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# CYTOTOXICITY AND ACTIN DEPOLYMERIZING ACTIVITY OF APLYRONINE A, A POTENT ANTITUMOR MACROLIDE OF MARINE ORIGIN, AND THE NATURAL AND ARTIFICIAL ANALOGS

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**Abstract:** The artificial analogs of aplyronine A (**1**), a potent cytotoxic and antitumor macrolide, were synthesized and the structure-activity (cytotoxicity and actin depolymerizing activity) studies were performed; the side chain portion in **1** was found to play a key role in both biological activities.


We isolated[1] aplyronine A (**1**) as a potent cytotoxic and antitumor substance from the sea hare *Aplysia kurodai* and determined its absolute stereostructure,[2] which was confirmed by its total synthesis.[3] Actin is one of the most abundant and common proteins in the cytoskeleton and regulates various cell functions, such as muscle contraction, cell motility, and cell division. Recently, aplyronine A (**1**) was found to inhibit the polymerization of globular actin (G-actin) to fibrous actin (F-actin) and depolymerize F-actin to G-actin by severing.[4] To date, very few antitumor substances have been reported that interact with actin and thus, aplyronine A (**1**) is regarded to be a new type of antitumor substance in terms of its mode of action. Previously, mycalolide B,[5] a cytotoxic macrolide from a marine sponge, was reported to exhibit actin depolymerizing activity.[6]

