新規薬剤を志向した生理活性物質の探索

Search for biologically active compounds to fill the demand for new drugs

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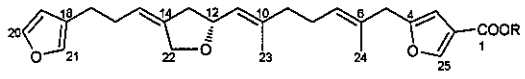
Infectious diseases are still a major cause of deaths worldwide. Both the number of infectious diseases in human beings and the frequency of outbreaks have increased over the years, with more than 3500 outbreaks foreseen by 2020. Diseases with long histories like yellow fever, dengue, plague, and influenza still exist, and resources are deployed to overcome these. In addition, new infectious agents have appeared such as coronavirus, henipavirus, and avian influenza virus. Drug discovery programs against some diseases have resulted in clinical medicines, however there remain diseases without effective drugs or vaccines.

In our collaborative projects for finding new bioactive molecules, we screened extracts of marine macroorganisms and microorganisms collected around Okinawa Prefecture, Japan, against penicillin binding proteins (PBPs) of drug-resistant bacteria, adenovirus (AdV), and dengue virus (DeNV) in parallel with cytotoxicity against cultured cells. We discuss structures and biological activity of isolated compounds.

1. Anti-PBP molecules

As a lipophilic extract of the sponge *Luffariella variabilis* showed enzyme inhibition against PBP, it was separated to give new compounds **1** and **2**.[1] After obtaining the planar structures of **1** and **2** with spectral analyses, the sole configuration of **1** was elucidated by comparing experimental specific rotation value with that calculated. The absolute configuration of δ-lactone of **2** was elucidated with ECD, while the relative stereochemistry of decaline portion was confirmed by NOE study. The whole absolute stereochemistry of **2** was determined by NOE study on stable conformers with DFT calculation. Compound **1** showed IC_{50} 47.5 μM against PBP and cytotoxicity at IC_{50} 16.8 μM, while compound **2** showed cytotoxicity at IC_{50} 1.0 μM.



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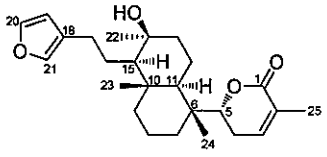

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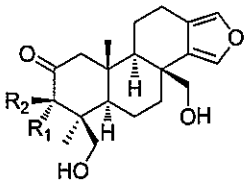
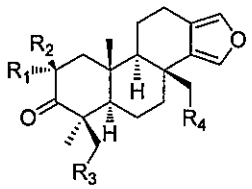
2. Anti-AdV molecules

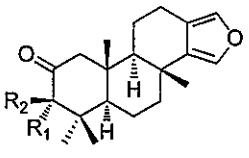
An extract of the sponge *Hyatella* aff. *intestinalis* showed moderate inhibition against AdV. After isolation work, a series of new and known spongian diterpenes **4-12** were isolated.[2] Their structures were elucidated mainly by NMR study. The absolute configuration of compound **5** was determined by ECD analysis. Three new compounds **4**, **5** and **6** did not show significant antiviral activity, while known spongiatriol (**9**) and isospongiatriol (**7**) showed antiviral activity at IC_{50} 17.0 and 52.0 μM, respectively.



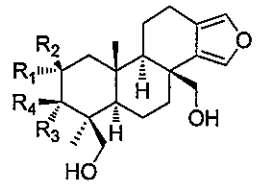
8 R_1 = H, R_2 = OH
9 R_1 = OH, R_2 = H



4 R_1= OH, R_2 = R_3 = R_4 = H
7 R_1= R_3 = R_4 = OH, R_2 = H
10 R_1= R_2 = R_4 = H, R_4 = OH



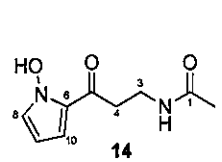
5 R_1 = H, R_2 = OH
11 R_1 = OH, R_2 = H



6 R_1 = R_3 = OAc, R_2 = R_4= H
12 R_1 = R_3 = H, R_2 = R_4 = OH

3. Anti-DeNV molecules

Our collaborators screened marine microbial extracts against DeNV, and four of them were examined by us. As results, two new (**14** and **18**) and six known compounds **13**, **15-17**, **19-20** were characterized. Compound **14** was spectroscopically elucidated as a pyrrole derivative having *N*-hydroxyl moiety. Compound **18** showed similar signals to those of cycloheximide (**16**) in ^{1}H NMR spectrum. The stereochemistry of compound **18** is based on its NOE and comparison to reference data. Among the isolates, cycloheximide (**16**) and anisomycin (**17**) showed strong antiviral activity at IC_{50} 0.02 and 0.03 μM, respectively.



