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Facile synthesis of diazido-functionalized biaryl compounds as radioisotope-free photoaffinity probes by Suzuki–Miyaura coupling

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ABSTRACT

Suzuki–Miyaura coupling of 3-azido-5-(azidomethyl)phenylboronic acid pinacol ester with various aryl bromides affords corresponding diazido-functionalized biaryl compounds in good yields. This approach provides an easy access to radioisotope-free photoaffinity probes possessing biaryl structure. By using this method, we prepared a novel diazido-functionalized dantrolene analog, which showed selective inhibitory effect on physiological Ca^{2+} release (PCR) from sarcoplasmic reticulum (SR) in mouse skeletal muscle without affecting Ca^{2+}-induced Ca^{2+} release (CICR).

1. Introduction

Photoaffinity labeling is a useful technique to determine target biomolecules of a bioactive compound.[1] Usually, photoaffinity probe consist of two functional groups. One is a photoreactive group, which enables to form a new covalent bond between the probe and its target molecule by photoreaction. Another one is a detectable tag to distinguish the photolabeled molecule from unlabeled ones. On the occasion of preparation of a photoaffinity probe, these functional groups are required to be introduced to the original compound without diminishing its bioactivity to obtain a reliable result. Representative photoreactive groups are aromatic azido, diazirinyl, and benzophenone derivatives, which generate highly reactive species such as nitrene, carbene, and excited carbonyl, respectively, by irradiation of light. The most widely used indicator is a radioisotope (RI) such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, and ^{125}I. The use of biotin-anchored probe, photoaffinity biotinylation,[2] is an excellent alternative, which can avoid the use of hazardous RIs. In this manner, biotin unit is employed as a detectable tag taking advantage of its extremely high affinity toward avidin, thereby allowing direct analysis or purification of photolabeled biomolecules.

We previously established a different protocol for RI-free photoaffinity labeling.[3] In this scheme, an alkyl azido group, which was proved to be relatively photostable under the photoreactive conditions of aryl azide and trifluoromethyldiazirine, was used as a post-modifiable group to introduce an arbitrary detectable tag such as a fluorescent unit. This method is based upon the bioorthogonal character of azido group as well as some efficient azido-targeting reactions such as Staudinger–Bertozzi ligation[4] and Huisgen 1,3-dipolar cycloaddition, click chemistry.[5,6] Our method only requires an introduction of an azidomethyl group to the probe with low polarity and small in size, which minimizes the adverse effects on the intrinsic biological activity and selectivity of the original compound. Indeed, the effectiveness of this approach was demonstrated by the fluorescent detection of photolabeled HMG-CoA reductase using a diazido-functionalized cerivastatin derivative, photovastatin CAA1 (**1**) (Fig. 1).[3a] We also prepared a diazidobenzylated dantrolene derivative, GIF-0430 (**2**), which exhibit a selective inhibitory effect on the physiological Ca^{2+} release (PCR) process involved in the contraction of skeletal muscle, and succeeded in the fluorescent detection of its target proteins.[3b] In both studies, 3-azido-5-(azidomethyl)benzyl derivatives were used as efficient photoaffinity probes (Fig. 1). These probes were designed that the all three substituents on the benzene ring to be placed in *meta*-positions in relation to each other to avoid unnecessary interactions.

In order to prepare these type of probes, especially those with biaryl structure, more straightforwardly, we conceived

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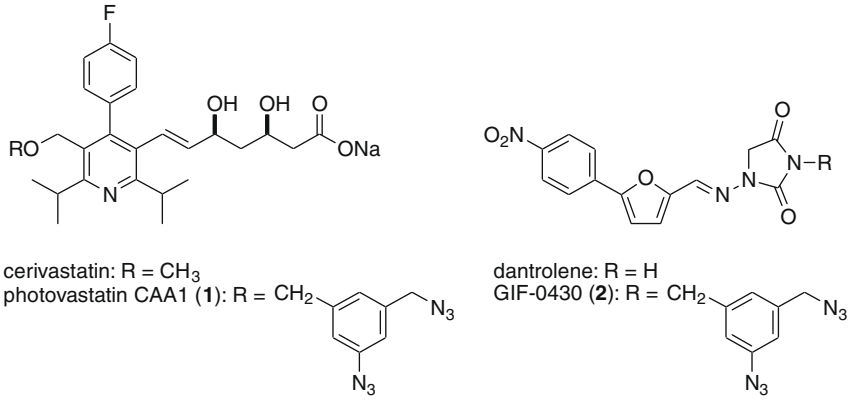