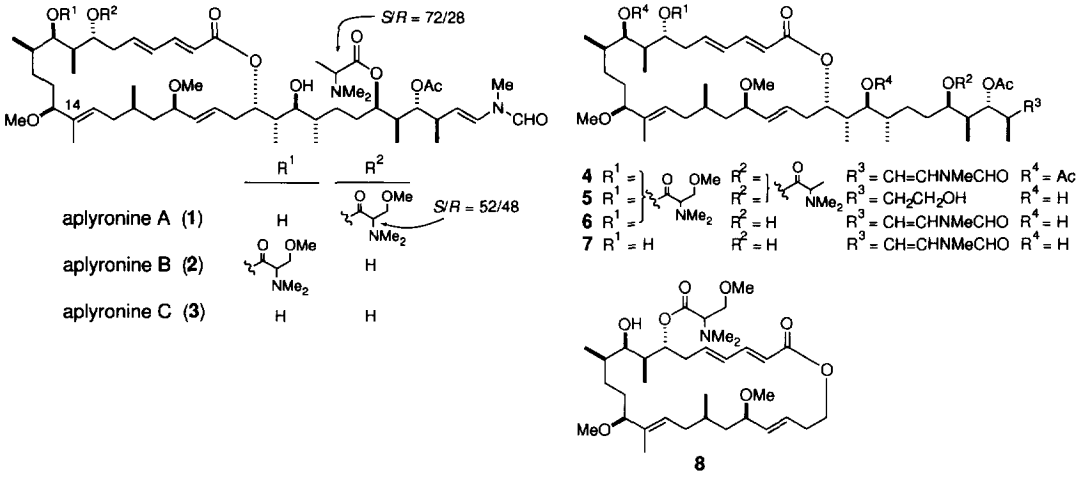


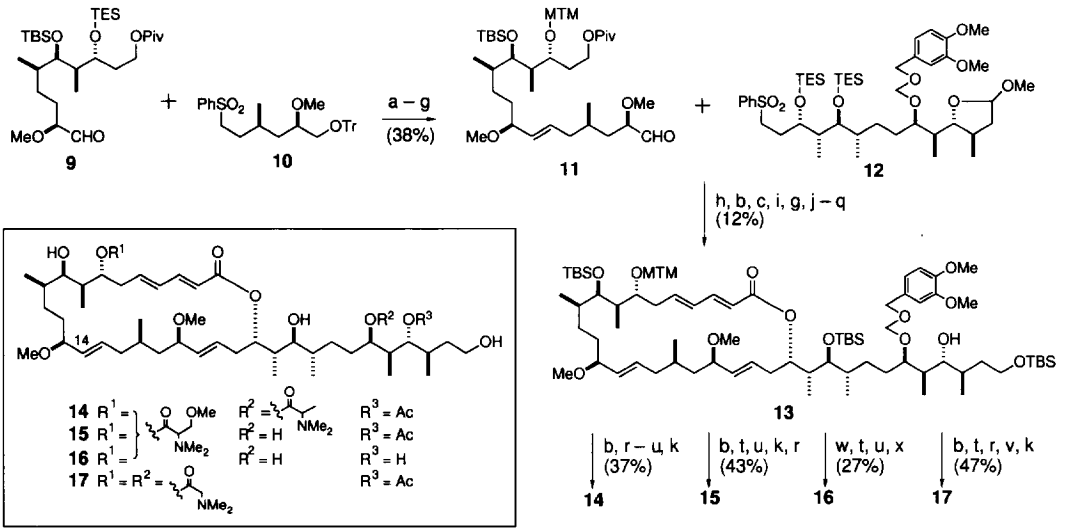

**Figure 1.** Natural aplyronines and the previously synthesized artificial analogs.

We investigated the structure-cytotoxicity relationships of aplyronine A (**1**) by using the natural and artificial analogs of **1**,[3d] and it was found that (1) the trimethylserine moiety, the two hydroxyl groups, and the side chain portion in aplyronine A (**1**) play an important role in its strong cytotoxicity, and (2) the *N*-formyl enamine part and the dimethylalanine moiety in **1** are not important for its strong cytotoxicity.[3d] So far, no information is available on the structure-actin depolymerizing activity relationships of **1**. We report here the synthesis of the artificial analogs of aplyronine A (**1**), describe the additional structure-cytotoxicity relationships of **1**, and also describe the structure-actin depolymerizing activity relationships of **1**. Figure 1 illustrates the natural aplyronines and the previously synthesized analogs of aplyronine A (**1**).[3d]

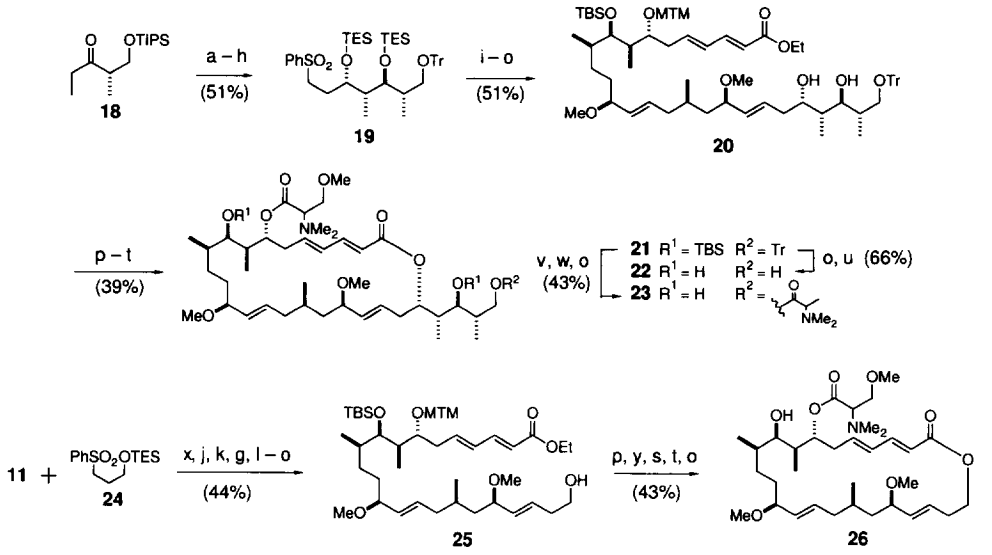
## Chemical Synthesis

**Synthesis of the artificial analogs that lack the C14 methyl group.** In the synthesis of aplyronine A (**1**), the stereoselective construction of the trisubstituted olefin at C14 was a difficult task to achieve. Since the C14 methyl group seemed to be unimportant for the biological activities of aplyronine A (**1**), we attempted to synthesize four analogs that lack the C14 methyl group, **14** – **17**, which were more readily accessible by organic synthesis than **1**.

