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Synthesis of Heterocyclic Triads by Pd-Catalyzed Cross-Couplings and Evaluation of Their Cell-Specific Toxicity Profile

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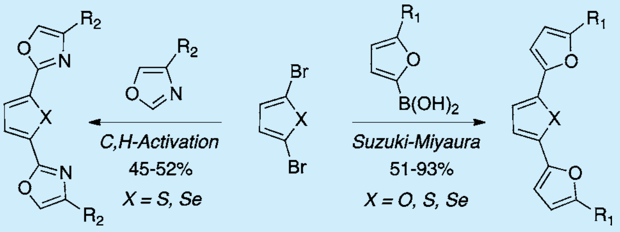
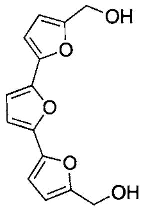
Supporting Information

ABSTRACT: Two complementary approaches for the preparation of linked 5-membered heterocycles were developed. The Pd-catalyzed Suzuki–Miyaura cross-coupling with halogenated furan, thiophene, and selenophene led to higher overall yields, but C,H-bond activation was a more efficient strategy for the coupling at C(2) of oxazoles. Potency and selectivity of the final hydroxymethyl products in renal (A498), lung (NCI-H226), kidney (CAKI-1), and breast (MDA-MB-468, MCF7) carcinoma cell lines were determined.



Five-membered heterocycles represent approximately half of the top 20 most common aromatic ring systems present in biologically active compounds.[1] They also frequently occur in natural products.[2] Their relative ease of synthesis from readily available precursors and their steric and electronic plasticity contribute to this popularity. As an extension of our synthetic studies on anticancer furans,[3] thiophenes,[4] oxazoles,[5] and thiazoles,[6] we became interested in the attractive cell-specific toxicity profile (i.e., the hyperselective growth inhibition) of terfuran **1**, in comparison to the broad-based toxicity of the thiophene-furan heterocyclic triad RITA (**2**, Figure 1).[7] The latter compound, also known as NSC 652287, has been shown to exhibit potent antiproliferative activity in various cancer cell lines, in particular in the renal cell line A498.[8] The mechanism of action of **2** has been the topic of much speculation. An interaction between **2** and the *N*-terminal domain of p53 was suggested to interfere with HDM2 binding and degradation of p53.[9] Other p53-mediated functions such as the regulation of pro- and antiapoptotic genes are also influenced by **2**, in addition to induction of autophagy, downregulation of c-Myc, cyclin E and *β*-catenin oncogene expression, and inhibition of the PI3K/Akt signaling pathway.[10] Among a series of analogues of **2**, terthiophene **3** was recently found to exhibit only weak activity against a p53 knockout cancer cell line but activated p53 and triggered apoptosis in wild-type HCT116 at low nanomolar concentrations.[11] The primary alcohol group in these compounds proved to be essential for bioactivity and possibly acts as a prodrug that is metabolized to the active agent within the cell.[12] Interestingly, the corresponding esters and aldehydes were significantly less active in the National Cancer Institute NCI-60 screen.[13]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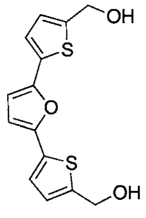
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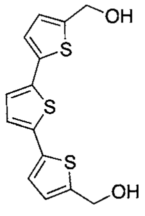
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Figure 1. Structures of selective antiproliferative furan and thiophene triads **1**, RITA (*r*eactivation of p53 and *i*nduction of *t*umor cell *a*poptosis, **2**), and **3**.

With the goal of improving synthetic accessibility and enabling further SAR studies of heterocyclic triads such as terfuran **1**,[7,11,14] which provides hyperselective inhibition of exceptionally responding cells, we developed two alternative strategies to attach substituted heterocycles to a dihalogenated core. First, we utilized the Suzuki–Miyaura cross-coupling of commercially available furan boronic acid **5a** and readily accessible dibrominated heterocycles **4a–c** to prepare dialdehydes **6a–c** (Scheme 1). After screening several Pd(0)/ligand combinations, we found that $Pd_2(dba)_3$ and tri-*tert*-butylphosphine under microwave conditions provided the best yield of 51% for **6a**.[15] Other ligands such as JohnPhos, triisopropylphosphine, and tricyclohexylphosphine gave yields <25% of **6a**. We also attempted to achieve a monocoupling of **5a** in the presence of an excess of dibromide **4a** and ligand XPhos;[16] however, bis-coupling product **6a** remained the major