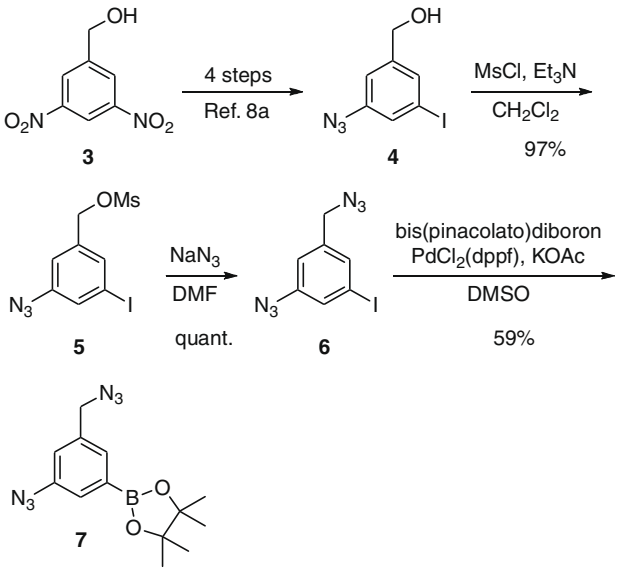
Figure 1. Structures of cerivastatin, dantrolene, and their corresponding RI-free photoaffinity probes.

the idea of introducing 3-azido-5-(azidomethyl)benzene unit by aryl–aryl coupling reaction. In this article, we describe the facile approach using Suzuki–Miyaura cross-coupling,[7] a well-established reaction to prepare diverse biaryl compounds, and its application to the synthesis of a novel diazido-functionalized dantrolene analog as an RI-free photoaffinity probe with specific bioactivity.

2. Results and discussion

2.1. Synthesis of the common intermediate

We prepared 3-azido-5-(azidomethyl)phenylboronic acid pinacol ester (**7**) as the common intermediate for Suzuki–Miyaura coupling (Scheme 1). Firstly, 3-azido-5-iodobenzyl alcohol (**4**) was prepared in four steps from commercially available 3,5-dinitrobenzyl alcohol (**3**) as we reported previously.[8a] The mesylation of **4** followed by treatment with sodium azide afforded diazido-functionalized iodobenzene derivative **6**. The Pd(0)-catalyzed coupling reaction of bis(pinacolato)diboron under standard condition[9] with **6** gave desired **7**.



Scheme 1. Synthesis of common intermediate **7** for Suzuki–Miyaura coupling.

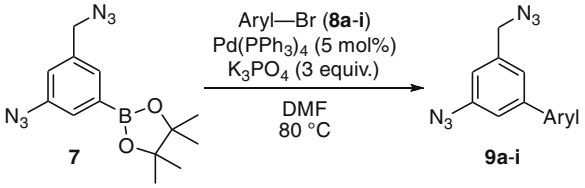
2.2. Suzuki–Miyaura coupling

With a common intermediate **7** in hand, we examined the cross-coupling reaction with various aryl bromides. First of all, the conventional reaction conditions for Suzuki–Miyaura coupling was applied to the reaction of boron reagent **7** with the most simple aryl bromide, bromobenzene (**8a**). Namely, the mixture of **8a** and **7** (1.2 equiv) in the presence of 5 mol% $Pd(PPh_3)_4$ and K_3PO_4 (3 equiv) in DMF was heated at 80 °C under nitrogen atmosphere. The reaction for 3 h gave the desired coupling product in 70% isolated yield (Table 1, entry 1). Under this condition, no side products such as compounds arose from reduction of azido groups were obtained. The reaction with iodobenzene slightly increased the yield to 80%. All other reactions examined using various heteroaryl bromide also gave desired coupling products in good yields (Table 1). For instance, the reaction with pyridyl (entry 2), pyrimidyl (entry 3), thienyl (entry 4), isoquinolinyl (entry 5), and indolyl (entries 6 and 7) substrates, which are typical heteroaromatic compounds often included in bioactive drug's structure, have proceeded smoothly. The reaction was not retarded in the presence of formyl or phenolic hydroxy groups (entries 4 and 8). The reaction for substrate **8i**, which did not proceed under the standard condition, was found to undergo efficiently by using a different phosphine ligand such as PCy_3 (entry 9).

2.3. Design and synthesis of a novel diazido-functionalized dantrolene analog

Dantrolene is a hydantoin derivative, which is known as a muscle relaxant used as an only drug for malignant hyperthermia.[10,11] In skeletal muscle, dantrolene inhibits two kinds of Ca^{2+} release from sarcoplasmic reticulum (SR): physiological Ca^{2+} release (PCR) and Ca^{2+}-induced Ca^{2+} release (CICR). Although the physiological role of the latter mode of Ca^{2+} release is not well understood, PCR from SR in skeletal muscle is controlled by a Ca^{2+}-releasing channel on the SR membrane, which is called skeletal-type ryanodine receptor (RyR1), whose function is regulated by a signal from the dihydropyridine receptor (DHPR), a voltage sensor in the cell membrane.[12] Although there is an established consensus that DHPR and RyR1 locate face-to-face together in the triad junction, the question of whether the signal from DHPR is transmitted to RyR1 directly or indirectly via undetermined molecules, is still controversial.[13,14] In order to elucidate the molecular mechanism involved in the Ca^{2+}-related processes of skeletal muscle, we have been developing chemical probes based on the structural modification of dantrolene.[3b,8] Of these, the RI-labeled photoaffinity probe, [^{125}I]GIF-0082 ([^{125}I]-**10**), whose structure include azidobenzylated unit showed highly selective inhibitory effect on PCR without

