Isolation of Salsolinol, a Tetrahydroisoquinoline Alkaloid, from the Marine Sponge Xestospongia cf. vансоestи as a Proteasome Inhibitor

Yumiko NAGASAWA,* a Reiko UEOKA,a Rumi YAMANOKUCHI,a Naoki HORIUCHI,b Tsuyoshi IKEDA,a Henki ROTINSULU,c,d Remy E. P. MANGINDAAAN,e Kazuyo UKAI,d Hisayoshi KOBAYASHI,f Michio NAMIKOSHI,e Hiroshi HIROTAb,e Hideyoshi YOKOSAWAb and Sachiko TSUKAMOTO*a,b

a Graduate School of Pharmaceutical Sciences, Kumamoto University; Kumamoto 862–0973, Japan; b Graduate School of Science, Chiba University; Inage-ku, Chiba 263–8522, Japan; c Faculty of Agriculture, Universitas Pembangunan Indonesia; Manado 95361, Indonesia; d Tohoku Pharmaceutical University; Aoba-ku, Sendai 981–8588, Japan; e Faculty of Fisheries and Marine Science, Sam Ratulangi University; Kampus Bahu, Manado 95115, Indonesia; f Institute of Molecular and Cellular Biosciences, University of Tokyo; Bunkyo-ku, Tokyo 113–0032, Japan; g RIKEN Genomic Sciences Center; Tsurumi-ku, Yokohama 230–0045, Japan; and h School of Pharmacy, Aichi Gakuin University; Chikusa-ku, Nagoya 464–8650, Japan.

Received October 7, 2010; accepted November 16, 2010; published online November 18, 2010

Salsolinol (1), a tetrahydroisoquinoline alkaloid, was isolated from the marine sponge Xestospongia cf. vансоestи collected in Indonesia as a proteasome inhibitor, along with three salsolinol derivatives, norsalsolinol (2), cis-4-hydroxysalsolinol (3), and trans-4-hydroxysalsolinol (4). Compounds 1 and 2 inhibited the chymotrypsin-like activity of the proteasome with IC50 values of 50 and 32 μg/ml, respectively, but 3 and 4 showed no inhibitory effect even at 100 μg/ml.

Key words tetrahydroisoquinoline alkaloid; proteasome; chymotrypsin-like activity; marine sponge

The proteasome functions as a proteolytic machine in the ubiquitin-dependent proteolytic pathway called the ubiquitin-proteasome system.1,2 The 26S proteasome consists of two subcomplexes, the 20S core particle (the 20S proteasome) and the 19S regulatory particle, and a client protein is degraded by the proteolytic active sites of the 20S proteasome after polyubiquitination. The 20S proteasome is classified as a threonine protease that contains two pairs of three different sites, which catalyze chymotrypsin-like, trypsin-like, and caspase-like activities. Since the level of proteasome activity is increased especially in tumor cells, it is reasonable to develop specific compounds targeting the ubiquitin-proteasome system for cancer treatment. The recent approval of bortezomib (PS-341, Velcade®), a synthetic proteasome inhibitor, for the treatment of relapsed multiple myeloma has opened the way to the discovery of drugs targeting the proteasome.3,4 Significantly, bortezomib is effective against various tumor cells that are resistant to conventional chemotherapeutic agents.5) Currently, three proteasome inhibitors, salinosporamide A (NPI-0052),5–7 carfilzomib (PR-171),9,10 and CEP-18770,11,12 are undergoing phase I and II clinical trials. So far, structurally-diverse proteasome inhibitors have been developed by chemical synthesis and also by searching natural sources and chemical libraries as drugs for the clinical treatment of cancer and also as molecular tools for the investigation of cellular events.13

During a search for natural products exhibiting biological activity, we screened extracts of natural sources for inhibitory activity against the proteasome. To date, based on the inhibition of the chymotrypsin-like activity of the proteasome, we isolated agosterols,14 mycalolides,15 cis-hinokiresiol,16 and aaptamine derivatives17 from natural sources as proteasome inhibitors. Here, we report the isolation of salsolinol (1) and its derivatives (2—4) from the marine sponge Xestospongia cf. vансоestи, and their two biological activities, inhibitory activity against the chymotrypsin-like activity of the proteasome and cytotoxic activity.

