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Discovery of a new class of cinnamyl-triazole as potent and selective inhibitors of aromatase (cytochrome P450 19A1)

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ABSTRACT

Synthesis of a novel class of natural product inspired cinnamyl-containing 1,4,5-triazole and the potent inhibition of human aromatase (CYP 450 19A1) by select members is described. Structure–activity data generated provides insights into the requirements for potency particularly the inclusion of an aryl bromide or chloride residue as a keto-bioisostere.

Aromatase inhibitors (AI's), such as exemestane **1**, anastrozole **2** and letrozole **3**, have emerged over the last few decades as alternatives to the selective estrogen receptor modulating agent tamoxifen **4**, in the treatment of hormone-dependent breast cancer. Approximately 75% of postmenopausal breast cancer patients have hormone-dependent (estrogen-dependent) breast cancer,[1] now the leading cancer amongst women.[2] Tamoxifen **4** (Fig. 1) served as the standard anti-estrogen in treating such tumors for many years.[3a–f] Tamoxifen and its metabolites function as selective estrogen receptor modulators (SERMs).[3f] Their partial antagonist action

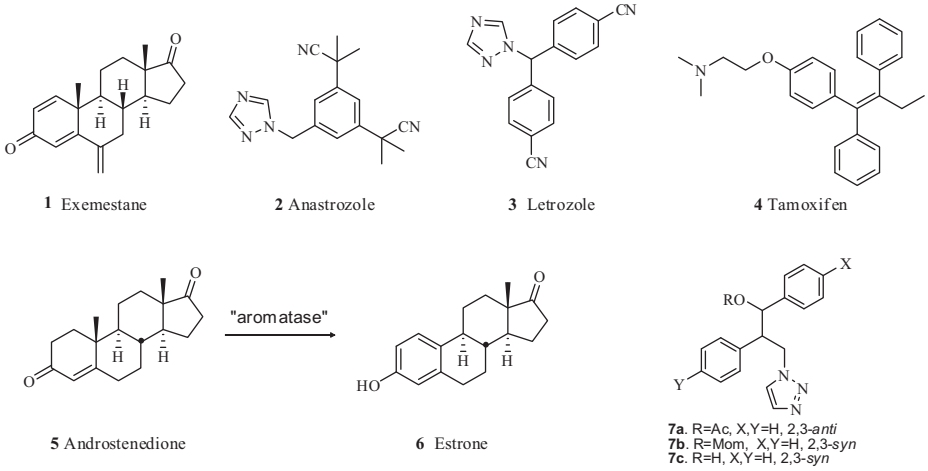

Figure 1. Structures of currently used AIs **1**–**3**, and the SERM agent tamoxifen **4**. Example of aromatase conversion of **5** to **6** and structure of recently described potent AIs **7** employing aryl halide ketone bioisosteres.

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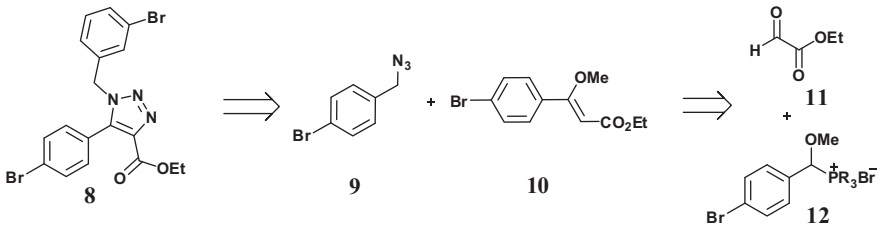


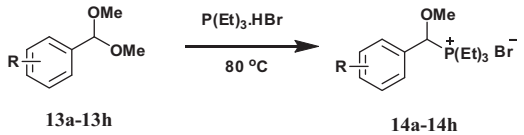
Figure 2. Cinnamyl-triazole core structure **8** and retrosynthetic analysis based on the Wittig reaction of alkoxyphosphonium salt **12**.

in breast tissue prevents estrogen binding and resulting downstream effects that include cancer cell proliferation and the activation of survival and anti-apoptosis pathways.[3d,e]

AIs are inhibitors of human cytochrome P450 19A1 enzyme complex (CYP19A1), the rate-limiting enzyme involved in the oxidative decarboxylation of the C19 methyl group in androgens such as testosterone and androstenedione **5** leading to the estrogens estradiol and estrone **6**, respectively, (Fig. 1).[4] Irreversible Type-I aromatase inhibitors (steroidal) such as exemestane **1** as well as reversible, nonsteroidal type-II inhibitors exemplified by anastrozole **2** and letrozole **3**, are currently approved AIs for the treatment of metastatic estrogen-dependent breast cancer.[1a,5a–e]

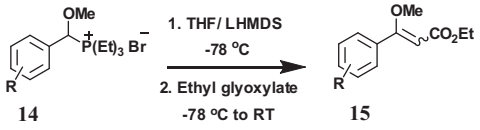
Despite their clinical success, current AIs are associated with issues such as osteoporosis, joint pain, reproductive problems and androgenic side effects. These compounds also partly inhibit cytochromes 1A1, 1A2, 2D6, 2C8/9 and 3A4, all of which are involved in the metabolism of xenobiotics, thus increasing the likelihood of drug–drug interactions. These factors necessitate the discovery and development of structurally novel, potent and selective AIs for the next generation treatment of ER positive breast cancer.

