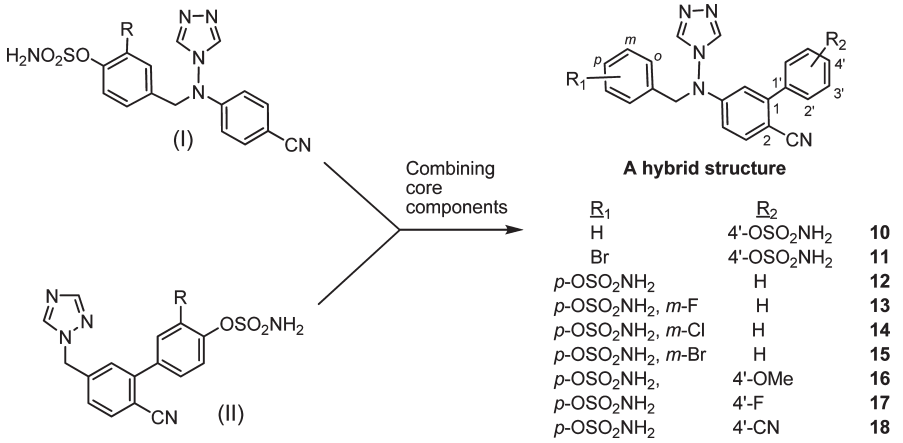


Figure 2. Derivatives **10**−**18** of a hybrid structure designed to possess dual inhibition against aromatase and STS by combining core components of the two leading 4-((4-bromobenzyl)-[1,2,4]-triazol-4-ylamino)benzonitrile-based (I) and biphenyl-based (II) DASIs.

out with the crystal structure of STS. There are pockets and areas around the docked molecules in regions away from the sulfamate moiety in the enzyme active site where further interactions could be engineered.

Based on these observations, we scrutinized our four classes of DASI and reasoned that introducing an additional moiety to an existing DASI could be realized by combining the core components of the two leading 4-((4-bromobenzyl)-[1,2,4]-triazol-4-ylamino)benzonitrile-based (I) and biphenyl-based (II) DASIs to form a series of hybrid structures as shown in Figure 2.

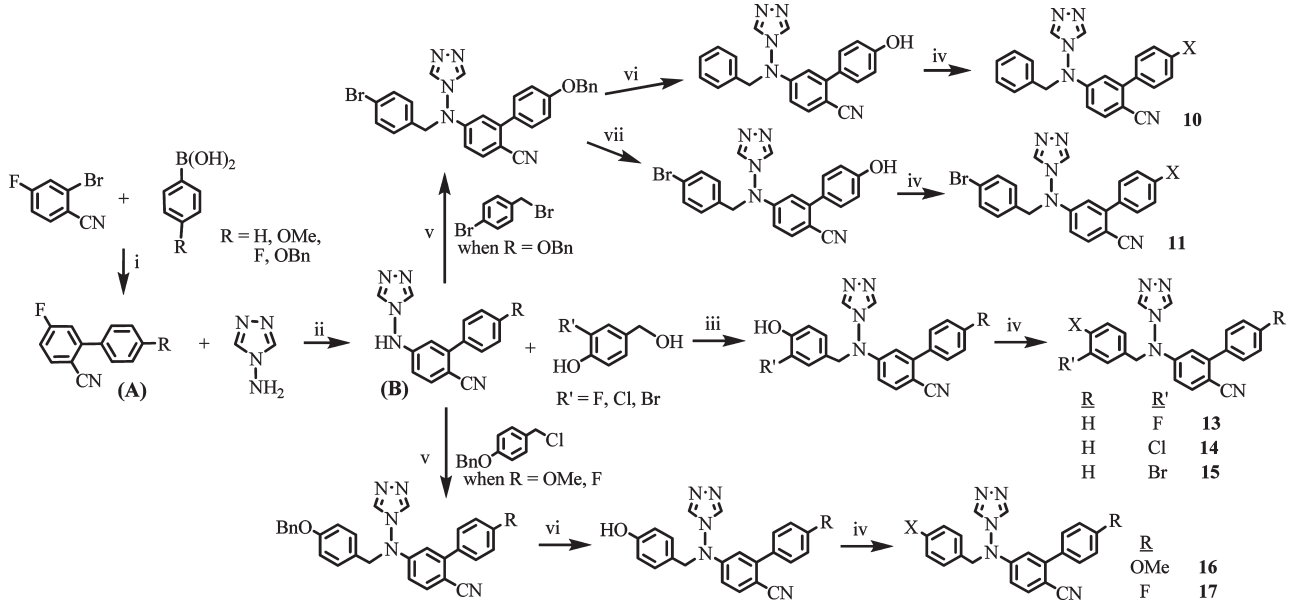
The synthesis of sulfamate-bearing hybrid structures **10**, **11**, and **13**−**17** (see Scheme 1) started with the formation of biphenyl intermediate (**A**) by reacting 2-bromo-4-fluorobenzonitrile with the required boronic acid under Suzuki cross-coupling conditions. Upon performing a nucleophilic aromatic substitution on (**A**) with 4*H*-1,2,4-triazol-4-amine, the resulting secondary amine was alkylated by an appropriately substituted benzyl halide. As required, the hybrid intermediate formed was deprotected to give the parent phenol, which was sulfamoylated to the corresponding sulfamate under established conditions.[26] A different approach was adopted for the synthesis of **12** and **18** (see Scheme 2). The key difference was the formation of a secondary amine by nucleophilic aromatic substitution, followed by conducting a Suzuki cross-coupling reaction on the resulting brominated intermediate.