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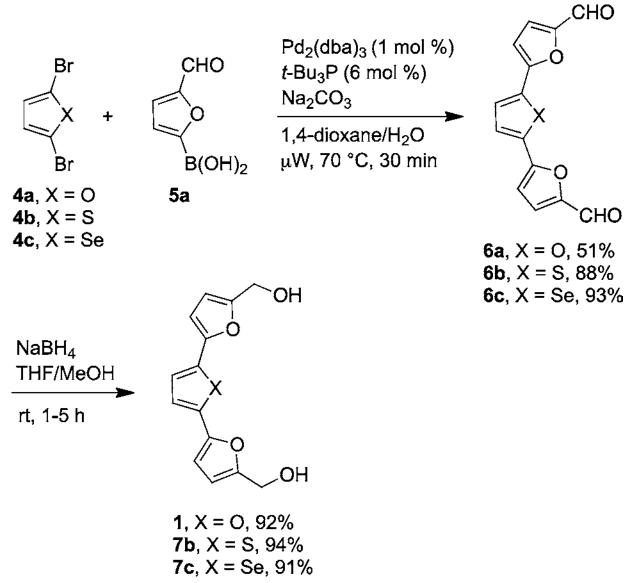
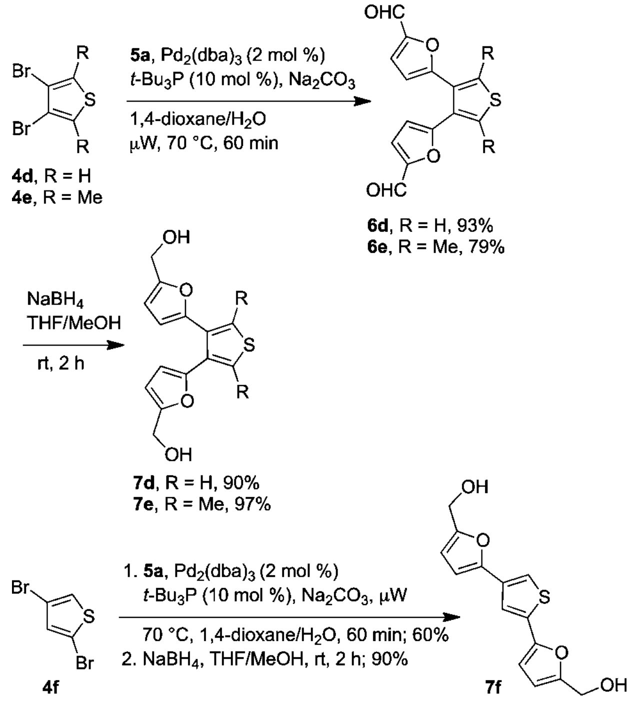
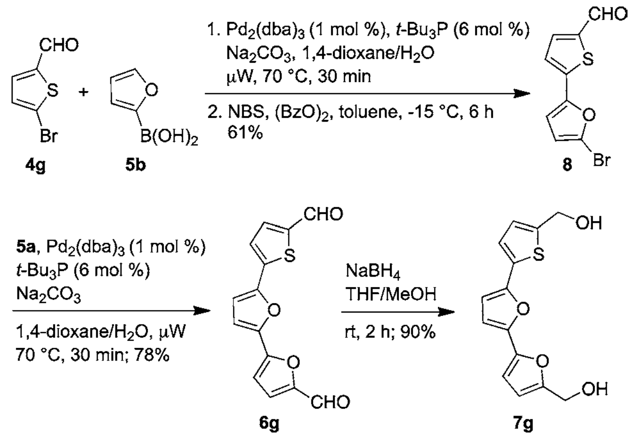
Scheme 1. Tandem Suzuki–Miyaura Cross-Coupling of 2,5-Dibrominated Furan, Thiophene, and Selenophene and Reduction to Diols



Scheme 2. Tandem Suzuki–Miyaura Cross-Coupling of 3,4- and 2,4-Dibrominated Thiophene and Reduction to Diols



Scheme 3. Sequential Suzuki–Miyaura Cross-Coupling of 5-Bromothiophene and Reduction to Diol



product. In comparison, $Pd(PPh_3)_4$ with tetra-*N*-butylammonium bromide as an additive mainly gave the monocoupled product.[17]

Bis-couplings at the 2,5-positions of dibrominated thiophene **4b** and selenophene **4c** were achieved in excellent yields under the conditions optimized for furan **4a** (Scheme 1). Interestingly, yellow precipitates of triads **6b** and **6c** formed at room temperature immediately after addition of the reaction components, and aldehyde **6b** was obtained in >70% yield after stirring overnight. These mild conditions can improve the scalability of the cross-coupling process. While the coupling proceeds well even at room temperature, microwave heating, greatly expedited the completion of the reaction without causing decomposition. Finally, reduction with $NaBH_4$ furnished the alcohols **1** and **7b**,**c** in excellent yields.