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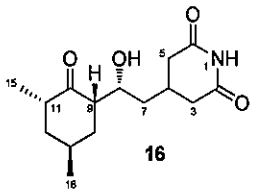

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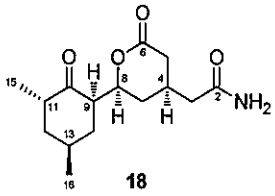

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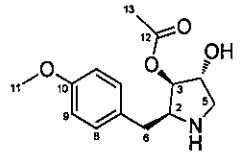

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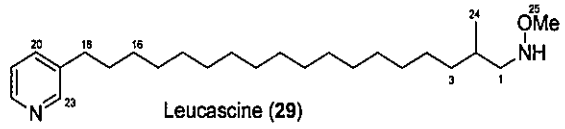
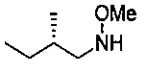
Due to the small amount of compounds **14** and **18**, we recultured the bacteria. Although the bacteria did not produce the same compounds, they produced other compounds **21-26** instead. Compound **26** was elucidated as a new compound, while others were identified as diketopiperazines.

4. Cytotoxic molecules

A new pyridine alkaloid named leucascine (**29**) was isolated from the sponge *Leucascus protogenes*. After spectral interpretation, the structure was elucidated as an analogue of cribochalines. To determine the sole configuration, Mosher's method (*R*/*S*-MPA) was applied to give amides **30-31**. A methyl signal (H-24) of **30** (**31**) appeared in a ratio of 2.8:1 (1:2.8) probably due to the presence of sp (synperiplanar) and ap (antiperiplanar) conformers. To confirm the phenomena, we tried to prepare model amides **35-36** from **34**. The amide **35** (**36**) showed signal separation, however, the ratio 1.6:1.4 (1.4:1.6) was quite different possibly due to racemization during preparation. Further trial is on the way. Compound **29** showed cytotoxicity at IC_{50} 9.2 μM.



Leucascine (**29**)



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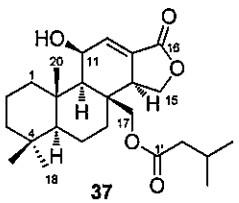
Four spongian diterpenes **37-40**, showing cytotoxicity at IC_{50} 3.1, 1.9, 8.4, and 3.1 μM, respectively, have been isolated from the sponge *Dysidea* cf. *arenaria*.[3] We also characterized a new sesquiterpene **42**[4] and isospongian diterpenes **45-46**.[5]



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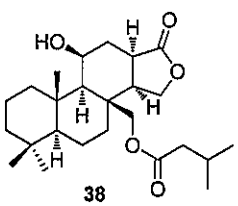

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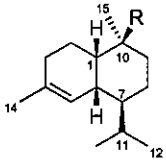

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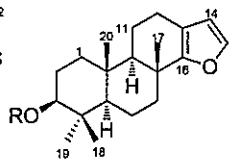
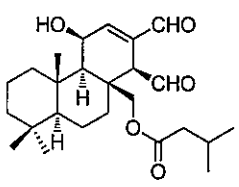
44 R = NCS



45 R = H
46 R = Ac



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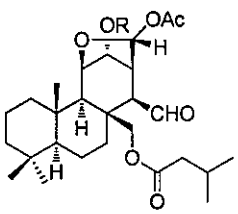
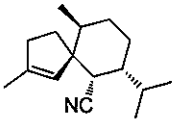


40 R = Ac
41 R = H



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References:
1. *Marine Drugs* **2017**, *15*, 249.
2. *Chem. Pharm. Bull.* **2017**, 65, 874-877.
3. *Chem. Pharm. Bull.* **2016**, *64*, 272-275.
4. *Nat. Prod. Commun.* **2015**, *10*, 1913-1914.
5. *Nat. Prod. Commun.* **2017**, *12*, 1011-1012.