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[^{125}I]-*N*-[(3-Azido-5-iodo)benzyl]dantrolene and [^{125}I]-*N*-{[3-Iodo-5-(3-trifluoromethyl-3*H*-diazirin-3-yl)]benzyl}dantrolene: Photoaffinity Probes Specific for the Physiological Ca^{2+} Release from Sarcoplasmic Reticulum of Skeletal Muscle

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Abstract—In order to capture and identify key molecules that regulate the release of Ca^{2+} from the sarcoplasmic reticulum (SR) of skeletal muscle, we designed specific photoaffinity probes based on the structural modification of dantrolene. Thus, GIF-0082 and GIF-0276 possessing azido- and trifluoromethyldiazirinyl-benzyl groups, respectively, at the hydantoin moiety were found to have a highly selective inhibitory effect on physiological Ca^{2+} release (PCR) without affecting Ca^{2+}-induced Ca^{2+} release (CICR). Successful realization of the sharp discrimination between PCR and CICR has led to the creation of [^{125}I]GIF-0082 and [^{125}I]GIF-0276, which were synthesized by substituting a stannyl group with ^{125}I in the corresponding phenylstannane precursors.

Muscle contraction is provoked by the release of Ca^{2+} into the cytoplasm from sarcoplasmic reticulum (SR), the intracellular Ca^{2+} store.[1] In skeletal muscle, physiological Ca^{2+} release (PCR) from SR is controlled by the ryanodine receptor (RyR1), a Ca^{2+}-releasing channel on the SR membrane, whose function is directed by the signal from the dihydropyridine receptor (DHPR), a voltage sensor in the cell membrane.[1,2] However, the precise molecular mechanism by which Ca^{2+} is released, in particular the question of whether the signal from DHPR is transmitted to RyR1 directly or via undetermined molecules, remains unclear.[3,4] The possibility that proteins may act as regulatory molecules in such a bioprocess is still under debate.[3,4] RyR1 not only plays a role as a PCR channel but also can facilitate Ca^{2+}-induced Ca^{2+} release (CICR), although the physiological significance of the CICR process is not clear in skeletal muscle.[2,3] Accordingly, an agent which acts specifically on PCR or CICR would be useful to elucidate the process by which Ca^{2+} is released. Therefore, we have designed specific photoaffinity probes[5] capable of discriminating PCR from CICR by the structural modification of dantrolene (**1**).

Compound **1**, which is used clinically in the treatment of malignant hyperthermia,[6,7] inhibits abnormal Ca^{2+} release from SR[8] and inhibits both PCR and CICR in normal skeletal muscle.[3,9] Our goal was to design specific photoaffinity probes by introducing a photoreactive functional group either to the phenyl ring or the hydantoin moiety of **1**. Derivatives **2**, **3**, and **4**, with azido, benzoyl, and 3-trifluoromethyl-3*H*-diazirin-3-yl groups on the phenyl ring, respectively, were either ineffective or non-selective.[10,11] On the other hand, the hydantoin derivatives, GIF-0082 (**5**) and GIF-0276 (**6**), exhibited a specific inhibitory effect on PCR without affecting CICR, as shown in Figure 1.[9,12,13] Here, three substituents, a photoreactive group, an iodo group (introduced as a latent ^{125}I group), and a methylene group, were placed to satisfy *meta* configurations with