Table 1
Synthesis of diazido-functionalized biaryl compounds by Suzuki–Miyaura coupling



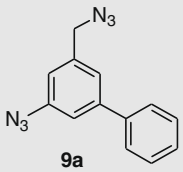
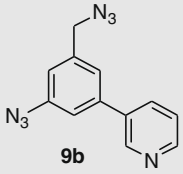
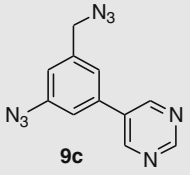
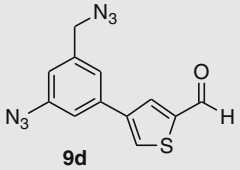
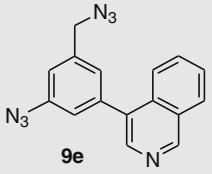
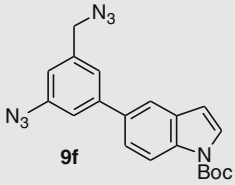
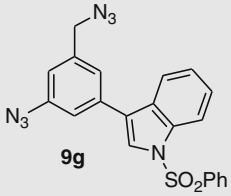
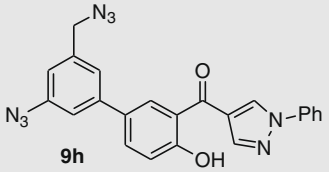
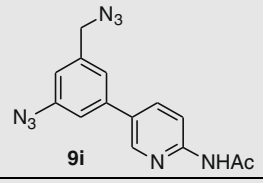
Entry	9	Time (h)	Yield (%)
1	 9a	3	70 (80)[a]
2	 9b	3	85
3	 9c	4	82
4	 9d	3.5	75
5	 9e	3.5	75
6	 9f	4	81
7	 9g	3	81
8	 9h	3	83

Table 1 (*continued*)

Entry	9	Time (h)	Yield (%)
9	 9i	20	90[b]

[a] Reaction with iodobenzene.
[b] Reaction conditions: 2 mol% $Pd_2(dba)_3$, PCy_3 (0.04 equiv), K_3PO_4 (3 equiv), 1,4-dioxane–H_2O, 100 °C.

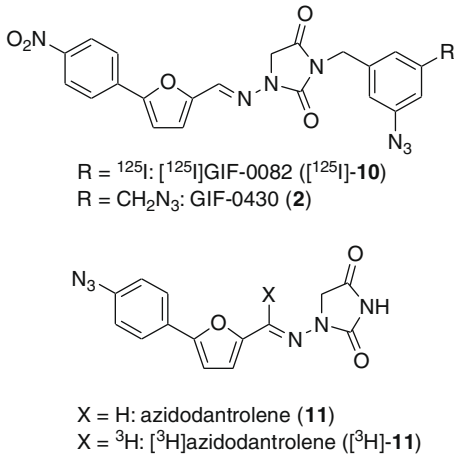
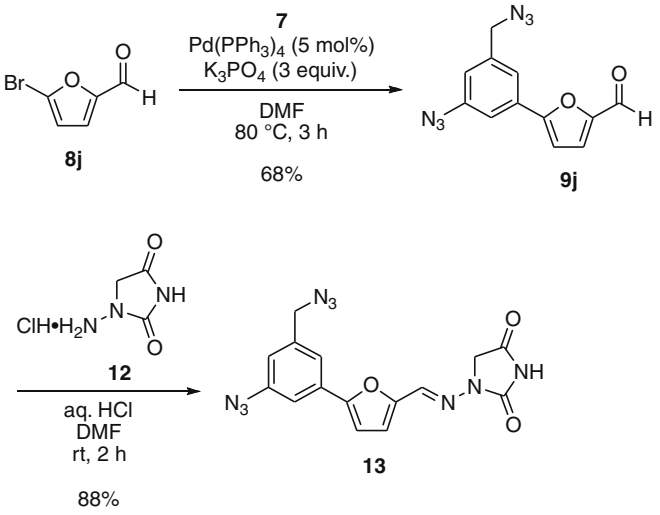


Figure 2. Photoaffinity probes based on dantrolene.

affecting CICR (Fig. 2).[8a,c] By using this selective inhibitor, we succeeded in the photolabeling of target protein candidates and characterized the primary structure of one target protein purified from an independent muscle preparation using molecular weight stratification by SDS–PAGE analysis. To make the direct analyses of photolabeled proteins possible, we prepared GIF-0430 (**2**), where the ^{125}I group of [^{125}I]-**10** is switched to an azidomethyl group as a latent detectable group.[3b] Compound **2** also showed selective PCR inhibitory effect and photolabeling experiment using this probe enabled fluorescent detection of target proteins, which showed good agreement with the result where RI-probe [^{125}I]-**10** was employed. Unexpectedly, the RyR1, which exhibits higher M_r value (>350,000), was not photolabeled by neither RI-probe [^{125}I]-**10** nor RI-free probe **2**. This is in marked disagreement with previous reports using tritium labeled azido congener of dantrolene, which is referred to as [^{3}H]azidodantrolene ([^{3}H]-**11**) (Fig. 2).[15] Although the reason for the discrepancy is presently unclear, it may be attributed to the lack of an inhibitory effect of [^{125}I]-**10** and **2** on the CICR mechanism.[3b,8a,c] Another possible reason is the difference of the position of photoreactive azido groups between our probes, [^{125}I]-**10** and **2**, and [^{3}H]-**11**.