The extent of *in vitro* inhibition of STS and aromatase activity produced by sulfamoylated compounds was assessed using JEG-3 human choriocarcinoma cells, which were chosen because these cells constitutively express both enzymes maximally.[19,24] In our hands, these enzyme activities remain stable during repeated subculturing over several months. The results are shown in Table 1. AI **1** and DASIs **2**, **3**, **8**, and **9**, which were tested previously in the same assay, are included as reference.

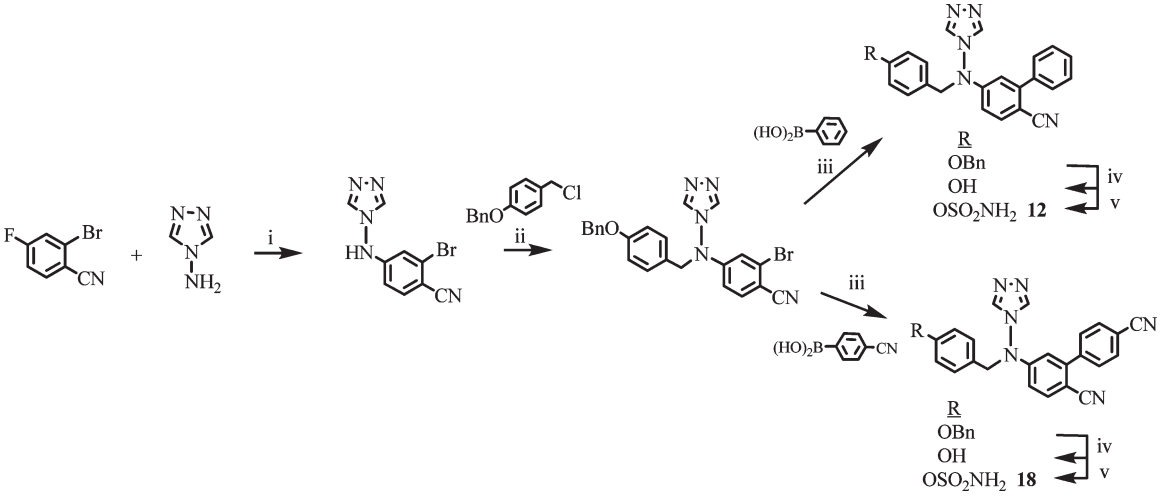
The modification of the DASI **8** by replacing the methylene bridge between the triazolyl group and the biphenyl scaffold with either a benzylamine or *para*-bromobenzylamine moiety significantly increases the potency of the resulting compounds against aromatase. Hybrids **10** (IC_{50}: 0.1 nM) and **11** (IC_{50}: 0.05 nM) are 1 and 2 orders of magnitude, respectively, more potent than **8** (IC_{50}: 2 nM) as AIs. However, the opposite effect is rendered with regard to STS inhibition. A substantial reduction in potency against STS is observed for **10** and **11** (both IC_{50}s: 2900 nM, cf. 35 nM for **8**). This suggests that the introduction of either a benzylamine or *para*-bromobenzylamine moiety to **8** results in productive interactions of **10** and **11** with the active site of aromatase but not with that of STS. Steric hindrance may be a contributory factor to the poor STS inhibition observed for **10** and **11**.

The introduction of a phenyl ring (either unsubstituted or with a substituent at the *para*-position) to the 4-((4-bromobenzyl)-[1,2,4]-triazol-4-ylamino)benzonitrile-based DASI **2** at the *ortho*

Scheme 1. Syntheses of Sulfamates (10, 11 and 13−17): (A) and (B) Are Key Intermediates; (i) $[Pd(dba)_2]_3$; (ii) KO^{t}Bu/DMSO; (iii) $SOCl_2$, K_2CO_3/DMF; (iv) $ClSO_2NH_2$/DMA; (v) NaH/DMF; (vi) Pd-C/MeOH/THF; (vii) AcOH/conc HCl (2:1, v/v), 1 h @ 100 °C, X = OSO_2NH_2



Scheme 2. Syntheses of Sulfamates (12 and 18): (i) KO^{t}Bu/DMSO; (ii) NaH/DMF; (iii) $[Pd(dba)_2]_3$; (iv) Pd-C/MeOH/THF; (v) $ClSO_2NH_2$/DMA



position to the cyano group renders a prominent increase in aromatase inhibition of most resulting hybrid compounds (IC_{50}'s: 0.015−0.75 nM vs 0.82 nM of **3** and 0.5 nM of **9**). Likewise, apart from **18** and to a lesser extent with **12**, **16**, and **17**, a significant increase in STS inhibition is observed for those hybrid compounds. This finding clearly demonstrates that the formation of hybrid structures has, *inter alia*, rendered a positive effect on the binding to the active site of both aromatase and STS, contributing toward the increased inhibitory activities observed for the molecules.