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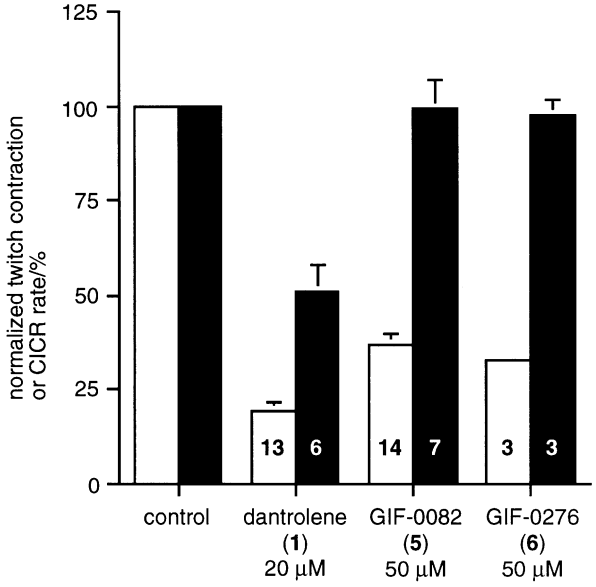
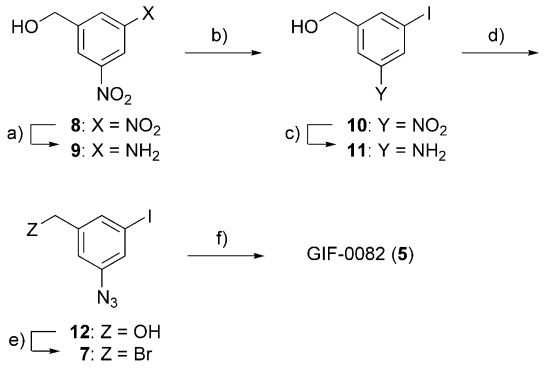


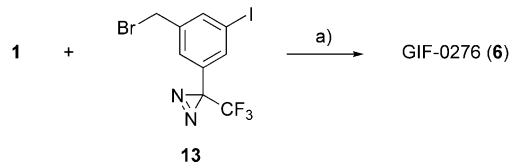
Figure 1. Effects of dantrolene (**1**), GIF-0082 (**5**), and GIF-0276 (**6**) on twitch contraction (open column) and CICR rate (filled column) of mouse skeletal muscle. For the methods of biological evaluation, see ref 9. The number of experiments (*n*), indicated in the column, of compounds **1** and **5** for twitch contraction were increased from that reported in ref 9b.

one another to avoid unnecessary interactions with neighboring functional groups.

GIF-0082 (**5**) was obtained by alkylation of **1** with benzyl bromide **7**, derived from commercial 3,5-dinitrobenzyl alcohol (**8**) (Scheme 1).[12] Thus, the partial reduction of one of the nitro groups of **8** by $SnCl_2$ under acidic conditions resulted in **9**. The amino group of **9** was converted to an iodo group by in situ diazo-iododediazotization to give **10**.[14] The remaining nitro group of **10** was changed to an azido group by reduction with $SnCl_2$ in ethanol followed by diazo-azidodediazotization to give **12**. Bromination of the benzyl alcohol **12** by the conventional method using CBr_4/PPh_3 produced the benzyl bromide **7** and, finally, the coupling of **1** with **7** in DMF gave the desired **5**.[12] Similarly, the diazirinyl-type



Scheme 1. Synthesis of GIF-0082 (**5**): (a) $SnCl_2$, concd. HCl, EtOH, 0°C to rt, 22 h, 57%; (b) $NaNO_2$, AcOH–H_2O (9:1), 0 °C; then NaI, 15 min, 67%; (c) $SnCl_2$, EtOH, 70 °C, 2.5 h, 91%; (d) $NaNO_2$, AcOH–H_2O (9:1), 0 °C; then NaN_3, 15 min, 95%; (e) CBr_4, PPh_3, CH_2Cl_2, rt, 2 h, 95%; (f) **1**, DMF, rt, 14.5 h, 85%.

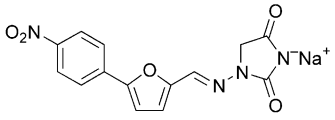


Scheme 2. Synthesis of GIF-0276 (**6**): (a) DMF, rt, 14.5 h, 67%.