To make these discrepancies clearer, we were interested in preparing the dantrolene analog such as **13**, which is a diazido congener of dantrolene. Because **13** has non-N-substituted hydantoin moiety unlike [^{125}I]-**10** and **2**, it is expected to own similar polarity with dantrolene and [^{3}H]-**11**. Compound **13** also possesses photoreactive azido group on the phenyl ring same as [^{3}H]-**11**. Therefore we anticipated that **13** could be used as a different type of RI-free photoaffinity probe instead of **2**. The compound **13** with small substituents on two *meta*-positions of the benzene ring was designed also because this type of compound was expected to show selective inhibitory effect on PCR. In fact, we previously have shown



Scheme 2. Synthesis of diazido-functionalized dantrolene analog **13**.

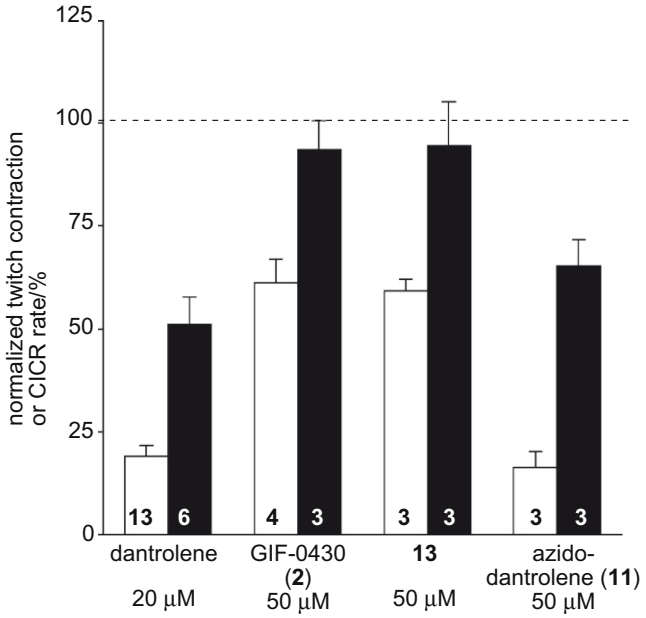


Figure 3. Effects of dantrolene, GIF-0430 (**2**), **13**, and azidodantrolene (**11**) on twitch contraction (open column) and CICR rate (filled column) of mouse skeletal muscle. For the methods of biological evaluation, see Ref. 8. The number of experiments (n) are indicated in the column. The data of dantrolene and GIF-0430 (**2**) are cited from Refs. 8a, and 3b, respectively.

that both 3-nitro and 3-methoxy congeners of dantrolene are highly selective inhibitor for PCR.[8d]

As shown in Scheme 2, the compound **13** could be easily prepared by applying above-mentioned synthetic method of diazido-functionalized biaryl compounds. Thus, Suzuki–Miyaura coupling of commercially available 5-bromo-2-furaldehyde (**8j**) with diazido boronic acid pinacol ester **7** afforded desired coupling product **9j** in 68% yield.[16] Finally, treatment of furaldehyde **9j** with 1-aminohydantoin hydrochloride (**12**) under acidic condition furnished dantrolene analog **13**.

2.4. Evaluation of effects on Ca^{2+} release

We examined the effect of diazido-functionalized dantrolene analog **13** on two kinds of Ca^{2+} release from SR of mouse skeletal muscle, PCR and CICR, according to the methods we previously reported.[8] The effects on PCR were estimated by comparing the tension of twitch contraction of intact mouse skeletal muscle at room temperature before and after treatment with the analogs.[8] The effects on CICR were evaluated at room temperature by measuring the rates of CICR in saponin-treated skinned muscle fibers of mouse skeletal muscle by using Fura-2 as a Ca^{2+} indicator under Mg^{2+}-free conditions at 1 μM Ca^{2+}.[8] For comparison, we also prepared azidodantrolene (**11**)[15a] and evaluated its effect on PCR and CICR. The effects of these compounds including those of dantrolene and GIF-0430 (**2**) are shown in Figure 3 with normalized values. As can be seen, compound **13** showed moderate but selective inhibitory effect on PCR with almost no effect on CICR, which is comparable to GIF-0430 (**2**). On the other hand, 50 μM dose of azidodantrolene (**11**) inhibited both PCR and CICR significantly to a similar extent of that of 20 μM dose of dantrolene. This result indicates that the novel probe **13** can be used as promising photoaffinity probe to elucidate selectively the target protein involved in the PCR mechanism and possibly can make some contribution to clarify the mechanism of action of dantrolene on Ca^{2+} releasing processes in skeletal muscle cells.