The best AIs in this series of compounds are **14**−**16**. Their IC_{50} values are similar in the low picomolar range (15 pM for **14** and **16**, and 18 pM for **15**). As anticipated for **14** and **15**, because of past observations in other series of DASIs, the introduction of a halogen *ortho* to the sulfamate group, in particular with Cl and Br, increases aromatase inhibition.[18−20,24] This can be attributed to the increase in lipophilicity effected by halogens on the molecule. It is not clear why **16** is equally potent against aromatase given the fact that, unlike **14** and **15**, it does not contain a halogen adjacent to the sulfamate group. The tentative explanation is that the *para*-methoxy group of **16** provides a significant contribution to the binding of the compound to the active site of aromatase. For **17** and **18**, they are some 2- and 3-fold less active as AI than **12**, respectively. The common feature in **17** and **18** is that they both possess an electron-withdrawing group (a fluoro atom in **17** and a cyano group in **18**) at the 4′-position which conjugates with the cyano group at the 2-position of the biphenyl scaffold. It is possible that such conjugation might weaken the ability of the cyano group at the

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Table 1. *In Vitro* Inhibition in JEG-3 Cells of Aromatase and STS Activity by Sulfamoylated Compounds 10−18, with Aromatase Inhibitor 1 and DASIs 2, 3, 8, and 9 Included as Reference

compd	aromatase IC_{50} (nM)	STS IC_{50} (nM)
1	0.5 ± 0.03[b]	n.d.[a]
2	100 ± 7.8[b]	277 ± 29[b]
3	0.82 ± 0.3[b]	39 ± 4.2[b]
8	2.0 ± 0.20[c]	35.0 ± 5.0[c]
9	0.50 ± 0.01[c]	5.50 ± 0.5[c]
10	0.1 ± 0.02	2900 ± 180
11	0.05 ± 0.01	2900 ± 100
12	0.21 ± 0.03	17 ± 2
13	0.15 ± 0.03	2.3 ± 0.9
14	0.015 ± 0.005	0.83 ± 0.15
15	0.018 ± 0.01	0.13 ± 0.01
16	0.015 ± 0.001	22 ± 6
17	0.47 ± 0.18	55 ± 6
18	0.75 ± 0.005	240 ± 60

[a] n.d.: not determined. [b] Reference 19. [c] Reference 24.

2-position to function as a hydrogen bond acceptor, which has been attributed to be an important factor for strong binding of nonsteroidal AIs bearing a cyano group similar to that of letrozole.[27]

As shown in Table 1, **18** is relatively the weakest STS inhibitor among its congeners (**12**−**17**). Since hybrids **12**, **16**, and **17** have IC_{50} values against STS of the same order of magnitude, this suggests that a fluoro atom or a methoxy group, but not a cyano group, substituted at the 4′-position is better tolerated by STS in this subgroup of compounds. The strongest STS inhibitors are those derivatives which have a halogen *ortho* to their sulfamate group. This has been a characteristic observed in our previous work.[18−20,21,23,24,28,29] The most potent inhibitor against STS in the series is the brominated **15**, whose IC_{50} value is 0.13 nM, which is two orders and one order of magnitude more potent than the 4-((4-bromobenzyl)-[1,2,4]-triazol-4-ylamino)benzonitrile-based DASI **3** (IC_{50}: 39 nM) and the biphenyl-based DASI **9** (IC_{50}: 5.5 nM), respectively. This further demonstrates the productive contribution of the additional phenyl ring to inhibitory activity and the advantage of hybrid structure.

In conclusion, a novel series of DASIs has been designed by combining the core components of two leading series of DASIs, namely 4-((4-bromobenzyl)-[1,2,4]-triazol-4-ylamino)benzonitrile-based and biphenyl-based series. Several resulting derivatives show a very high level of dual inhibition against aromatase and STS *in vitro*. The improvement observed compared with the best leading DASIs is significant and substantial, making this new inhibitor class worthy of further optimization and arguably the most promising to date for preclinical studies. This work clearly validates the "hybrid" strategy and demonstrates that structurally novel and distinctive DASIs with improved dual inhibitory activities can be designed. It is now warranted to further develop these "hybrid" compounds and explore their full potential as therapeutic agents for the treatment of hormone dependent cancers such as that of the breast.

■ ASSOCIATED CONTENT

Supporting Information. Syntheses of compounds **14**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

Overall strategy and core structures were designed by L.W.L.W. and B.V.L.P. in consultation with C.B. C.B. synthesized the compounds, and A.P. carried out their biological evaluation. The paper was written by L.W.L.W. and B.V.L.P. in consultation with all authors.

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■ ABBREVIATIONS

AI, aromatase inhibitor; DASI, dual aromatase-sulfatase inhibitor; DMA, *N*,*N*-dimethylacetamide; HDBC, hormone-dependent breast cancer; STS, steroid sulfatase; STX64, 6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[*c*]chromen-3-yl sulfamate

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