These analogs were prepared by a synthetic strategy similar to that for aplyronine A (**1**) from the intermediate **9**[3d] (Scheme 1). Julia olefination[8] between aldehyde **9** and sulfone **10**[3d] followed by a sequence




**Scheme 1.** (a) **10**, BuLi, THF, –78 °C, then $MgBr_2$, **9**, –78 °C; (b) $Ac_2O$, DMAP, pyridine, 23 °C; (c) 5% Na–Hg, $Na_2HPO_4$, MeOH, 0 °C; (d) AcOH, $H_2O$, THF, 23 °C; (e) DMSO, $Ac_2O$, AcOH, 23 °C → 40 °C; (f) $HCO_2H$, $Et_2O$, 28 °C; (g) Dess-Martin periodinane, pyridine, $CH_2Cl_2$, 23 °C; (h) **12**, BuLi, THF, –78 °C, then $MgBr_2$, **11**, –78 °C; (i) DIBAL, $CH_2Cl_2$, –78 °C; (j) LDA, $(EtO)_2P(O)CH_2CH{=}CHCO_2Et$, THF, –45 °C → 0 °C; (k) HF•pyridine, pyridine, THF, 23 °C; (l) LiOH, $H_2O$, MeOH, 23 °C; (m) 2,4,6-trichlorobenzoyl chloride, DMAP, $Et_3N$, $CH_2Cl_2$, 23° C; (n) TBSCl, imidazole, DMF, 58 °C; (o) HCl, $H_2O$, DME, 23 °C; (p) $NaBH(OMe)_3$, MeOH, 23°C; (q) TBSCl, $Et_3N$, DMAP, $CH_2Cl_2$, 23°C; (r) DDQ, $CH_2Cl_2$, *t*-BuOH, phosphate buffer (pH 6), 23 °C; (s) *N,N*-dimethylalanine (*S*/*R* = 3/2), DCC, CSA, DMAP, $CH_2Cl_2$, 23 °C; (t) $AgNO_3$, 2,6-lutidine, $H_2O$, THF, 30 °C; (u) *N,N,O*-trimethylserine (*S*/*R* = 5/2), DCC, CSA, DMAP, $CH_2Cl_2$, 35 °C; (v) *N,N*-dimethylglycine, DCC, CSA, DMAP, $CH_2Cl_2$, 23 °C; (w) TESCl, imidazole, DMF, 23°C; (x) HCl, $H_2O$, dioxane, 50 °C.




**Scheme 2.** (a) $Sn(OTf)_2$, $Et_3N$, $CH_2Cl_2$, –78 °C, then 3-(benzyloxy)propanal, –78 °C → –20 °C; (b) $Me_4NBH(OAc)_3$, MeCN, AcOH, –20 °C; (c) $H_2$, 20%$Pd(OH)_2$–C, dioxane, 23 °C; (d) $(PhS)_2$, $Bu_3P$, DMF, 0 °C → 23 °C; (e) $Bu_4NF$, THF, 23 °C; (f) TrCl, pyridine, 50 °C; (g) TESCl, imidazole, DMF, 50 °C; (h) mCPBA, $NaHCO_3$, $CH_2Cl_2$, 0 °C → 23 °C; (i) **19**, BuLi, THF, –78 °C, then $MgBr_2$, **11**, –78 °C; (j) $Ac_2O$, DMAP, pyridine, 23 °C; (k) 5% Na–Hg, $Na_2HPO_4$, MeOH, 0 °C; (l) DIBAL, $CH_2Cl_2$, –78 °C; (m) Dess-Martin periodinane, pyridine, $CH_2Cl_2$, 23 °C; (n) LDA, $(EtO)_2P(O)CH_2CH{=}CHCO_2Et$, THF, –40 °C → 0 °C; (o) HF•pyridine, pyridine, THF, 23 °C; (p) LiOH, MeOH, $H_2O$, 23 °C; (q) 2,4,6-trichlorobenzoyl chloride, DMAP, $Et_3N$, $CH_2Cl_2$, 23 °C; (r) TBSCl, imidazole, DMF, 60 °C; (s) $AgNO_3$, 2,6-lutidine, $H_2O$, THF, 30 °C; (t) *N,N,O*-trimethylserine (*S*/*R* = 5/2), DCC, CSA, DMAP, $CH_2Cl_2$, 35 °C; (u) HCl, $H_2O$, dioxane, 50 °C; (v) $HCO_2H$, $Et_2O$, 26 °C; (w) *N,N*-dimethylalanine (*S*/*R* = 3/2), DCC, CSA, DMAP, $CH_2Cl_2$, 23 °C; (x) **24**, BuLi, THF, –78 °C, then $MgBr_2$, **11**, –78 °C; (y) $PPh_3$, DEAD, toluene, –10 °C → 4 °C.