Specimens of Xestospongia cf. vансоestи were collected in Indonesia. The EtOH extract of the sponge was evaporated, and the aqueous residue was extracted with EtOAc and then n-BuOH. The n-BuOH (6.0 g) and H2O fractions, which showed inhibitory activity against the chymotrypsin-like activity of the proteasome and cytotoxicity, were subjected to octadecyl silyl (ODS) column chromatography and ODS HPLC to afford salsolinol (1, 191.8 mg, 0.048% wet weight), norsalsolinol (2, 4.96 mg, 0.0012%), cis-4-hydroxysalsolinol (3, 1.35 mg, 0.00034%), and trans-4-hydroxysalsolinol (4, 3.68 mg, 0.00092%) (Chart 1).

The 1H-NMR spectrum of salsolinol (1) showed a doublet methyl signal at δ 1.58 (d, J=6.4 Hz), two pairs of methylene signals at δ 2.86/2.95 and 3.30/3.46, and a methine signal at δ 4.10 (2H, s). The 13C-NMR spectrum of 1 showed six hydrocarbon carbons at δ 129.7 (CH2), 130.1 (CH2), 130.9 (CH2), 133.3 (CH), 135.5 (CH), and 146.0 (CH), and the latter two indicated to be oxygenated by chemical shifts. Analysis of 2D-NMR data strongly suggested that 1 was 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol).18 The 1H- and 13C-NMR spectra of norsalsolinol (2) indicated that the methyl group at C-1 in 1 is replaced with a hydrogen atom in 2 to be a methylene group, δ10 4.10 (2H, s) and δ11 4.96 (2H, t, J=6.4 Hz).
43.9 (CH$_3$), which was supported by the FAB-MS spectrum. Thus, 2 was identified as 6,7-dihydroxy-1,2,3,4-tetrahydro-isoquinoline (norsalsolinol).\textsuperscript{19} The molecular formulas of the cis- (3) and trans-4-hydroxysalsolinols (4), C$_{10}$H$_{13}$NO$_3$, were established by high resolution (HR)-FAB-MS and both isomers were found to contain an additional oxygen atom when compared to 1. Their NMR spectra were similar to those of 1, except for the absence of the methylene group in 1 when compared to 3 and 4. Analysis of 2D-NMR data indicated that 3 and 4 were 4-hydroxy derivatives of 1. Although 3 and 4 have yet to be isolated from natural sources, they have been chemically synthesized.\textsuperscript{20} From the NMR data, 3 and 4, isolated from the sponge, were identified as cis and trans isomers, respectively. The specific rotation of 1, [\(\alpha\)]$_{D}$ 0, indicated a racemic mixture, as confirmed by flat circular dichroism (CD) curves from 200 to 400 nm. The racemic character of 1 could be rationalized by considering its biosynthetic pathway (Chart 2), in which it is generated by the condensation of dopamine and acetaldelyde. On the other hand, 3 and 4 would be biosynthesized by the condensation of (–)-noradrenaline and acetaldelyde, and so have the 4R configuration (Chart 2). We prepared 3 and 4 from (–)-noradrenaline and measured the specific rotations. However, the values of the synthetic 3 and 4 were almost zero and we could not determine the absolute configurations on the basis of the values.

