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Bis-aryloxadiazoles as effective activators of the aryl hydrocarbon receptor

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ABSTRACT

Bis-aryloxadiazoles are common scaffolds in medicinal chemistry due to their wide range of biological activities. Previously, we identified a 1,2,4-bis-aryloxadiazole that blocks mammary branching morphogenesis through activation of the aryl hydrocarbon receptor (AHR). In addition to defects in mammary differentiation, AHR stimulation induces toxicity in many other tissues. We performed a structure activity relationship (SAR) study of 1,2,4-bis-aryloxadiazole to determine which moieties of the molecule are critical for AHR activation. We validated our results with a functional biological assay, using desmosome formation during mammary morphogenesis to indicate AHR activity. These findings will aid the design of oxadiazole derivative therapeutics with reduced off-target toxicity profiles.

Small molecule libraries are widely used as a tool in chemical biology,[1] both to probe biological pathways and to develop new therapeutics. However, the success of chemical library screening efforts is limited by library composition and size. One strategy to produce a large number of drug-like compounds is to use scaffolds that have previously generated biologically active chemicals.[2] In particular, the oxadiazole nucleus has been used extensively as a scaffold in drug development[3] due to the range of activities reported for its derivatives, including antimicrobial, anticancer, anti-inflammatory, and antiviral effects.[4–7]

As a heteroaromatic ring, oxadiazoles can be prepared as several constitutional isomers. The 1,2,4-oxadiazole isomer has been used in numerous pharmacologic drugs, including metabotropic glutamate subtype 5 receptor antagonists,[8] sphingosine-1-phosphate-1 receptor agonists,[9] and anticancer apoptosis inducers.[10] Additionally, we previously identified a derivative of this isoform as a potent compound that blocks mammary branching morphogenesis.[11] In our assay, 1,2,4-bis-aryloxadiazole **1** (referred as 1023 in our previous communication) was the lead compound identified in a chemical genetic screen for molecules that block mammary branching morphogenesis. Further analysis showed **1** had an EC_{50} of 1.2 ± 0.050 μM and blocked branching through activation of the aryl hydrocarbon receptor (AHR).

In addition to influencing mammary branching, AHR agonists also block differentiation and lactation in the mammary gland[12–14] and exhibit a wide range of toxic effects in other tissues.[15,16] Our previous observations that compound **1** potently activated AHR suggested that other 1,2,4-oxadiazole derivatives may display unwanted drug effects due to AHR stimulation. Given the structural relationship of these derivatives to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a known carcinogen and environmental toxin that also activates AHR, we performed structure–activity relationship (SAR) studies of **1** to identify key elements of the molecule that contribute to AHR activation. A library of bis-aryloxadiazoles was prepared by Lewis-acid mediated coupling of benzoyl chlorides with benzamidoximes (Scheme 1). The activity of each analog was determined by measuring expression of the AHR target gene, *Cyp1a1*,[17,18] in HC11 mammary epithelial cells (MECs) treated for 48 h with 10 μM compound.

We initially made systematic modifications on the C-ring of **1** (Table 1). Based on a previous homology model,[11] this ring was predicted to form charge/polar interactions with amino acid residues His-291 and Gln-383 in the AHR binding pocket. Our results indicated that replacing the *o*-Cl substituent with an amino group (compound **2**) increased AHR activity ~5-fold, as shown by

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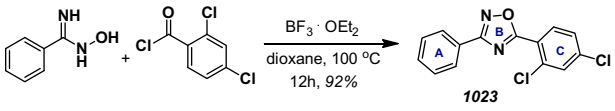
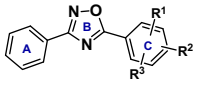


Scheme 1. General strategy for synthesis of bis-aryloxadiazoles.

Table 1
SAR study of the C-ring of 1,2,4-bis-aryloxadiazole



Compound	R^1	R^2	R^3	Relative *Cyp1a1*
1	*o*-Cl	*p*-Cl	H	128.53 ± 0.17
2	*o*-NH_2	*p*-Cl	H	638.80 ± 0.14
3	*o*-NO_2	*p*-CF_3	H	0.36 ± 0.15
4	*o*-NO_2	*p*-OMe	H	0.42 ± 0.25
5	*o*-NO_2	*p*-OH	H	0.25 ± 0.14
6	*o*-NO_2	*p*-OCO_2Me	H	0.39 ± 0.16
7	*o*-NO_2	*p*-*O*-Propargyl	H	2.43 ± 0.19
8	*o*-NO_2	p-F	H	122.64 ± 0.12
9	*o*-NH_2	*p*-CF_3	H	58.63 ± 0.15
10	*o*-Cl	*p*-Cl	*m*-Cl	25.79 ± 0.17

Expression of an AHR response gene, *Cyp1a1*, was measured in HC11 MECs treated with 10 μM compound for 48 h.

increased *Cyp1a1* gene expression with compound **2**. In contrast, placement of an electron-withdrawing group (NO_2) at the *ortho* position of the C-ring dramatically decreased AHR activity (compounds **3**–**7**). These results suggested that the C-ring of bis-aryloxadiazole is compatible with an electroneutral or protic-polar substitution that can be stabilized by hydrogen bonding with His-291 (Fig. 1). This was confirmed by replacing the nitro group at the *ortho* position of compound **3** with an amino group (compound **9**), which partially restored *Cyp1a1* gene expression.