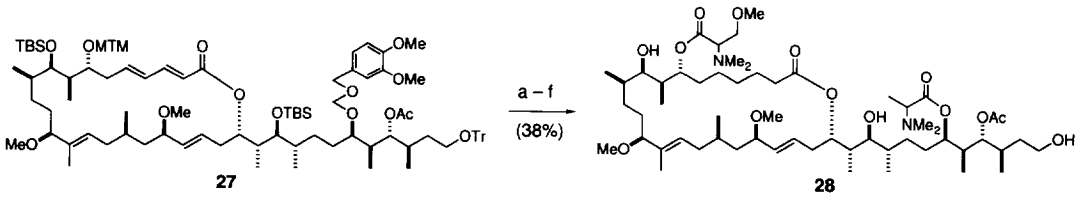
of reactions gave aldehyde **11** as shown in Scheme 1. Again, Julia olefination between aldehyde **11** and sulfone **12**[3d] and subsequent reactions including Yamaguchi lactonization[9] led to lactone **13**. Lactone **13** was transformed into analogs **14** – **17** as shown in Scheme 1.

**Synthesis of the artificial analogs with the shorter side chain and the artificial analog without the side chain.** To investigate the relationships between the length of the side chain portion and the biological activities of the artificial analogs, three analogs, **22**, **23**, and **26**, were prepared (Scheme 2).

The side chain segment **19** was prepared from ketone **18**[10] by employing the Paterson aldol reaction[10] as a key step. The analogs with the shorter side chain, **22** and **23**, were prepared from **19** by a strategy similar to that for aplyronine A (**1**). The synthesis of the artificial analog without the side chain, **26**, was effected using the same strategy as for analog **8**[3d] except for the macrolactonization method (Scheme 2).

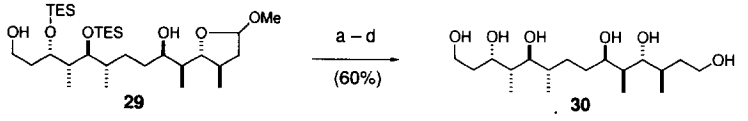
**Synthesis of the tetrahydro analog and the artificial analog that only consists of the side chain.** The tetrahydro analog that lacks the conjugated diene system, **28**, was synthesized from the synthetic intermediate **27**[3d] of aplyronine A (**1**) by employing $NaBH_4$–$NiCl_2$[11] reduction as a key step (Scheme 3).

The artificial analog that only consists of the side chain, **30**, was prepared from the synthetic intermediate **29**[3d] in four steps (Scheme 4).




27 28

**Scheme 3.** (a) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH, $CH_2Cl_2$, 0 °C; (b) DDQ, $CH_2Cl_2$, *t*-BuOH, phosphate buffer (pH 6), 23 °C; (c) *N,N*-dimethylalanine (*S/R* = 3/2), DCC, CSA, DMAP, $CH_2Cl_2$, 23 °C; (d) $AgNO_3$, 2,6-lutidine, $H_2O$, THF, 30 °C; (e) *N,N,O*-trimethylserine (*S/R* = 5/2), DCC, CSA, DMAP, $CH_2Cl_2$, 35 °C; (f) HF•pyridine, pyridine, THF, 23 °C.

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**Scheme 4.** (a) $Ac_2O$, DMAP, pyridine, 23 °C; (b) HCl, $H_2O$, DME, 23 °C; (c) $NaBH_4$, EtOH, 0 °C; (d) NaOMe, MeOH, 23 °C.