3. Conclusion

In summary, we have established a simple method to prepare diazido-functionalized biaryl compounds by Suzuki–Miyaura coupling. This method would allow rapid access to RI-free photoaffinity probes of bioactive compounds especially with phenyl–heteroaryl structure, which are commonly-seen in drug-like molecules. Indeed, by using this method, we prepared a novel diazido-functionalized dantrolene analog **13**, which showed selective inhibitory effect on PCR from SR in mouse skeletal muscle without affecting CICR. Actual photolabeling studies using **13** along with design and synthesis of different kinds of RI-free photoaffinity probes based on this strategy are currently in progress.

4. Experimental

4.1. Chemistry

General: All chemical reagents were used as received. Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm) silica-gel plates (MERCK, Silica Gel 60 F_{254}, Cat. No. 1.05715). Column chromatography was conducted using silica-gel (Kanto Chemical Co., Inc., Silica Gel 60 N, spherical neutral, mesh 40–50 μm, Cat. No. 37563-85). Melting points (mp) were measured on a YANACO MP-J3 instrument and are uncorrected. ^1H and ^{13}C NMR were obtained with Varian UNITY Plus 400 or Varian MERCURY 300 spectrometers. $CDCl_3$ (CIL) and DMSO-d_6 (CIL) were used as solvents for obtaining NMR spectra. Chemical shifts (δ) are given in parts per million (ppm) downfield from $(CH_3)_4Si$ (δ 0.00 for ^1H NMR in $CDCl_3$) or solvents (δ 7.27 and 2.49 for ^1H NMR in $CDCl_3$ and DMSO-d_6, respectively, and δ 77.0 and 39.5 for ^{13}C NMR in $CDCl_3$ and DMSO-d_6, respectively), as internal references with coupling constants (J) in hertz (Hz). The abbreviations s, d, m, and br signify singlet, doublet, multiplet, and broad, respectively. IR spectra were measured by diffuse reflectance method on a Shimadzu IRPrestige-21 spectrometer attached with DRS-8000A with the absorption band given in cm^{-1}. Elemental analyses were performed with a YANACO CHN CORDER MT-5 at the Center for Advanced Materials Analysis (Suzukakedai), Technical Department, Tokyo Institute of Technology. Mass spectra (MS) and high-resolution mass spectra (HRMS) were measured on a JEOL JMS-700 mass spectrometer under electron impact ionization (EI) or positive fast atom bombardment (FAB^+) conditions at the Center

for Advanced Materials Analysis (Suzukakedai), Technical Department, Tokyo Institute of Technology.

Caution: Azido-containing compounds are presumed to be potentially explosive. Although we have never experienced such an explosion with azido-functionalized compounds used in this study, all manipulations should be carefully carried out behind a safety shield in a hood.

4.1.1. 3-Azido-5-iodobenzyl methanesulfonate (5)

To a solution of 3-azido-5-iodobenzyl alcohol (**4**)[8a] (2.69 g, 9.78 mmol) in CH_2Cl_2 (80 mL) was successively added triethylamine (3.40 mL, 24.4 mmol) and methanesulfonyl chloride (1.50 mL, 19.4 mmol) at 0 °C. After stirring for 45 min at the same temperature, to the mixture was added water and the mixture was extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with brine (×1), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 2/1) to give **5** (3.35 g, 97.0%) as a pale yellow solid; mp 77–79 °C; TLC R_f = 0.32 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.02 (s, 3H), 5.14 (s, 2H), 7.01 (dd, 1H, *J* = 1.6, 2.0 Hz), 7.39 (dd, 1H, *J* = 1.6, 2.0 Hz), 7.51 (dd, 1H, *J* = 1.6, 1.6 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 38.2, 68.9, 94.8, 118.3, 128.4, 133.5, 137.0, 141.9; IR (KBr, cm^{-1}) 513, 530, 698, 737, 754, 804, 837, 864, 907, 930, 955, 970, 991, 1173, 1213, 1263, 1298, 1337, 1381, 1439, 1572, 1593, 2118, 3026, 3406; HRMS (EI) *m*/*z* 352.9340 (M^+, $C_8H_8IN_3O_3S$ requires 352.9331).

4.1.2. 1-Azido-3-azidomethyl-5-iodobenzene (6)

To a solution of **5** (2.85 g, 8.07 mmol) in DMF (20 mL) was added sodium azide (2.10 g, 32.3 mmol) at room temperature and the mixture was stirred for 1.5 h at the same temperature. To this was added water and the mixture was extracted with EtOAc (×3). The combined organic extracts were successively washed with water (×3) and brine (×1), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 15/1) to give **6** (2.41 g, 99.5%) as a pale yellow oil; TLC R_f = 0.78 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.31 (s, 2H), 6.93 (dd, 1H, *J* = 1.5, 2.0 Hz), 7.34 (dd, 1H, *J* = 1.5, 2.0 Hz), 7.43 (dd, 1H, *J* = 1.5, 1.5 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 53.4, 94.8, 117.9, 127.6, 133.2, 139.1, 141.8; IR (KBr, cm^{-1}) 675, 698, 814, 845, 1202, 1246, 1292, 1339, 1433, 1442, 1560, 1595, 2100, 2328, 2359, 3065, 3370; HRMS (EI) *m*/*z* 299.9618 (M^+, $C_7H_5IN_6$ requires 299.9620).