Thus, we isolated salsolinol (1), norsalsolinol (2), cis-4-hydroxysalsolinol (3), and trans-4-hydroxysalsolinol (4) from the marine sponge Xestospongia cf. vансoehst collected in Indonesia. Proteasome inhibitory and cytotoxic activities of 1 and 2 are listed in Table 2. Compounds 1 and 2 inhibited the chymotrypsin-like activity of the proteasome with IC$_{50}$ values of 50 and 32 \(\mu\)g/ml, respectively, and also showed cytotoxicity against human cervix epithelioid carcinoma (HeLa) cells with IC$_{50}$ values of 17 and 7 \(\mu\)g/ml, respectively. Compounds 3 and 4 showed no proteasome inhibitory activity even at 100 \(\mu\)g/ml and no cytotoxicity against HeLa cells at 50 \(\mu\)g/ml. In addition, the cytotoxicity of 1 was tested against murine leukemia (L1210), human amnion (FL), human oral epidermoid carcinoma (KB), and human lung adenocarcinoma (A549) cell lines. The respective IC$_{50}$ values were 8, 13, 20, and 27 \(\mu\)g/ml.

Salsolinol (1) is detected in human and animal brains, and is thought to be synthesized from dopamine.\textsuperscript{21} Kicha et al. isolated 1 as salts with steroidal sulfates from the starfish Lestherias nanimensis chelifera,\textsuperscript{18} and reported that the R-isomer was predominantly incorporated into the salts. Liu et al. found the ratio of (R)- to (S)-1 to be 1.6 in the cerebral ganglion of Aplysia californica and 1.07 in a dried banana, by gas chromatography-mass spectrometry with cyclodextrin chiral columns.\textsuperscript{25} In addition, 1 has been isolated from Asclepiadaceae,\textsuperscript{23} Papaveraceae,\textsuperscript{23} and Aristolochiaceae\textsuperscript{24} plants. However, this is the first time that 1 has been isolated from a marine sponge and it should be noted that its yield, 0.048% (wet weight), is relatively high. As 2 was detected in dopamine-rich areas of the human brain\textsuperscript{25,26} and both 3 and 4 have been only chemically synthesized,\textsuperscript{20} this is the first isolation of 2–4 as secondary metabolites from a natural source.

In mammalian brains, salsolinol (1) has neurotoxic effects in dopaminergic cells, and its metabolites, including its N-
methylated derivative N-methylsalsolinol, are implicated in the etiopathogenesis of Parkinson’s disease.\textsuperscript{21} In dopaminergic
glioblastoma SH-SY5Y cells, I causes both apoptosis and necrosis.\textsuperscript{27} Despite its neurotoxic activity, I induces the
release of prolactin from the anterior lobe of the pituitary gland by binding an unknown receptor,\textsuperscript{1,12,30} implying that it functions as a neuremodulator.

In this study, we first found that salsolinol (\(1\)) and its deri-

Experimental

Optical rotations were determined with a JASCO P-1020 polarimeter in
\(H_2O\). CD spectra were measured on a JASCO J-720WI spectropolarimeter in
MeOH. NMR spectra were recorded on a Bruker Avance 500 NMR spec-
trometer in CD\(_3\)OD or D\(_2\)O. Chemical shifts in CD\(_3\)OD were referenced to
the residual solvent peaks, \(\delta_{3.3}\) and \(\delta_{4.90}\). In D\(_2\)O, chemical shifts of
\(^1\)H-NMR were referenced to the peak of residual solvent at \(\delta_{4.65}\), and those of 
\(^1\)C-NMR to the peak of dioxygen at \(\delta_{6.65}\). Mass spectra were measured on a JMX AX-500 or JMS HX-110 mass spectrometer.

Extraction and Isolation

The marine sponge was collected at a depth of 10 m in
North Sulawesi, Indonesia, in September 2006 and soaked in
EtOH immediately. The sponge was identified as Xestospongia cf. vanessai.
A voucher specimen (RMNH POR. 4807) has been deposited in the Na-
tional Museum of Natural History, The Netherlands. The sponge (400 g, wet
weight) was extracted with EtOH. The extract was evaporated, and the aque-
ous residue was extracted with EtOAc and then n-BuOH fraction. The n-BuOH frac-
tion (6.0 g) and H\(_2\)O fraction showed inhibitory activity against the
chymotrypsin-like activity of the proteasome and cytotoxicity. The n-BuOH fraction
was partitioned between hexane and 90% MeOH-H\(_2\)O, and the methanolic fraction (5.0 g) was subjected to ODS column chromatography
with a step-wise gradient using H\(_2\)O and MeOH. The fraction eluted with
methanolic fraction (5.0 g) was subjected to ODS column chromatography
with our findings, it should be noted that dopamine inhibits a
dopaminergic neuron death possibly through the inhibition of the pro-
teasome. Thus, it can be inferred that I induces dopaminergic
neuron death possibly through the inhibition of the pro-
teasome.