In addition to the *ortho* position, the *para* group of the C-ring also influenced AHR activity. Specifically, *p*-CF_3 (compound **9**) showed significantly lower *Cyp1a1* gene expression compared to *p*-Cl (compound **2**). This result is likely due to the electron withdrawing and sterically bulky nature of CF_3. Surprisingly, *p*-F (compound **8**) offset the decreased activity of *o*-NO_2 observed in other analogs. This may be explained by the small size of *p*-F, which would decrease van der Waals repulsion and contribute to aromatic stabilization through charge interaction. Together, modifications on the C-ring suggested bis-aryloxadiazole requires subtle

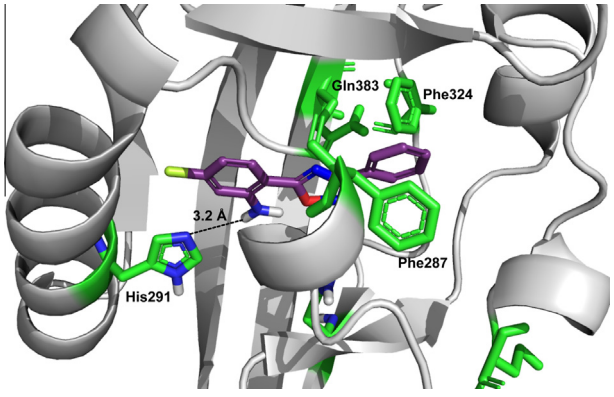
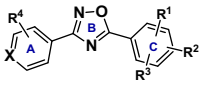


Figure 1. Homology model structure of human AHR (gray) and compound **2** (purple), with residues predicted to contribute to compound binding shown in green. Hydrogen bonding between the amino group at the *ortho* position of the C-ring and His-291 is predicted to stabilize binding.

Table 2
SAR study of the A-ring of 1,2,4-bis-aryloxadiazole



Compound	X	R^1	R^2	R^3	R^4	Relative *Cyp1a1*
11	CH	*o*-Cl	*p*-Cl	H	*m*-CF_3	1.84 ± 0.28
12	CH	*o*-Cl	*p*-Cl	H	*m*-CO_2Me	0.18 ± 0.49
13	CH	*o*-F	H	H	*m*-CO_2Me	0.29 ± 0.74
14	CH	*o*-F	H	H	*m*-CO_2H	3.71 ± 0.22
15	CH	*o*-Cl	*p*-Cl	H	*o*-Cl	5.91 ± 0.18
16	CH	*o*-Cl	*p*-Cl	H	*m*-Cl	1.59 ± 0.30
17	CH	*o*-Cl	*p*-Cl	H	*p*-Cl	8.15 ± 0.14
18	CH	*o*-Cl	*p*-Cl	H	*p*-*O*-Propargyl	0.66 ± 0.26
19	CH	*o*-NO_2	*p*-Cl	H	*p*-*O*-Propargyl	0.29 ± 0.35
20	CH	*o*-NH_2	*p*-Cl	H	*p*-*O*-Allyl	0.62 ± 0.16
21	CH	*o*-NH_2	*p*-Cl	H	*p*-*O*-Propargyl	0.38 ± 0.42
22	N	*o*-Cl	*m*-Cl	*p*-Cl	H	0.53 ± 0.28
23	N	*o*-Cl	*p*-Cl	H	H	0.37 ± 0.13
24	N	*o*-NO_2	*p*-Cl	H	H	8.25 ± 0.13
25	N	*o*-NO_2	*p*-OAc	H	H	0.35 ± 0.41

Expression of an AHR response gene, *Cyp1a1*, was measured in HC11 MECs treated with 10 μM compound for 48 h.

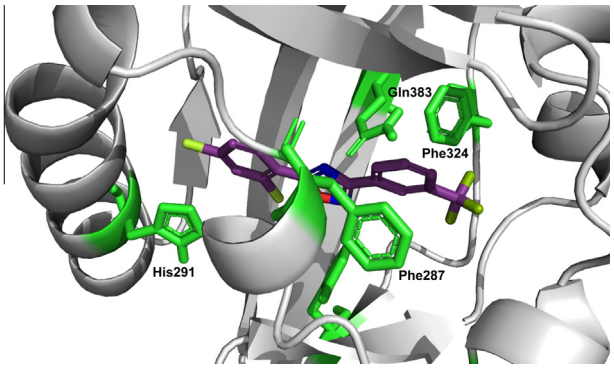


Figure 2. Homology model structure of human AHR (gray) and compound **11** (purple). Residues predicted to contribute to compound binding are shown in green. Steric interactions of the A-ring with Phe324 and Phe287 lead to decreased AHR activation.

electronic demand at the *ortho* and *para* positions and tight van der Waals radii at the *para* position to elicit significant AHR activation.

Next, we extended our SAR study to the A-ring of bis-aryloxadiazole (Table 2). Previous modeling studies[11,19] suggested this portion of the molecule binds within a tight hydrophobic cavity of AHR and is stabilized by aromatic π-stacking of Phe-324 and Phe-287. As a result, we hypothesized that functional groups on the A-ring of bis-aryloxadiazole able to distort this π-stacking would also diminish AHR activation (Fig. 2). Supporting this hypothesis, we previously showed that addition of *m*-CF_3 to the A-ring (compound **11**) dramatically decreased AHR activity.[11] Similarly, polar carbomethoxy or carboxylate substituents at R_4 (compounds **12**–**14**) showed low *Cyp1a1* gene expression, irrespective of identities at R_1 and R_2. Importantly, these substitution patterns are seen in lead compounds for the treatment of nonsense mutation disorders (e.g., Ataluren).[20]

In addition to *meta* substitutions, we altered other positions of the A-ring and modified the A-ring itself. Substitution of CF_3 with Cl at different positions resulted in only subtle AHR activation, with *p*-Cl showing the highest *Cyp1a1* gene induction (compound **15**–**17**). Similarly, larger *para*-substituents on the A-ring (compounds **18**–**21**) or substitution of the A-ring with a heterocycle