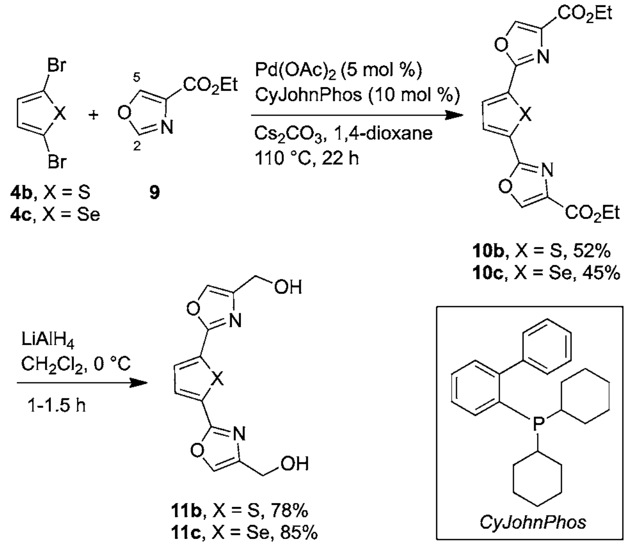
Regioisomeric thiophene analogues were obtained by bis-coupling of **5a** to 3,4-dibromothiophene (**4d**), 2,5-dimethyl-3,4-dibromothiophene (**4e**), and 2,4-dibromothiophene (**4f**) (Scheme 2). Due to the lower reactivity at C(3) of thiophene,[18] we increased catalyst loadings and equivalents of boronic acid **5a** in order to facilitate the formation of bis-coupled products. Finally, $NaBH_4$ reduction furnished the diols **7d**–**f**. NMR analysis of the reaction mixture of analogue **7d** showed signs of decomposition after mild heating at ca. 50 °C, presumably due to side reactions as a consequence of the high reactivity of the unsubstituted thiophene C(2) and C(5) positions.

Lead structure **1** and analogues **7b**–**f** could be rapidly assembled using these optimized Suzuki–Miyaura conditions, adding two identical heterocyclic end groups to the core structure in one cross-coupling step. Moreover, a related stepwise approach was readily identified that allowed a variation of the terminal heterocycles and access to desymmetrized products. Commercially available bromothiophene **4g** and furan boronic acid **5b** were combined at C(5) and C(2), respectively, and the furan C(5) position was subsequently brominated with *N*-bromosuccinimide and benzoyl peroxide[19] to yield aldehyde **8** in 61% yield over the two steps (Scheme 3). Cross-coupling of **8** with furan boronic acid **5a** led to the bisfuranylthiophene triad **6g** in 78% yield, and reduction of the two aldehyde functional groups with $NaBH_4$ provided diol **7g** in 90% yield.

A significant further diversification of the scaffold of the heterocyclic triads **1** and **7b**–**g** was envisioned by an isosteric replacement of the terminal furan and thiophene rings with oxazole moieties. However, while the requisite ethyl 2-iodo-4-oxazole carboxylate building block was readily prepared,[20] neither Suzuki–Miyaura nor Negishi cross-couplings provided more than trace amounts of the desired products.[21]

The challenge of functionalizing and coupling oxazoles[22] can be addressed more directly with a regioselective arylation of a C(2)-unsubstituted oxazole. Hoarau's group, for example, reported a successful arylation of both the C(2)- and C(5)-positions of commercially available **9** using palladium catalysis.[23] The electron-withdrawing nature of the ester group in **9** was found to be important for the reactivity of the oxazole, particularly for the activation of the C(2) carbon−hydrogen bond. While our attempts to couple the alcohol and aldehyde derivatives of **9** under these conditions were unsuccessful, we found that reactivity is also influenced by the position of the ester substituent on the oxazole ring. When we attempted a coupling with ethyl 5-oxazole carboxylate, only 5% of the bis-coupled product was isolated, reinforcing the apparent need for an electron-withdrawing group at C(4). We were pleased to find that **9** underwent a C(2)-selective tandem C−H bond activation/cross-coupling with both **4b** and **4c** under Hoarau's conditions to furnish esters **10b** and **10c** in satisfactory yields (Scheme 4). Finally, $LiAlH_4$ reduction provided access to the desired alcohols **11b** and **11c** in good yields.