4.1.3. 2-[3-Azido-5-(azidomethyl)phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (7)

Under N_2 atmosphere, to a solution of **6** (2.68 g, 8.93 mmol) in DMSO (15 mL) were successively added bis(pinacolato)diboron (3.17 g, 12.5 mmol), potassium acetate (2.45 g, 25.0 mmol), and $PdCl_2$(dppf)·CH_2Cl_2 (221 mg, 271 μmol) at room temperature. The mixture was heated at 80 °C and stirred for 4.5 h. After cooling to room temperature, to the mixture was added 1 M aqueous HCl solution and extracted with EtOAc (×3). The combined organic extracts were successively washed with saturated aqueous $NaHCO_3$ solution (×1), water (×3) and brine (×2), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 20/1) to give **7** (1.59 g, 59.3%) as a pale yellow solid; mp 46–48 °C; TLC R_f = 0.77 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 12H), 4.35 (s, 2H), 7.05 (dd, 1H, *J* = 1.8, 2.2 Hz), 7.45 (dd, 1H, *J* = 0.4, 2.2 Hz), 7.51 (dd, 1H, *J* = 0.4, 1.8 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.8 (4C), 54.1, 84.2 (2C), 121.4, 124.6, 130.7, 136.7, 140.2 (the carbon adjacent to boron was not observed); IR (KBr, cm^{-1}) 706, 735, 851, 905, 968, 1113, 1144, 1165, 1206, 1288, 1308, 1339, 1369, 1429, 1470, 1587, 2108, 2932, 2980, 3431; HRMS (EI) *m*/*z* 300.1508 (M^+, $C_{13}H_{17}BN_6O_2$ requires 300.1506).

4.1.4. 3-Azido-5-(azidomethyl)biphenyl (9a)

4.1.4.1. A representative procedure for Suzuki–Miyaura coupling. Under N_2 atmosphere, to a solution of **7** (117 mg, 390 μmol) in DMF (3.5 mL) was successively added bromobenzene (**8a**) (51.0 mg, 325 μmol), $Pd(PPh_3)_4$ (17.4 mg, 15.1 μmol), and potassium phosphate *n*-hydrate (191 mg, 900 μmol) at room temperature. The mixture was heated at 80 °C and stirred for 3 h. After cooling to room temperature, to the mixture was added 2 M aqueous HCl solution and extracted with EtOAc (3). The combined organic extracts were successively washed with saturated aqueous $NaHCO_3$ solution (×1), water (×3), and brine (×1), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 15/1) to give **9a** (57.1 mg, 70.2%) as a pale yellow oil; TLC R_f = 0.75 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.41 (s, 2H), 6.96 (dd, 1H, *J* = 1.4, 1.4 Hz), 7.20 (dd, 1H, *J* = 1.4, 1.7 Hz), 7.30 (dd, 1H, *J* = 1.4, 1.7 Hz), 7.37–7.59 (m, 5H); IR (KBr, cm^{-1}) 696, 725, 762, 851, 1261, 1325, 1346, 1423, 1454, 1497, 1576, 1595, 2100, 2926, 3036, 3061; MS (EI) *m*/*z* 250.

4.1.5. 3-[3-Azido-5-(azidomethyl)phenyl]pyridine (9b)

Colorless oil; TLC R_f = 0.25 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.44 (s, 2H), 7.03 (dd, 1H, *J* = 1.5, 1.7 Hz), 7.18 (dd, 1H, *J* = 1.7, 1.7 Hz), 7.26 (dd, 1H, *J* = 1.5, 1.7 Hz), 7.40 (ddd, 1H, *J* = 0.9, 4.9, 7.9 Hz), 7.86 (ddd, 1H, *J* = 1.5, 2.3, 7.9 Hz), 8.64 (dd, 1H, *J* = 1.5, 4.9 Hz), 8.83 (dd, 1H, *J* = 0.9, 2.3 Hz); IR (KBr, cm^{-1}) 712, 806, 851, 1022, 1184, 1261, 1327, 1346, 1402, 1445, 1485, 1595, 2104; MS (EI) *m*/*z* 251.

4.1.6. 5-[3-Azido-5-(azidomethyl)phenyl]pyrimidine (9c)

Colorless solid; TLC R_f = 0.13 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.47 (s, 2H), 7.10 (dd, 1H, *J* = 1.6, 1.6 Hz), 7.17 (dd, 1H, *J* = 1.6, 1.6 Hz), 7.29 (dd, 1H, *J* = 1.6, 1.6 Hz), 8.95 (s, 2H), 9.26 (s,1H); IR (KBr, cm^{-1}) 681, 692, 725, 854, 1188, 1261, 1356, 1408, 1557, 1607, 2110; MS (EI) *m*/*z* 252.