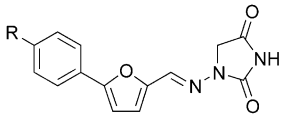
probe GIF-0276 (**6**) was prepared from **1** and known benzyl bromide **13**[15] (Scheme 2).[12]

A conspicuous biological property of **5** and **6** led to the syntheses of the corresponding ^{125}I-labeled compounds, [^{125}I]GIF-0082 ([^{125}I]-**5**) and [^{125}I]GIF-0276 ([^{125}I]-**6**). Since it is better to introduce a radioisotope as late as possible, we planned to apply conventional radioiododestannylation[16] at the final stage of the synthesis. Thus, the iodo derivatives, **5** and **6**, were once converted to the corresponding stannanes, **14** and **15**, respectively, by palladium(0)-catalyzed stannylation with bis(tri-*n*-butyltin) (Scheme 3).[12] The resulting stannyl precursor underwent radioiododestannylation[16,17] by mixing about a 600-fold excess of stannanes with commercial 10 mCi $Na^{125}I$ solution (Perkin–Elmer™, NEZ-033A) in the presence of Chloramine-T (~150-fold excess) followed by standing at room temperature overnight. An extractive work up with EtOAc followed by purification by silica-gel column chromatography produced the desired [^{125}I]-**5** and [^{125}I]-**6** in 61–97 and 31% radiochemical yields, respectively, based on $Na^{125}I$.[18]

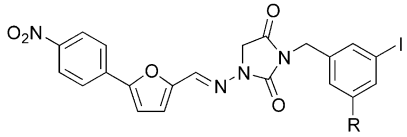
Thus, based on the molecular design and biological evaluation, we succeeded in developing specific photoreactive inhibitors for the PCR process, which is involved in the excitation–contraction (E–C) coupling[1,2] in skeletal muscle. The use of these probes for photoaffinity labeling, and for capturing novel molecules and determining their structures and functions, will be reported in due course.[19]



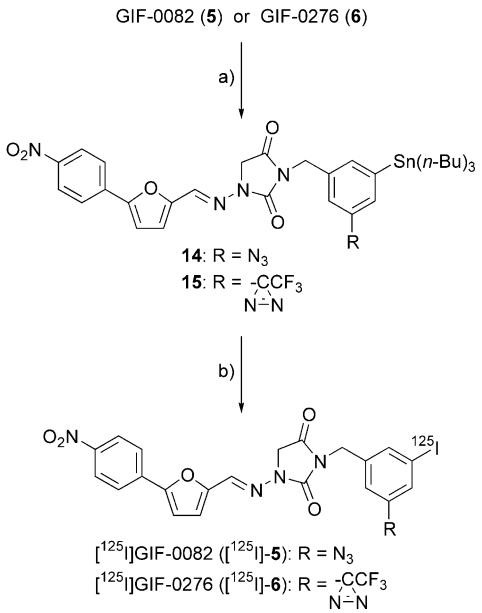
dantrolene (**1**)



2: R = N_3
3: R = Bz
4: R = -CCF3, N=N



GIF-0082 (**5**): R = N_3
GIF-0276 (**6**): R = -CCF3, N=N



Scheme 3. Synthesis of [^{125}I]GIF-0082 ([^{125}I]-**5**) and [^{125}I]GIF-0276 ([^{125}I]-**6**): (a) (n-$Bu_3Sn)_2$, $(Ph_3P)_4Pd$ (cat.), DME, 90 °C, (**14**; 22 h, 55%, **15**; 5.5 h, 41%); (b) Na^{125}I, Chloramine-T, DMF–H_2O (3:1), room temperature, ([^{125}I]-**5**; 17 h, 61–97%, [^{125}I]-**6**; 20 h, 31% radiochemical yields).