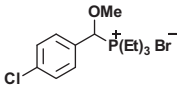
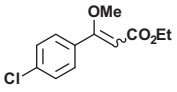
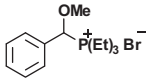
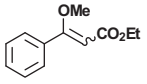
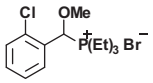
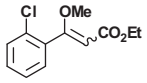
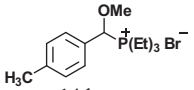
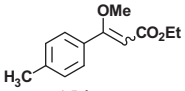
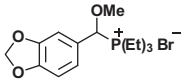
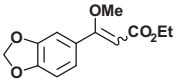
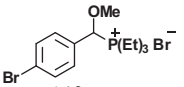
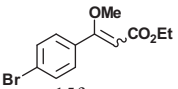
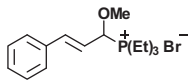
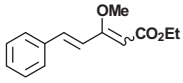
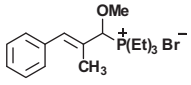
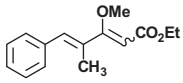
Natural products and structural analogues have proven to be valuable sources in the search for lead compounds as nonsteroidal AIs. In particular, natural products from the cinnamic and coumaric acid pathways, including cinnamates, chalcones, flavanones/flavones, isoflavones and stilbenes[6] have been shown to be privileged structures amongst naturally occurring AIs. Work in our own laboratories resulted in the discovery of natural flavones[6c] that exhibited potent aromatase inhibitory activity as well as a series of alkaloids and synthetic derivatives that demonstrated selective activity against aromatase and other cytochrome P450s.[7] This work resulted in the discovery[8a] of potent triazole-containing AIs based on a common 5-component pharmacophore **7** (Fig. 1). A key aspect of the optimization in this work was the inclusion of aryl bromide residues as carbonyl-mimics at positions corresponding the keto groups on androstenedione.[8b] The use of aryl halides as ketone bioisosteres resulted in increased aromatase inhibition to 20 nM. In continuance of this work, we desired to explore a rapidly accessible system capable of mimicking the androstenedione core and allowing incorporation of aryl halide groups at the critical positions. Consideration of both natural product and synthetic AI structures led us to postulate the cinnamyl-triazole core molecule **8** (Fig. 2) as a potential new lead for developing AIs. In this Letter, we report the synthesis of an initial mini-panel of compounds based on this cinnamyl-triazole core and discovery of their potent aromatase activities.



Scheme 1. Synthesis of alkoxyphosphonium salts **14a**–**14h**.

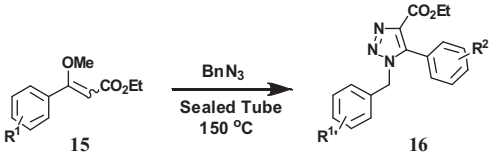
Table 1
Conversion of α-methoxy phosphonium salts to β-methoxycinnamate 'alkyne equivalents'

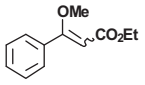
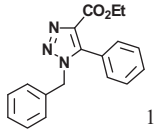
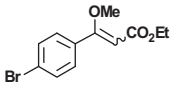
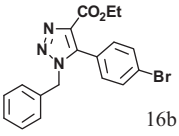
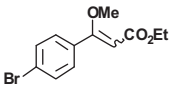
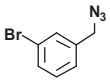
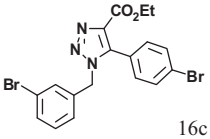
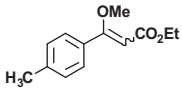
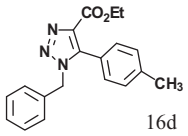
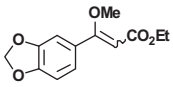
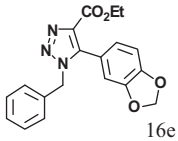
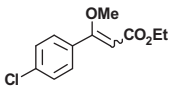
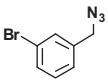
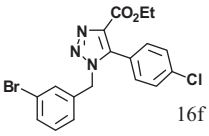
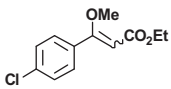
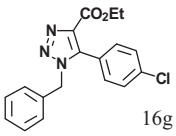
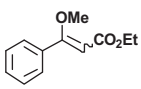
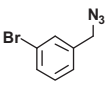
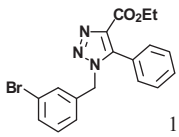


Entry	Alpha-alkoxy P salts	Beta-alkoxy acrylate	Isolated yield(%)
1	 14a	 15a	80
2	 14b	 15b	85
3	 14c	 15c	82
4	 14d	 15d	80
5	 14e	 15e	85
6	 14f	 15f	88
7	 14g	 15g	52
8	 14h	 15h	40

