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3,5-Diarylazoles as novel and selective inhibitors of protein kinase D

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ABSTRACT

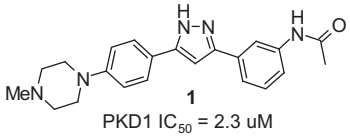
The synthesis and preliminary studies of the SAR of novel 3,5-diarylazole inhibitors of Protein Kinase D (PKD) are reported. Notably, optimized compounds in this class have been found to be active in cellular assays of phosphorylation-dependant HDAC5 nuclear export, orally bioavailable, and highly selective versus a panel of additional putative histone deacetylase (HDAC) kinases. Therefore these compounds could provide attractive tools for the further study of PKD / HDAC5 signaling.

Cardiac hypertrophy is a common response to stress signals in the heart that arise from a variety of cardiovascular disorders, including myocardial infarction and chronic hypertension.[1] Prolonged hypertrophy has been recognized as a major determinant of heart failure,[2] a disease of increasing prevalence and high morbidity and mortality.

Recently, it has been reported that histone deacetylase 5 (HDAC5), acts as a signal responsive repressor of cardiac hypertrophy when it is co-localized with transcription factors, such as MEF2, in the nucleus.[3] In response to stress signals, phosphorylation of HDAC5 by one or more HDAC kinases triggers its export from the nucleus into the cytoplasm and releases the repression of MEF2 responsive genes.[4] This is accompanied by an increase in cardiomyocyte cell size, assembly of sarcomeres, and the activation of a 'fetal' program of gene expression, leading to pathological cardiac hypertrophy. Protein kinase D 1 (PKD1) has been implicated as an HDAC kinase mediating the subcellular localization and signaling of HDAC5 in cardiac tissue.[5,6] Therefore, inhibition of PKD1 would be predicted to block the nuclear export of HDAC5 and blunt the hypertrophic response to stress in cardiac tissue, potentially providing a novel therapy for heart failure.

High throughput screening against recombinant PKD1 identified 3,5-diarylpyrazole **1** as a novel kinase inhibitor scaffold with moderate activity (Figure 1). The synthesis and preliminary SAR studies of benzamide analogs of this chemotype, which resulted in the development of potent, selective, and orally available small molecule inhibitors of PKD, are reported herein.[7]

The synthesis of 3,5-diarylazole PKD inhibitors is shown in Schemes 1–5. The 3,5-diarylpyrazole core[8] of compounds **4a** (Scheme 1), **4b** (Scheme 4), and **4c** (Scheme 5) was constructed via Claisen condensation of a suitably substituted acetophenone (**2a–c**) and dimethyl isophthalate **3**, followed by further condensation of the resultant diketone with hydrazine. Analogously, 3,5-diarylisoxazoles[9] could be formed by reaction of the diketone products of the Claisen condensation with hydroxylamine (not shown). However, this approach generally resulted in mixtures of 3,5-isoxazole regioisomers. Therefore, the 3,5-diarylisoxazole core of compounds **10a** (Scheme 3) and **10b** (Scheme 5) was constructed through the [3+2] cycloaddition of nitrile *N*-oxides (formed in situ from hydroximoyl chlorides **8a–b**) with substituted phenylacetylenes **9a–b**, which provided isoxazoles as single, predictable, isomers.



1

PKD1 IC_{50} = 2.3 uM

Figure 1. Initial 3,5-diarylazole hit.

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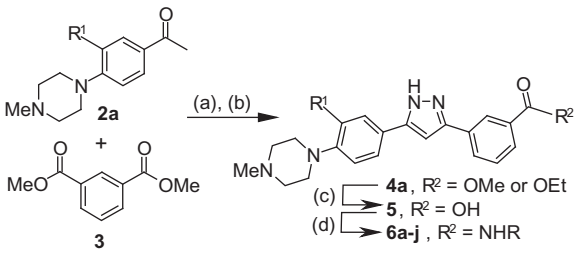
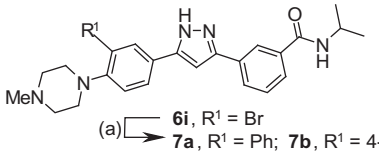
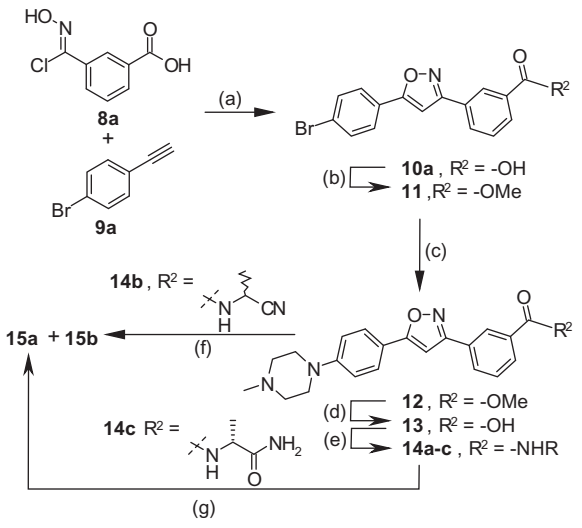


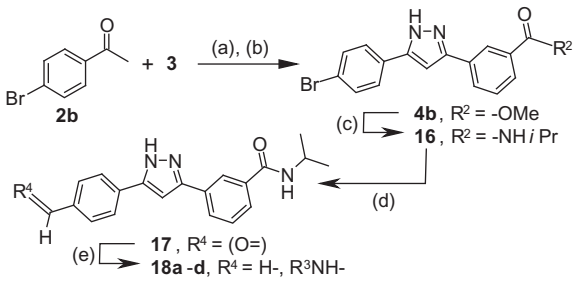
Scheme 1. R and R^1 are defined in Table 1. Reagents: (a) NaH, THF or NaOEt, EtOH; (b) hydrazine, EtOH; (c) LiOH, THF:H_2O; (d) HATU, R-NH_2, DiPEA, DMF.



