Isolation of Tribromoacetamide from an Okinawan Alga and Biological Activities of Its Analogs

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Tribromoacetamide was isolated from the Okinawan alga *Wrangelia* species, and the biological activities of this compound and its analogs were investigated.

In our continuing search for bioactive agents from marine organisms using unique bioassay systems, tribromoacetamide (**1**) was found to be an active component of the extract of the alga *Wrangelia* sp. to prevent biofilm formation.[1] It was isolated for the first time as a marine natural product. We describe herein the isolation and biological activities of **1** and its analogs.

The EtOAc soluble material of MeOH extract of the alga *Wrangelia* sp. (600 g), collected in Okinawa, was partitioned between hexane and the 70% EtOH. Using the bioassay system,[1] the material obtained from 70% EtOH portion was chromatographed on ODS silica gel (MeOH-H_2O) and silica gel ($CHCl_3$) to give tribromoacetamide (**1**, 2.1 mg), which was identical with an authentic sample: ^{13}C NMR ($CDCl_3$) δ 36.7, 166.9; EIMS m/z 293, 295, 297, 299 (M^+). This compound inhibited the growth of the attaching bacteria *Rhodospirillum salexigens* at an IC_{50} of 4.9 $\mu g/cm^2$.

During the course of investigating the biological activities of the synthetic analogs[2] of **1**, we found that some analogs possessed moderate cytotoxicity against P388 leukemia (Figure 1). All analogs exhibited weaker cytotoxicities than **1**. Trichloroacetamide derivatives **30**–**32**, and **34** showed weak cytotoxicity, and the dibromo derivative **29** exhibited weaker activity than the tribromo derivative **14**, indicating that the tribromoacetyl group is important to the cytotoxicity of haloacetamide derivatives. Considering the weaker cytotoxicities of **6**–**8**, the hydrophobicity of *N*-substituents might play an important role in cytotoxicity. From a comparison of the cytotoxicities of **10**–**15**, it followed that the low electron density of the *N*-benzylic substituents might be important. Therefore, the cytotoxicities of the picolyl derivatives **25**–**27** were investigated and found to be stronger than that of the corresponding benzyl derivative **11**. Among them, 3-picolyl derivative **26** exhibited the strongest activity. The weaker cytotoxicities of **23** and **24** indicated that the longer chain length decreases the activity. A comparison of the cytotoxicities of the NMe derivatives, **13**–**15**, **19**–**21**, and **24**, with those of the NH derivatives, **10**–**12**. **16**–**18**, and **23**, suggested that the NMe group is not so important to the cytotoxicity. Considering the availability and acute toxicity,[3] we chose *N*-benzyl-*N*-methyltribromoacetamide (**14**) as one of the best tribromoacetamides for further biological studies.

To investigate the possibility of **14** as an antitumor agent candidate, the bioactivities concerning the modes of antitumor activity were examined. As a result, **14** was found to possess an inhibitory activity[4] on microtubule assembly at 10^{-5} M and to exhibit an inhibitory activity[5] against tumor invasion into basement membranes as strong as that of doxorubicin (Table 1). Furthermore, compound **14** showed an apoptosis-inducing activity, i.e., morphological change and DNA fragmentation; however, it is weak (over 1 $\mu g \cdot mL^{-1}$).[6]

The lability of the amide bond in **14** may decrease the biological activities. To obtain the stable analogs of **14** against enzymatic hydrolysis of the amide moiety, we designed and synthesized the ketone analog **35**.[7] Coupling reaction of 3-phenylpropanal with tribromomethyl lithium and subsequent Dess–Martin oxidation gave tribromoketone **35** (62%, 2 steps). Compound **35** exhibited a cytotoxicity against P388 leukemia with an IC_{50} of 0.32 $\mu g \cdot mL^{-1}$, which suggested that tribromoketone derivatives might work as mimics of tribromoacetamides. Preliminary investigation of the biological property revealed that **35** inhibited topoisomerases I and II with IC_{50} values of 10–30 μM and ca. 30 μM, respectively.[8] Further biological studies of **35** and its derivatives are now in progress.

Table 1. Invasion-inhibiting activity of tribromoacetamide **14**.

Concentration /$\mu g \cdot mL^{-1}$	Relative value of invasion cell number[a]	
	14	Doxorubicin
0	100	100
0.0001	107	
0.001	102	
0.01	97	
0.1	97	
1	43	32

[a]Human fibrosarcoma HT-1080 was incubated in the upper compartment of the transwell chamber assembly fitted with a Matrigel-coated filter for 4 h, and the invasion cells that passed through the filter were visually counted under a microscope. See Ref. 5.