The principal method for preparing 1,2,3-triazoles is the copper(I) catalyzed 1,3-dipolar stepwise cycloaddition of an azide onto an alkyne (Click Reaction).[11] The direct synthesis of 1,2,3-triazoles under metal-free thermal cycloaddition conditions (Huisgen reaction) is less explored. In addition to alkynes, access to 1,2,3-triazoles from the cycloaddition of azides onto heteroatom-substituted alkenes is an alternative route to functionalized triazoles. Examples of such 'alkyne equivalents' include enol

Table 2
Regiocontrolled thermal Huisgen cycloaddition of benzyl azides onto β-methoxycinnamates leading to potent AIs



Entry	Beta-alkoxy acrylate	Benzyl azide	Click Product	Isolated Yield (%)	Ki (uM)	pKi
1	 15b		 16a	95	1.41	5.85
2	 15f		 16b	95	0.64	6.20
3	 15f		 16c	96	0.02	7.66
4	 15d		 16d	57	0.71	6.15
5	 15e		 16e	40	0.71	6.15
6	 15a		 16f	40	0.03	7.60
7	 15a		 16g	44	0.36	6.45
8	 15b		 16h	48	0.15	6.84

ethers, enamines, vinylogous amides, vinyl amides, and vinyl sulfoxides. The cycloadditions of azides onto functionalized alkenes typically requires higher temperatures than alkynes, but such alkenes do possess advantages such easy access from carbonyl compounds and high regioselectivity in the cycloaddition due to the polarized nature of the alkene.

Following a recently described method for the synthesis of α-alkoxy-phosphonium salts (Scheme 1), from the reaction of a dialkylacetal and a trialkylphosphine hydrobromide salt,[9a] we prepared the series of α-methoxybenzyl phosphonium salts **14a**–**h**. These salts were obtained in high yield (92–98%) without need of chromatographic purification through simply removing methanol under high vacuum. We next investigated α-methoxy ylide formation and olefination of the range of substituted phosphonium salts with ethyl glyoxylate. Dark red solutions of the ylides were generated in THF at −78 °C using lithium bistrimethylsilylazide. Efficient

olefination occurred in all cases upon addition of ethyl glyoxylate and the corresponding methoxycinnamate **15a–h** were purified by silica gel chromatography and isolated in 40–88% yields and good (*E*) selectivity. (*E*/*Z* ratio ranging between 70:30 and 80:20) as summarized in Table 1. These β-alkoxycinnamates are relatively stable to air but are readily hydrolysed to the corresponding β-keto esters in high yield upon treatment with acids.

Thermal cycloaddition of the series of methoxycinnamates with benzylic azides occurred smoothly in a sealed tube at 150 °C, leading to the desired triazoles **16a–h** in fair to excellent yield. The cycloadditions were seen to be completely regioselective, leading only to the 1,4,5 trisubstituted 1,2,3-triazoles. Regiochemical assignments are in accord with earlier examples confirmed via X-ray crystallographic analysis.[10i] The well-known Cu-catalyzed azide alkyne cycloaddition leads to 1,4-triazoles and requires employment of a terminal alkyne.[11] Thus in addition to complete regiocontrol, the use of vinyl ethers allows access to functionalized triazoles that are not available from the conventional click process.

The series of eight novel cinnamyltriazoles **16a–h** described in Table 2 were screened for activity against recombinant human aromatase via kinetic monitoring of the conversion of *O*-dibenzylfluorescein benzyl ester (DBF) substrate to fluorescein by-product.[8a] Fluorometric measurement of emission was made at 535 nm after excitation at 485 nm utilizing ketoconazole as a positive control.[6c] The overall results of the screening are shown in Table 2. To our delight, several analogs proved to be potent inhibitors, displaying a 100-fold range in potencies ranging from the μM range down to 20 nM, Table 2. The most interesting examples proved to be the dibromo compound **16c** and the corresponding bromochloro-analog **16f** that exhibited potencies of 20 and 30 nM, respectively. The positions of halogenation approximate the location of the carbonyl groups in natural substrates such as androstenedione. These results add further to the previous postulate of arylbromide/chloride residues as bioisosteres[8b] in the development of stable analogs as potent AI's. In addition to potency, the discovery of this new pharmacophore is significant in view of the rapid synthetic entry to the series in only four chemical steps following the sequence aldehyde to dimethylacetal **13**, to alkoxyphosphonium salt **14**, to methoxycinnamate **15** and finally triazole **16**. The final thermal Huisgen reaction with benzylic (or other) azide as the fourth (last) step leading to **16** allows significant late-stage diversity for further optimization in this new lead series.

In summary, we report a new natural product-inspired heteroaromatic 1,4,5-substituted cinnamyltriazole pharmacophore, examples of which display potent aromatase inhibitory activity. The core structure can be rapidly prepared in four linear steps from readily available aldehydes and the synthesis incorporates a regiospecific, diversity oriented thermal Huisgen reaction as the last step. The most potent AIs within this new series incorporate aryl halide residues at positions mimicking the carbonyl substituents of the natural enzyme substrates. Further development of potent and selective AIs based on this novel pharmacophore are currently under investigation.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.07.083.

References and notes

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