4.1.7. 4-[3-Azido-5-(azidomethyl)phenyl]thiophene-2-carboxaldehyde (9d)

Pale yellow solid; TLC R_f = 0.60 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.42 (s, 2H), 6.98 (dd, 1H, *J* = 1.5, 2.0 Hz), 7.18 (dd, 1H, *J* = 2.0, 2.0 Hz), 7.31 (dd, 1H, *J* = 1.5, 2.0 Hz), 7.90 (dd, 1H, *J* = 1.4, 1.4 Hz), 8.02 (d, 1H, *J* = 1.4 Hz), 9.90 (d, 1H, *J* = 1.4 Hz); IR (KBr, cm^{-1}) 665, 849, 1188, 1221, 1248, 1302, 1339, 1354, 1425, 1541, 1595, 1670, 2108; MS (EI) *m*/*z* 284.

4.1.8. 4-[3-Azido-5-(azidomethyl)phenyl]isoquinoline (9e)

Pale yellow solid; TLC R_f = 0.53 (*n*-hexane/EtOAc = 2/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46 (s, 2H), 7.11 (dd, 1H, *J* = 1.5, 1.7 Hz), 7.15 (dd, 1H, *J* = 1.7, 1.7 Hz), 7.24 (dd, 1H, *J* = 1.5, 1.7 Hz), 7.67 (ddd, 1H, *J* = 1.2, 6.8, 8.2 Hz), 7.72 (ddd, 1H, *J* = 1.6, 6.8, 8.2 Hz), 7.85 (dd, 1H, *J* = 1.2, 8.2 Hz), 8.07 (dd, 1H, *J* = 1.6, 8.2 Hz), 8.47 (s, 1H), 9.29 (s, 1H); IR (KBr, cm^{-1}) 700, 754, 785, 856, 893, 1209, 1223, 1254, 1275, 1308, 1331, 1344, 1391, 1439, 1591, 2106; MS (EI) *m*/*z* 301.

4.1.9. 5-[3-Azido-5-(azidomethyl)phenyl]-1-*tert*-butoxycarbonyl-1*H*-indole (9f)

Pale yellow oil; TLC R_f = 0.80 (*n*-hexane/CH_2Cl_2 = 3/2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.69 (s, 9H), 4.42 (s, 2H), 6.63 (d, 1H, *J* = 3.5 Hz), 6.95 (dd, 1H, *J* = 1.6, 1.6 Hz), 7.25 (dd, 1H, *J* = 1.6, 1.6 Hz), 7.35 (dd, 1H, *J* = 1.6, 1.6 Hz), 7.52 (dd, 1H, *J* = 1.7, 8.8 Hz), 7.64 (d, 1H, *J* = 3.5 Hz), 7.75 (d, 1H, *J* = 1.7 Hz), 8.21 (d, 1H,

J = 8.8 Hz); IR (KBr, cm^{-1}) 729, 766, 818, 854, 1024, 1086, 1138, 1157, 1240, 1282, 1321, 1340, 1368, 1449, 1479, 1593, 1603, 1732, 2106, 2978; MS (EI) m/z 389.

4.1.10. 3-[3-Azido-5-(azidomethyl)phenyl]-1-(phenylsulfonyl)-1*H*-indole (9g)

Pale yellow solid; TLC R_f = 0.50 (n-hexane/EtOAc = 3/1); ^1H NMR (400 MHz, $CDCl_3$) δ 4.43 (s, 2H), 7.00 (dd, 1H, J = 1.7, 2.0 Hz), 7.22 (dd, 1H, J = 1.7, 1.7 Hz), 7.31–7.36 (m, 2H), 7.41 (ddd, 1H, J = 0.9, 7.2, 8.2 Hz), 7.46–7.51 (m, 2H), 7.55–7.60 (m, 1H), 7.75 (ddd, 1H, J = 0.9, 0.9, 8.2 Hz), 7.75 (s, 1H), 7.94–7.98 (m, 2H), 8.08 (ddd, 1H, J = 0.9, 0.9, 8.2 Hz); IR (KBr, cm^{-1}) 571, 592, 685, 729, 746, 972, 1090, 1111, 1134, 1177, 1242, 1277, 1292, 1310, 1371, 1447, 1593, 1607, 2106; MS (EI) m/z 429.