Scheme 4. Sequential Pd-Catalyzed C−H Bond Activation/Cross Coupling of Oxazoles with Dibromothiophene and Dibromoselenophene and Reduction to Diol



Diols **1**, **2**, **7b−e**,**g**, and **11b**,**c** as well as aldehyde **6c** were evaluated in the NCI 60-cell line panel[24] for selective anticancer activity.[25] Table 1 shows the concentrations required to achieve 50% growth inhibition (GI_{50}) in the two most sensitive cancer cell lines.

With the exception of oxazole-containing analogues **11b** and **11c**, which provided no significant cell growth inhibition at 10 μM concentration, the heterocyclic triads were, in general, more toxic to the renal cell line A498 than other cell lines (Table 1). Of the tested compounds, those with the 3,4-substitution pattern on a central thiophene, i.e., **7d** and **7e**, were the most potent, in addition to the desymmetrized triad **7g**, which combined two 2,5-substituted furans with a 2,5-substituted thiophene terminus. The latter compound is remarkably more potent (10×) than its sequence isomer **7b**. Overall, the cellular toxicity profiles of **7d**, **7e**, and **7g** were similar to compound **2**. On the other hand, compounds **7b** and **7c**, similar to **1**, were less potent but more hyperselective in the NCI 60-cell line panel (data not shown). Aldehyde **6c** also showed a significant 20-fold drop off in activity from the most sensitive cell line (A498) to the next most sensitive one (MCF7).

Table 1. Growth Inhibition in Renal (A498), Lung (NCI-H226), Kidney (CAKI-1), and Breast (MDA-MB-468, MCF7) Cancer Cell Lines

compd	NSC no.	GI_{50}[a] (μM)	GI_{50}[b] (μM)
1	672348	0.18 (A498)	0.58 (NCI-H226)
2	652287	0.012 (CAKI-1)	0.015 (A498)
7b	773097	0.13 (A498)	0.17 (MDA-MB-468)
6c	773393	0.30 (A498)	6.3 (MCF7)
7c	773392	0.17 (A498)	0.22 (MDA-MB-468)
7d	777422	0.015 (A498)	0.074 (MDA-MB-468)
7e	778301	0.016 (MDA-MB-468)	0.018 (A498)
7g	777196	0.016 (A498)	0.018 (MDA-MB-468)

[a] Most sensitive cell line. [b] Next most sensitive cell line.

In conclusion, we have developed and optimized two complementary synthetic protocols for the preparation of the nine heterocyclic triads **1**, **7b−g**, and **11b**,**c**. First, we identified a catalytic Pd system for the direct Suzuki−Miyaura tandem cross-coupling of commercially available furan boronic acids with dibromofuran, -thiophene, and -selenophene. In a variation of this protocol, the sequential cross-coupling of a bromothiophene provided the desymmetrized triad **7g**. Second, we utilized a Pd-catalyzed C,H-bond activation at C(2) of oxazole ester **9** to assemble the bis-oxazole triads **11b** and **11c** from dibromothiophene **4b** and dibromoselenophene **4c** in good yields and high synthetic efficiency. Biological evaluation provided a significant extension of the SAR information available for the parent terfuran **1** and allows for an examination of the effects of heterocyclic sequence and connectivity patterns on cancer cell toxicity. Ongoing studies, including in vivo xenograft models, aim to determine if a broad spectrum pattern of high cellular activity (as demonstrated by **2**, **7d**, **7e**, and **7g**) or lower cellular activity paired with hyperselectivity (as demonstrated by **1**, **6c**, **7b**, and **7c**) are predictive of a greater therapeutic index in this series.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and spectral data for all new or previously incompletely characterized compounds, including copies of ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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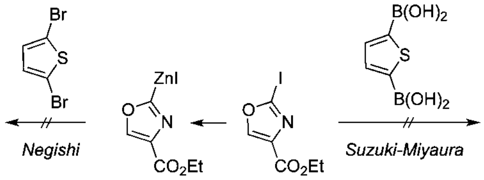
(21) Overview of attempted cross-couplings:



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(25) The biological evaluation of additional analogues, as well as in vivo studies, are pending and will be reported in due course.