Scheme 2. Reagents and conditions: (a) Aryl$B(OH)_2$, Pd(dppf)Cl_2, K_2CO_3 (aq), 1:10 EtOH in PhMe, 110 °C.



Scheme 3. Unless otherwise indicated, R is defined in Table 1. Reagents and conditions: (a) DiPEA, CH_2Cl_2; (b) TMSCl, MeOH; (c) Pd$(OAc)_2$, BINAP, $Cs(CO_3)_2$, PhMe, reflux; (d) LiOH, THF:H_2O; (e) HATU, R-NH_2, DiPEA, DMF; (f) Chiral HPLC, Chiralpak IA column, 0.2% TEA:1% EtOH; ACN; (g) TFAA, Et_3N.



Scheme 4. Substituent R^3 is defined in Table 2. Reagents and conditions: (a) NaH, THF; (b) hydrazine, DiPEA; (c) $AlMe_2$, $NH_2(iPr)$; (d) i) BuLi, −78 °C; (ii) DMF, −78 °C; (e) NH_2-R^3, $Na(OAc)_3BH$.

Further elaboration of pyrazole ester **4a** to compounds **6a–j** was achieved through saponification to carboxylate **5**, followed by HATU mediated amide bond formation (Scheme 1).[10] Compounds **7a–b** were synthesized by a further Suzuki–Miyaura coupling of the bromide of **6i** with the respective aryl boronic acid (Scheme 2).[11]

Synthesis of the piperazine-containing isoxazole compounds **14a–c** (Scheme 3) required protection of bromo-acid **10a** as a methyl ester (**11**) before installation of the piperazine ring using Buchwald–Hartwig amination[12] conditions to provide intermediate **12**. By analogy to the route illustrated in Scheme 1, isoxazole ester **12** was saponified to provide carboxylic acid **13**, which could be coupled with amines to provide amides **14a–c**. Chiral, non-racemic compounds **15a–b** were resolved from the racemate **14b** by standard HPLC techniques on a chiral stationary phase. Alternatively, dehydration of chiral, non-racemic D-alanine amide **14c** proceeded with complete retention of enantiopurity to provide **15a**.[13] In addition to providing convenient access to enantiomerically pure (>98% ee) materials from a chiral pool source, this orthogonal strategy also allowed the assignment of the absolute stereochemistry of the aminonitrile enantiomers.

Pyrazole compounds **18a–d**, in which the piperazine ring has been replaced with a benzylic amine, were synthesized from bromo ester **4b** (Scheme 4). Amidation of the methyl ester was mediated by trimethylaluminum[14] to afford bromo-amide **16**. Lithium–halogen exchange followed by quenching with DMF provided aldehyde **17**, which was converted to benzylic amines **18a–d** by reductive amination. The benzylic amine of pyrazole **20** (Scheme 5, X = NH) was installed through an alternate sequence starting from dimethyl acetal **4c**. Hydrolysis of the acetal with aqueous acid provided aldehyde **19**, which was converted to a benzylic amine by reductive amination. Benzylamine-substituted isoxazole **20** (Scheme 5, X = O), was synthesized from compound **10b** by radical bromination of the tolyl group with NBS, followed by trapping of the benzyl bromide intermediate with amines.

Saponification of the methyl ester of **20** (Scheme 5, X = N or O) to carboxylate **21**, followed by amide bond formation provided compounds **22a–d**. Further transformation of the D-alanine amide of **22d** by dehydration with trifluoroacetic anhydride provided aminonitrile **23**, in which the benzylic amine was simultaneously protected by trifluoroacetic anhydride. The resulting trifluoroacetamide could be removed cleanly and chemoselectively by treatment with sodium borohydride in methanol[15] to provide compounds **24a–c** in excellent enantiopurity (>98% ee). Compound **24d**, the enantiomer of compound **24c**, was synthesized by the same methods and in similar enantiopurity by substituting L-alanine amide in the amide bond formation step from acid **21**.

Preliminary structure–activity relationship (SAR) data is provided in Tables 1 and 2. In general, equivalently substituted pyrazole- and isoxazole-derived compounds show similar potency as PKD1 inhibitors (cf. Table 1, **6c**, **14a**; Table 2, **24a**, **24b**).

In order to improve the moderate PKD1 inhibitory potency observed with **1** (Figure 1), a series of varied benzamide derivatives were synthesized (Table 1, **6a–i**). Alkyl amides (**6a–b**) show potency against PKD1 similar to **1**. However, an α-aminonitrile amide (**6c**) provides a significant (~18-fold) improvement in potency. While the nature of the interaction of the nitrile with the PKD1 binding site is unknown, the effect of this substituent appears to be quite specific, as amide substituents bearing other polar and/or hydrogen bond-accepting functionality (**6e–h**), or even an additional methylene spacer (β-aminonitrile **6d**), uniformly fail to provide increases in potency similar to the α-aminonitrile, and are, in some cases, even detrimental to PKD1 inhibitory activity compared to a simple alkyl amide (**6a–b**). The beneficial effect of the α-aminonitrile is also observed with the 3,5-diarylisoxazole core (**14a**, also cf. **22a**, **22b**).

Notably, a modest but consistent increase in potency is observed with addition of α-branching on the aminonitrile substitu-