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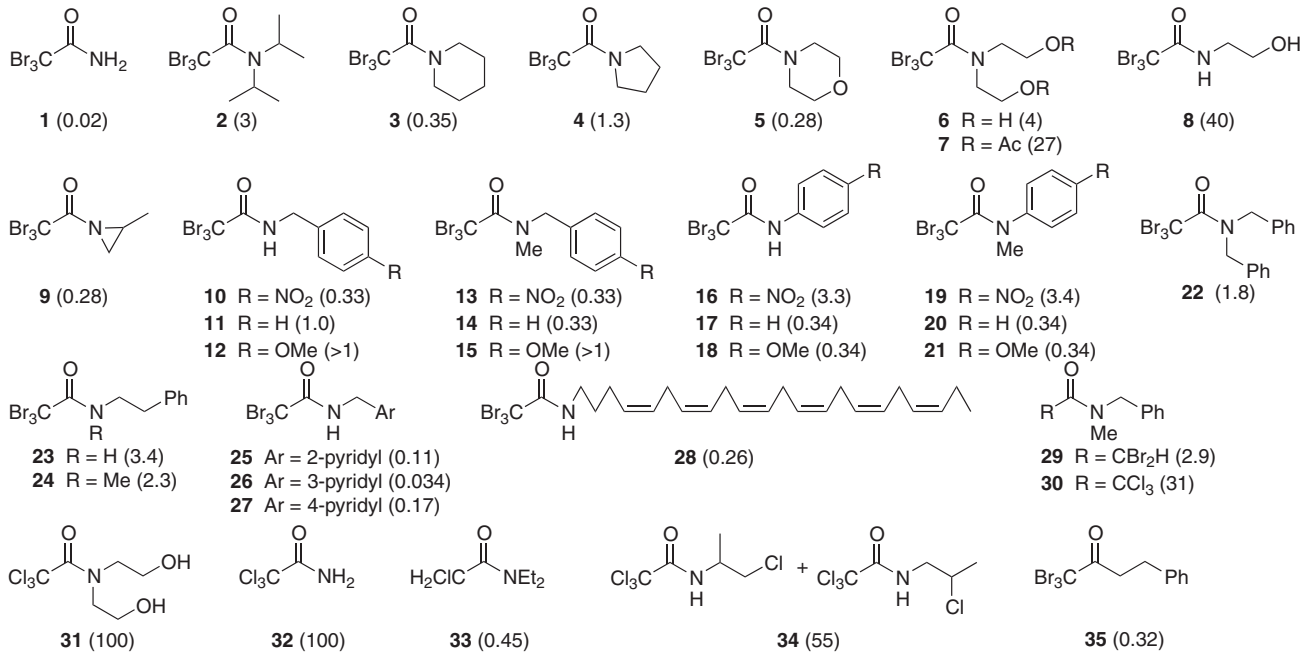

Figure 1. Cytotoxicity of synthetic analogs of tribromoacetamide (**1**). IC_{50} values ($\mu g \cdot mL^{-1}$) are in parenthese. Human leukemia P388 cells were treated with the test drug in RPMI 1640 supplemented with 5 μM 2-hydroxyethyldisulfide and 100 $\mu g \cdot mL^{-1}$ kanamycin sulfate. After incubation, the cells were counted by the MTT method, and the IC_{50} value was determined by the growth carve.