Acknowledgements

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10. Palnitker et al. recently reported the synthesis of tritium labeled **2** and identified 160/172 kDa proteins in procine/rabbit SR, which are the endogenously cleaved NH2-terminus parts of RyR1. We independently synthesized **2** and found it to be a non-selective inhibitor for both Ca^{2+} release modes as is **1**. (a) Palnitkar, S. S.; Bin, B.; Jimenez, L. S.; Morimoto, H.; Williams, P. G.; Paul-Pletzer, K.; Parness, J. *J. Med. Chem.* **1999**, *42*, 1872. (b) Paul-Pletzer, K.; Palnitkar, S. S.; Jimenez, L. S.; Morimoto, H.; Parness, J. *Biochemistry* **2001**, *40*, 531.
11. We intended to radiolabel these compounds by tritium according to ref 10a.
12. All new compounds were characterized by spectroscopic means. **5**: Yellow solid; TLC R_f = 0.63 (n-hexane/EtOAc = 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.31 (s, 2H), 4.68 (s, 2H), 6.97 (d, 1H, J = 3.8), 6.99 (d, 1H, J = 3.8), 7.07 (dd, 1H, J = 2.0, 1.5 Hz), 7.32 (dd, 1H, J = 1.5, 2.0 Hz), 7.56 (dd, 1H, J = 1.5, 1.5 Hz), 7.86–7.91 (AA′BB′, 2H), 8.01 (s, 1H), 8.25–8.29 (AA′BB′, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.6, 49.0, 94.8, 111.0, 115.7, 119.2, 124.4 (2C), 124.7 (2C), 127.8, 134.1, 135.18, 135.22, 138.6, 141.8, 147.1, 150.2, 152.6, 153.6, 166.0; IR (KBr, cm^{-1}) 851, 992, 1130, 1148, 1202, 1238, 1331, 1445, 1510, 1566, 1597, 1723, 1781, 2122; UV (EtOH, nm) λ_{max} (log ε) 298 (3.66), 309 (3.65), 381 (3.94). Anal. calcd for $C_{21}H_{14}N_7O_5I$: C, 44.15; H, 2.47; N, 17.16. Found: C, 43.93; H, 2.48; N, 17.14. **6**: Yellow solid; TLC R_f = 0.35 (n-hexane/EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 (s, 2H), 4.69 (s, 2H), 6.97 (d, 1H, J = 3.6 Hz), 6.99 (d, 1H, J = 3.6 Hz), 7.24 (dd, 1H, J = 1.2, 1.5 Hz), 7.50 (dd, 1H, J = 1.2, 1.5 Hz), 7.86 (dd, 1H, J = 1.5, 1.5 Hz), 7.86–7.90 (AA′BB′, 2H), 8.01 (s, 1H), 8.25–8.29 (AA′BB′, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ 26.7 ($^2J(^{19}F–^{13}C)$ = 41.1 Hz), 40.5, 47.5, 93.8, 110.4, 114.6, 120.6 ($^1J(^{19}F–^{13}C)$ = 273.1 Hz), 123.2 (2C), 123.5 (2C), 125.3, 129.9, 133.0, 133.8, 134.2, 137.4, 137.9, 145.6, 149.3, 151.5, 151.9, 165.3; ^{19}F NMR (372 MHz, $CDCl_3$) δ 12.65 (s, 3F); IR (KBr, cm^{-1}) 741, 754, 853, 1150, 1179, 1202, 1246, 1333, 1412, 1441, 1510, 1599, 1728, 1782; UV (EtOH, nm) λ_{max} (log ε) 309 (4.24), 323 (4.22), 379 (4.55). Anal. calcd for $C_{23}H_{14}N_6O_5F_3I$: C, 43.28; H, 2.21; N, 13.17. Found: C, 43.06; H, 2.21; N, 13.31.
13. The effect for PCR was evaluated by the twitch tension of intact mouse skeletal muscle. The CICR rates were quantified by the amount of Ca^{2+} released using microfluorometry of Fura-2, a Ca^{2+} indicator, in skinned fibers prepared from mouse skeletal muscle. For the methods of biological evaluation, see ref 9.
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17. Before handling the radioisotope, the reproducibility of the reaction was confirmed by examining iododestannylation under 'cold' conditions similar to the actual conditions used.
18. We confirmed from the TLC analysis that [^{125}I]-**5** was completely decomposed by photoirradiation by UV-light (254 nm, 16 W UV-lamp, from the distance of 1 cm) within 60 s under the actual photolabeling conditions. On the other hand, [^{125}I]-**6** required rather longer irradiation time (10 min) for complete decomposition even using the 100 W UV-lamp (365 nm).
19. Actually, we have succeeded in capturing novel molecules by using these specific photoaffinity probes. Their structures and functions will be reported separately in due course.