## Biological Activities and Discussion

**Structure-cytotoxicity relationships of natural aplyronines and the artificial analogs.** The cytotoxicity of natural aplyronines and the artificial analogs against HeLa $S_3$ cells is summarized in Table 1. Previously, the side chain portion of **1** was shown to be essential for its strong cytotoxicity.[3d] In the present study the effect of the side chain moiety of **1** on the cytotoxicity was examined in more detail; comparison of the cytotoxicity of **8**, **15**, and **22** revealed that not only the presence of the side chain but also the length of the side chain is crucial for the strong cytotoxicity. Comparison of the cytotoxicity of **5** and **28** revealed that the conjugated diene moiety is responsible for the strong cytotoxicity of **1**. Analog **17** that has two dimethylglycine groups is ≈1000-fold less cytotoxic than analog **14** that possesses dimethylalanine and trimethylserine groups. This finding indicates the importance of the structures of the amino acid residues for the cytotoxicity. The analogs that lack both the *N*-formyl enamine group and the dimethylalanine group, **15** and **16**, are less cytotoxic than the analogs that possess either the *N*-formyl enamine group or the dimethylalanine group, **5**, **6**, and **14**. The result indicates that either one of the aforementioned two groups is necessary for aplyronine analogs to exhibit the strong cytotoxicity. As expected, the C14 methyl group of **1** was shown to have no significant effect on the activity by comparison of the activity of **5** and **14**.

**Structure-actin depolymerizing activity relationships of natural aplyronines and the artificial analogs.** Actin depolymerizing activity of aplyronine A (**1**) and its natural and artificial analogs was determined by flow birefringence as shown in Table 1. The natural and artificial analogs that possess the side chain of the same length as in the case of aplyronine A (**1**) exhibit a strong activity comparable to that of **1**, whereas the analogs with the shorter side chain, **22** and **23**, are approximately 100 times less active than **1**, and the analog without the side chain, **26**, is inactive. It is noteworthy that even the analog that only consists of the side chain, **30**, exhibits actin depolymerizing activity although it is very weak. These results revealed that: (1) the side chain portion in **1** plays a key role in exhibiting the activity, and (2) the combination of the side chain portion and the macrolide moiety is essential for **1** to exhibit its potent activity. The C14 methyl group and the *N*-formyl

enamine part in aplyronine A (**1**) were shown to be unimportant for the strong activity of **1**, as in the case of the cytotoxicity of **1**, by comparison of the activity of **1**, **5**, and **14**. Other functional groups such as the amino acid residues, the two hydroxyl groups, and the conjugated diene moiety, proved to have little effect on the actin depolymerizing activity of **1**, in contrast to the cytotoxicity previously mentioned. The acetyl group in **15** was shown to be important to some extent for the activity by comparison of the activity of **15** and **16**.

In conclusion, structure-cytotoxicity relationships and structure-actin depolymerizing activity relationships of aplyronine A (**1**) were determined to a considerable extent. The side chain portion of aplyronine A (**1**) turned out to play a key role in both biological activities of **1**.

**Table 1.** Cytotoxicity and actin depolymerizing activity of natural aplyronines and the artificial analogs

| compound | cytotoxicity against HeLa $S_3$ cells | | actin depolymerizing activity[a] | |
|---|---|---|---|---|
| | $IC_{50}$ (ng/mL) | relative potency[b] | $IC_{50}$[c] (μM) | relative potency[b] |
| aplyronine A (**1**) | 0.48 [d] | 100 | 31 | 100 |
| aplyronine B (**2**) | 3.11 [d] | 15 | 33 | 94 |
| aplyronine C (**3**) | 21.2 [d] | 2.3 | 32 | 97 |
| **4** | 216 [d] | 0.22 | 57 | 54 |
| **5** | 1.72 [d] | 28 | 78 | 40 |
| **6** | 1.03 [d] | 47 | 35 | 86 |
| **7** | 113 [d] | 0.42 | 35 | 86 |
| **8** | 2100 [d] | 0.023 | n.d. | |
| **14** | 2.6 | 18 | 38 | 82 |
| **15** | 37 | 1.3 | 36 | 86 |
| **16** | 83 | 0.58 | 190 | 16 |
| **17** | >2000 | <0.02 | 49 | 63 |
| **22** | >2000 | <0.02 | 4500 [e] | 0.69 |
| **23** | n.d. | | 1800 | 1.7 |
| **26** | n.d. | | inactive | |
| **28** | 57 | 0.84 | 70 | 44 |
| **30** | n.d. | | 7600 [e] | 0.41 |