4.1.11. 5-[3-Azido-5-(azidomethyl)phenyl]-2-hydroxyphenyl 1-phenyl-1*H*-pyrazol-4-yl ketone (9h)

Yellow solid; TLC R_f = 0.53 (n-hexane/EtOAc = 3/1); ^1H NMR (400 MHz, $CDCl_3$) δ 4.42 (s, 2H), 6.97 (dd, 1H, J = 1.5, 2.0 Hz), 7.12 (dd, 1H, J = 1.5, 2.0 Hz), 7.18 (dd, 1H, J = 0.4, 8.6 Hz), 7.24 (dd, 1H, J = 1.5, 1.5 Hz), 7.38–7.43 (m, 1H), 7.49–7.55 (m, 2H), 7.72–7.78 (m, 3H), 8.08 (dd, 1H, J = 0.4, 2.3 Hz), 8.21 (d, 1H, J = 0.7 Hz), 8.53 (d, 1H, J = 0.7 Hz), 12.03 (s, 1H); IR (KBr, cm^{-1}) 758, 835, 953, 1221, 1238, 1288, 1308, 1329, 1348, 1404, 1447, 1460, 1491, 1504, 1541, 1595, 1626, 2104; MS (EI) m/z 436.

4.1.12. 2-Acetamino-5-[3-azido-5-(azidomethyl)phenyl] pyridine (9i)

Yellow solid; TLC R_f = 0.27 (n-hexane/EtOAc = 3/2); ^1H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H), 4.43 (s, 2H), 7.00 (dd, 1H, J = 1.5, 1.8 Hz), 7.14 (dd, 1H, J = 1.5, 1.8 Hz), 7.26 (dd, 1H, J = 1.8, 1.8 Hz), 7.89 (dd, 1H, J = 2.4, 8.7 Hz), 8.17 (s, 1H), 8.29 (dd, 1H, J = 0.6, 8.7 Hz), 8.47 (dd, 1H, J = 0.6, 2.4 Hz); IR (KBr, cm^{-1}) 839, 1260, 1308, 1321, 1375, 1452, 1543, 1593, 1607, 1701, 2110, 3021, 3067, 3244; MS (EI) m/z 308.

4.1.13. 5-[3-Azido-5-(azidomethyl)phenyl]-2-furaldehyde (9j)

Orange solid; TLC R_f = 0.31 (n-hexane/EtOAc = 3/1); ^1H NMR (400 MHz, $CDCl_3$) δ 4.43 (s, 2H), 6.90 (d, 1H, J = 3.7 Hz), 7.02 (dd, 1H, J = 1.6, 1.6 Hz), 7.34 (d, 1H, J = 3.7 Hz), 7.41 (dd, 1H, J = 1.6, 1.6 Hz), 7.54 (dd, 1H, J = 1.6, 1.6 Hz), 9.69 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 53.9, 108.8, 115.2, 119.2, 121.0, 123.2, 131.0, 138.4, 141.6, 152.2, 157.4, 177.3; IR (KBr, cm^{-1}) 764, 800, 856, 966, 1030, 1252, 1281, 1315, 1342, 1389, 1441, 1516, 1568, 1599, 1674, 2108; MS (EI) m/z 268; HRMS (FAB^+) m/z 269.0789 ($[M+H]^+$, $C_{12}H_9N_6O_2$ requires 269.0787).

4.1.14. ({5-[3-Azido-5-(azidomethyl)phenyl]furfurylidene} amino)imidazolidine-2,4-dione (11)

A solution of 1-aminohydantoin hydrochloride (**12**) (42.7 mg, 282 μmol) in 2.0 M aqueous HCl (0.5 mL, 1.0 mmol) was added to a solution of **9j** (50.4 mg, 188 μmol) in DMF (1.5 mL) at 0 °C and the mixture was stirred for 2 h at room temperature. To this was added water (ca. 10 mL) and the precipitate formed was filtered and washed well with water on a funnel. The collected solid was dried under reduced pressure to give **11** (60.5 mg, 88.1%) as a slightly yellow solid; TLC R_f = 0.42 (n-hexane/EtOAc = 1/2); ^1H NMR (400 MHz, DMSO-d_6) δ 4.43 (s, 2H), 4.56 (s, 2H), 6.97 (d, 1H, J = 3.6 Hz), 7.12 (br s, 1H), 7.28 (d, 1H, J = 3.6 Hz), 7.43 (br s, 1H), 7.57 (br s, 1H), 7.74 (s, 1H), 11.29 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6 + $CDCl_3$) δ 48.7, 52.9, 109.4, 113.7, 114.9, 117.9, 120.0, 131.4, 132.9, 138.4, 140.6, 149.5, 153.0, 153.2, 168.4; IR (KBr, cm^{-1}) 440, 465, 606, 687, 741, 779, 810, 856, 912, 949, 972, 1022, 1121, 1215, 1229, 1246, 1283, 1319, 1344, 1377, 1406, 1445, 1518, 1558, 1603, 1618, 1726, 1782, 1819, 2108, 3064, 3169; HRMS (FAB^+) m/z 366.1060 ($[M+H]^+$, $C_{15}H_{12}N_9O_3$ requires 366.1063).

5. Biological evaluation

See Ref. 8 and references cited therein.

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16. Compound **9j** was also obtainable by Stille coupling of 5-(tri-*n*-butylstannyl)-2-furaldehyde and 1-azido-3-azidomethyl-5-iodobenzene (**6**) in 84% yield (5 mol% $(Ph_3P)_2PdCl_2$/DME, 70 °C, 5 h), which is a general synthetic method of dantrolene analogs we previously reported. See Ref. 8d.