References and Notes

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2 The acetamides were prepared from the corresponding acetyl chlorides and amines. Some of *N*-methyl analogs, **13**, **15**, **20**, **21**, and **24**, were synthesized by *N*-methylation of the corresponding primary amides. Acetate **7** was prepared by acetylation of **6**. ^{1}H NMR data for acetamides ($CDCl_3$, otherwise noted): **4** δ 1.85–1.96 (m, 2H), 1.96–2.10 (m, 2H), 3.60–3.70 (m, 2H), 3.87–4.00 (m, 2H); **5** δ 3.78 (t, $J = 5.0$ Hz, 4H), 3.89 (m, 4H); **6** δ 2.62 (br s, 1H), 3.15 (br s, 1H), 3.61 (t, $J = 5.0$ Hz, 4H), 3.86 (br s, 2H), 3.91 (br t, $J = 5.0$ Hz, 2H); **7** δ 2.06 (s, 3H), 2.12 (s, 3H), 3.66 (t, $J = 5.6$ Hz, 2H), 3.71 (t, $J = 5.6$ Hz, 2H), 4.24 (t, $J = 5.6$ Hz, 2H), 4.26 (t, $J = 5.6$ Hz, 2H); **8** δ 1.76 (t, $J = 5.0$ Hz, 1H), 3.57 (q, $J = 5.0$ Hz, 2H), 3.85 (q, $J = 5.0$ Hz, 2H), 7.35 (br s, 1H); **9** δ 1.57 (d, $J = 6.6$ Hz, 3H), 3.44 (dd, $J = 6.1$, 7.8 Hz, 0.4H), 3.49 (dd, $J = 6.1$, 7.8 Hz, 0.6H), 3.76 (dd, $J = 3.6$, 6.1 Hz, 0.6H), 3.81 (dd, $J = 3.6$, 6.1 Hz, 0.4H), 4.25 (ddq, $J = 3.6$, 7.8, 6.6 Hz, 1H); **10** δ 4.68 (d, $J = 4.7$ Hz, 2H), 7.31 (br m, 1H), 7.51 (d, $J = 8.6$ Hz, 2H), 8.23 (d, $J = 8.6$ Hz, 2H); **12** δ 3.79 (s, 3H), 4.48 (d, $J = 5.9$ Hz,), 6.88 (d, $J = 8.3$ Hz, 2H), 7.12 (br m, 1H), 7.25 (d, $J = 8.3$ Hz, 2H); **13** δ 3.37 (br s, 3H), 4.87 (br s, 2H), 7.46 (d, $J = 8.6$ Hz, 2H), 8.24 (d, $J = 8.6$ Hz, 2H); **14** (−30 °C) δ 2.95 (s, 0.9H), 3.35 (s, 2.1H), 4.70 (s, 1.4H), 5.08 (s, 0.6H), 7.26–7.45 (m, 5H); **15** δ 3.25 (br s, 3H), 3.81 (s, 3H), 4.72 (br s, 2H), 6.82 (d, $J = 8.6$ Hz, 2H), 7.22 (d, $J = 8.6$ Hz, 2H); **17** δ 7.20–7.60 (m, 5H), 8.40–8.60 (br s, 1H); **18** δ 3.82 (s, 3H), 6.93 (br d, $J = 9.2$ Hz, 2H), 7.48 (br d, $J = 9.2$ Hz, 2H), 8.35–8.50 (br s, 1H); **19** δ 3.72 (s, 3H), 7.56 (d, $J = 8.7$ Hz, 2H), 8.30 (d, $J = 8.7$ Hz, 2H); **20** δ 3.60 (s, 3H), 7.23–7.43 (m, 5H); **21** δ 3.53 (br s, 3H), 3.82 (s, 3H), 6.91 (br d, $J = 8.6$ Hz, 2H), 7.23 (br d, $J = 8.6$ Hz, 2H); **22** (−30 °C) δ 4.60 (s, 2H), 4.95 (s, 2H), 7.10–7.44 (m, 10H); **23** δ 2.91 (t, $J = 6.6$ Hz, 2H), 3.63 (q, $J = 6.6$ Hz, 2H), 6.98–6.84 (br s, 1H), 7.38–7.20 (m, 5H); **24** δ 2.90–3.00 (br m, 2H), 3.22 (s, 3H), 3.80–3.60 (br m, 2H), 7.40–7.20 (m, 5H); **25** δ 4.68 (d, $J = 4.6$ Hz, 2H), 7.26 (br dd, 8.6, 6.3 Hz, 1H), 7.30 (br d, $J = 8.6$ Hz, 1H), 7.72 (td, $J = 8.6$, 1.7 Hz, 1H), 8.47 (br m, 1H), 8.59 (br d, $J = 6.3$ Hz, 1H); **26** δ 4.60 (d, $J = 5.9$ Hz, 2H), 7.32 (dd, $J = 7.9$, 4.6 Hz, 1H), 7.39 (br m, 1H), 7.71 (dd, $J = 7.9$, 1.7 Hz, 1H), 8.57 (d, $J = 1.7$ Hz, 1H), 8.58 (d, $J = 4.6$ Hz, 1H); **27** (CD_3OD) δ 4.54 (s, 2H), 7.41 (d, $J = 6.3$ Hz, 2H), 8.49 (d, $J = 6.3$ Hz, 2H); **28** δ 0.97 (t, $J = 7.1$ Hz, 3H), 1.71 (tt, $J = 7.0$, 7.1 Hz, 2H), 2.08 (dq, $J = 6.7$, 7.1 Hz, 2H), 2.18 (dt, $J = 7.1$, 7.1 Hz, 2H), 2.80–2.88 (m, 10H), 3.40 (dt, $J = 7.0$, 7.0 Hz, 2H), 5.28–5.48 (m, 12H), 6.88 (br s, 1H); **29** δ 3.00 (s, 1H), 3.11 (s, 2H), 4.63 (s, 1.3H), 4.72 (s, 0.7H), 6.18 (s, 0.3H), 6.23 (s, 0.7H), 7.20–7.44 (m, 5H); **30** δ 2.96 (s, 0.9H), 3.29 (s, 2.1H), 4.68 (s, 1.4H), 4.96 (s, 0.6H), 7.25–7.39 (m, 5H); **31** δ 1.74 (br s, 1H), 2.41 (br s, 1H), 3.42 (t, $J = 5.2$ Hz, 2H), 3.70 (t, $J = 8.1$ Hz, 2H), 3.83 (t, $J = 5.2$ Hz, 2H), 4.36 (t, $J = 8.1$ Hz, 2H); **34** δ 1.38 (d, $J = 6.8$ Hz, 0.3H), 1.56 (d, $J = 6.8$ Hz, 2.7H), 3.45 (ddd, $J = 5.4$, 8.3, 14.1 Hz, 0.9H), 3.63 (dd, $J = 3.4$, 11.2 Hz, 0.1H), 3.79 (ddd, $J = 3.9$, 6.8, 14.1 Hz, 0.9H), 3.79 (m, 0.1H), 4.23 (m, 0.9H), 4.32 (m, 0.1H), 6.80 (br s, 0.1H), 7.09 (br s, 0.9H).

3 $LD_{99} = 530$ mg/kg·mouse for **1**; >1000 mg/kg·mouse for **14**.

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