Proteasome Inhibition Assay

The fluorogenic compound Suc-Leu-Leu-Val-Tyr-MCA (Peptide Instru-
mens, Inc., Osaka, Japan) was used as a substrate for the chymotrypsin-like activity of the 20S proteasome preparation from human, erythrocyte (Biosearch, Inc.). The proteasome (0.5 \(\mu\)l in a mixture (100 \(\mu\)l) that contained 50 mM Tris–HCl, \(pH\) 7.8, 1 mM dithiothreitol, 5 mM ethylenediaminetetraacetic acid (EDTA), and 0.02% sodium dodecyl sulfate (SDS) was pre-incubated with test compounds at various concentrations at \(30^\circ\)C for 10 min. Then the substrate (10 \(\mu\)l) was added and the mixture was further incubated at \(30^\circ\)C for 16 h. The reaction was
stopped by adding 100 \(\mu\)l of 10% SDS and the fluorescence intensity owing to 7-amino-4-methylcoumarin (AMC) was measured (excitation, 360 nm; emission, 450 nm) with the microplate reader. The \(IC_{50}\), the concen-
tration required for 50% inhibition of the proteasome inhibitory activity, was
calculated from duplicate measurements.

Acknowledgments

We thank Dr. N. J. de Voogd of the National Mu-
museum of Natural History and Dr. R. W. M. van Soest of the University
of Amsterdam for identification of the sponge. This work was

References

4. Adams J., Behnke M., Chen S., Crichtshank A. D., Dick L. G., Gre-

7. Macherla V. R., Mitchell S. S., Manan R. R., Reed K. A., Chao T.-H.,
Nicholson B., Deyanat-Yazdi G., Ma B., Jensen P. R., Fenical W. F.,
Neuteboem S. T. C., Lam K. S., Palladino M. A., Potts B. C. M.,
8. Fenical W., Jensen P. R., Palladino M. A., Lam K. S., Lloyd G. K.,
9. Kish P. D. J., Chen Q. Y., Zhan X. C., Jones M. T., Polak J. K., Sun C.
M., Demo S. D., Bennett M. K., van Leeuwen F. W., Chan-Vahan-Khan A.
7091 (2009).
11. Dorsey B. D., Iqbal M., Chatterjee S. M., Gupta E., Bernardini R.,
Bennardelli C., Bassareggii A., Cassarà P. G., D’Arasso G., Feretti E.,
Demuri S., Oliva A., Pezzoni G., Allievi C., Sterrponi L., Ruggeri B.,
1078 (2008).
12. Sanchez E., Li, Steinberg J. A., Wang C., Shen J., Bonavida B., Li
Z. W., Chen H., Berenson J. R., Br. J. Haematol., 148, 569—581
(2009).
14. Tsukamoto S., Tatsuno M., van Soest R. W. M., Yokosawa H., Oikawa T.,
16. Tsukamoto S., Wakana T., Koimaru K., Yoshida T., Sato M., Oikawa T.,
17. Tsukamoto S., Yamanouchi R., Yoshitomi M., Sato K., Ikeda T.,
Rotinsulu H., Mangindaan R. E. P., de Voogd N. J., van Soest R. W.
18. Kicha A. A., Ivanichina N. V., Kalinovsky A. I., Dmitrenok P. S.,
19. Kobayashi H., Fukuhara K., Tada-Oikawa S., Yada Y., Hiraku Y.,