[a] Activity was monitored by measuring flow birefringence. For conditions of biological assay, see reference 12. [b] The relative potencies were calculated from the $IC_{50}$ values of the compounds (aplyronine A = 100). [c] $IC_{50}$ is the concentration required to depolymerize F-actin (40 μM) to 50% of its control amplitude. [d] Reference 3d. [e] Exact $IC_{50}$ value could not be obtained because of the limited solubility of the test compound.

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**References and Notes**


1. Yamada, K.; Ojika, M.; Ishigaki, T.; Yoshida, Y.; Ekimoto, H.; Arakawa, M. *J. Am. Chem. Soc.* **1993**, *115*, 11020.
2. (a) Ojika, M.; Kigoshi, H.; Ishigaki, T.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8501. (b) Ojika, M.; Kigoshi, H.; Ishigaki, T.; Nisiwaki, M.; Tsukada, I.; Mizuta, K.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8505. (c) Ojika, M.; Kigoshi, H.; Ishigaki, T.; Tsukada, I.; Tsuboi, T.; Ogawa, T.; Yamada, K. *J. Am. Chem. Soc.* **1994**, *116*, 7441.
3. (a) Kigoshi, H.; Ojika, M.; Suenaga, K.; Mutou, T.; Hirano, J.; Sakakura, A.; Ogawa, T.; Nisiwaki, M.; Yamada, K. *Tetrahedron Lett.* **1994**, *35*, 1247. (b) Kigoshi, H.; Ojika, M.; Ishigaki, T.; Suenaga, K.; Mutou, T.; Sakakura, A.; Ogawa, T.; Yamada, K. *J. Am. Chem. Soc.* **1994**, *116*, 7443. (c) Suenaga, K.; Ishigaki, T.; Sakakura, A.; Kigoshi, H.; Yamada, K. *Tetrahedron Lett.* **1995**, *36*, 5053. (d) Kigoshi, H.; Suenaga, K.; Mutou, T.; Ishigaki, T.; Atsumi, T.; Ishiwata, H.; Sakakura, A.; Ogawa, T.; Ojika, M.; Yamada, K. *J. Org. Chem.* **1996**, *61*, 5326.
4. Saito, S.; Watabe, S.; Ozaki, H.; Kigoshi, H.; Yamada, K.; Fusetani, N.; Karaki, H. *J. Biochem.* **1996**, *120*, 552.
5. Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1989**, *30*, 2809.
6. Saito, S.; Watabe, S.; Ozaki, H.; Fusetani, N.; Karaki, H. *J. Biol. Chem.* **1994**, *269*, 29710.
7. The numbering used in this paper corresponds to that of aplyronine A (**1**).
8. Julia, M.; Paris, J.-M. *Tetrahedron Lett.* **1973**, 4833.
9. (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367.
10. Paterson, I.; Tillyer, R. D. *Tetrahedron Lett.* **1992**, *33*, 4233.
11. Satoh, T.; Nanba, K.; Suzuki, S. *Chem. Pharm. Bull.* **1971**, *19*, 817.
12. Actin was extracted in cold water from the acetone-dried powder of rabbit skeletal muscle, purified,[13] and polymerized in 100 mM KCl, 5 mM Tris-HCl (pH 8.0), 0.2 mM ATP, and 1 mM DTT before use. The test compounds were dissolved in DMSO and added to the F-actin solution (40 μM). The incubated actin solutions (23 °C, 1 min) were analyzed in a micro FBR-Mark II apparatus (Wakenyaku Co., Kyoto, Japan) with rotation at 500 rpm. The final concentration of DMSO introduced in the reaction mixtures with the test compounds was 1% (v/v) except for compounds **22**, **23** and **26** (5-10%, v/v). The $IC_{50}$ values are mean values of at least three experiments.
13. (a) Ebashi, S.; Ebashi, F. *J. Biochem.* **1965**, *58*, 7. (b) Higashi-Fujime, S. *J. Biochem.* **1983**, *94*, 1539.


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