NOTE

Avarol Effectively Inhibits *Bacillus cereus* Growth

B. PEJIN^{1,*}, T. KARTALI², B. STANIMIROVIC³ and M. KARAMAN²

¹University of Belgrade, Institute for Multidisciplinary Research - IMSI, Department of Life Sciences, Kneza Viseslava 1, 11030 Belgrade, Serbia

²University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovica 2, 21000 Novi Sad, Serbia

³Institute MOL Ltd, Nikole Tesle 15, 22300 Stara Pazova, Serbia

*Corresponding author: E-mail: brspjn@gmail.com; boris.pejin@imsi.rs

Received: 21 March 2014;

Accepted: 15 April 2014;

Published online: 15 November 2014;

AJC-16338

This work extends *in vitro* screening of antibacterial activity of avarol on four selected pathogenic bacteria associated with psoriasis (*Bacillus cereus*, *Clostridium sporogenes*, *Enterococcus faecalis* and *Staphylococcus aureus*). Avarol exhibited a moderate activity with minimum inhibitory and minimum bactericidal concentrations ranged from 0.78-1.56 and 3.12-18.75 µg/mL, respectively. It proved to be more effective against *B. cereus* in comparison to the both positive controls applied, gentamycin and ampicillin. According to the experimental data, this marine natural product may inspire development of new antibiotics with an enhanced therapeutic index, at first place those targeting *B. cereus*.

Keywords: Porifera, *Dysidea avara*, Marine natural product, Antibacterial activity, Psoriasis.

Sponges are sessile organisms with a specific chemical defense of great interest for the natural product chemists. Indeed, their terpenoid quinones and hydroquinones have attracted the most attention¹.

Avarol, a marine sesquiterpenoid hydroquinone possessing a rearranged drimane skeleton, is the main secondary metabolite of the Mediterranean sponge *Dysidea avara* Schmidt^{2,3}. Pharmacological properties of this natural product include antibacterial, antifungal and antiviral activities⁴. It has shown to be active against a variety of Gram-positive bacterial and some fungal species. On the other hand, no antibacterial activity has been found for Gram-negative bacteria⁵⁻⁷.

Avarol exhibits antipsoriatic activity as well⁸. Indeed, recent studies performed in human keratinocytes showed that avarol decreased the production of skin-derived antileukoprotease (SKALP/Elafin) determined as index of psoriatic keratinocyte differentiation, without affecting cell proliferation. It is supposed that observed biological activity is partially mediated by the downregulation of TNF-α and NF-κB in psoriatic skin⁹. Psoriasis is a common skin disease characterized by epidermal hyperplasia, inflammation in dermis and epidermis and leukocyte infiltration¹⁰. However, its pathogenesis still remains unclear. Pathogenic organisms that have been associated with psoriasis include *Bacillus cereus*, *Clostridium* sp., *Enterococcus faecalis* and *Staphylococcus aureus*^{11,12}. Anthralin is among the most widely used drugs in

the treatment of psoriasis. However, its clinical efficacy is limited by the side-effects of irritation and staining of the uninvolved skin¹³.

The low toxicity in male mice (lethal dose for 10 % of the mice kill is 111 mg) and no side effects in men (at the dose of 3 mg/kg)^{14,15}, prompted us to extend *in vitro* antibacterial screening of avarol related to psoriasis. The screening has included four Gram-positive bacteria, namely *Bacillus cereus* ATCC 11778, *Clostridium sporogenes* ATCC 19404, *Enterococcus faecalis* ATCC 19433 and *Staphylococcus aureus* ATCC 25923. Avarol was isolated from the sponge *D. avara*, collected in the Bay of Kotor (Kotor, Montenegro) as previously reported^{2,3}, while its antibacterial activity was evaluated *in vitro* by a microdilution method¹⁶.

This marine natural product exhibited good activity against all bacteria tested with minimum inhibitory (MIC) and bactericidal (MBC) concentrations ranged from 0.78-1.56 and 3.12-18.75 µg/mL, respectively (Table-1).

The most sensitive bacterium was *B. cereus* with MIC/MBC values reached at 0.78 and 3.12 µg/mL, respectively. In comparison, both positive controls used, gentamycin and ampicillin, were less effective towards this strain (MIC/MBC values 2.50/10.00 µg/mL and 2.00/4.00 µg/mL, respectively). It's worth noting that avarol did show selectivity against the Gram-positive bacteria screened inhibiting *B. cereus* and *E. faecalis* in a bactericidal rather than in a bacteriostatic manner

TABLE-1
MINIMUM INHIBITORY (MIC) AND BACTERICIDAL (MBC) CONCENTRATIONS OF AVAROL

Bacteria	Avarol ^{xy}	Gentamycin ^{xy}	Ampicillin ^{xy}
<i>Bacillus</i>	0.78 ± 0.02	2.50 ± 0.03	2.00 ± 0.06
<i>cereus</i> ATCC 11778	3.12 ± 0.03	10.00 ± 0.02	4.00 ± 0.08
<i>Clostridium</i>	1.56 ± 0.06	2.50 ± 0.02	64.00 ± 0.06
<i>sporogenes</i> ATCC19404	18.75 ± 0.03	>20	>128
<i>Enterococcus</i>	1.56 ± 0.03	5.00 ± 0.06	16.00 ± 0.03
<i>faecalis</i> ATCC 19433	6.25 ± 0.06	>20	>128
<i>Staphylococcus</i>	1.56 ± 0.08	2.50 ± 0.06	8.00 ± 0.06
<i>aureus</i> ATCC 25923	12.50 ± 0.06	10.00 ± 0.03	>128

*MIC/MBC (µg/mL), ^ypositive control, ^xp < 0.05

(MBC/MIC = 4). This study confirms avarol potential related to psoriasis and indicates the need for the antibacterial activity screening at *in vivo* conditions. Indeed, its chemical structure may inspire development of new and more effective antibiotics targeting *B. cereus*, the bacterium known to cause chronic skin infections that are difficult to eradicate.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Research grants Nos. 172053 and 172058). The authors gratefully acknowledge Prof. Dusan Sladic (University of Belgrade, Faculty of Chemistry, Department of Organic Chemistry) and Dr. Irena Novakovic (University of Belgrade, ICTM, Center of Chemistry) for providing the sample of avarol.

REFERENCES

1. D. Sladic and M.J. Gasic, *Molecules*, **11**, 1 (2006).
2. L. Minale, R. Riccio and G. Sodano, *Tetrahedron Lett.*, **15**, 3401 (1974).
3. S. De Rosa, L. Minale, R. Riccio and G. Sodano, *J. Chem. Soc., Perkin Trans. 1*, 1408 (1976).
4. M.J. Gašić, *J. Serb. Chem. Soc.*, **53**, 229 (1988).
5. L. Cariello, L. Zanetti, V. Cuomo and F. Vanzanella, *Comp. Biochem. Physiol. B*, **71**, 281 (1982).
6. G. Seibert, W. Raether, D. Dogovic, M.J. Gašić, R.K. Zahan and W.E. Müller, *Zentralbl. Bakteriolog. Mikrobiol. Hyg. A*, **260**, 379 (1985).
7. B. Pejin, C. Iodice, G. Tommonaro and S. De Rosa, *J. Nat. Prod.*, **71**, 1850 (2008).
8. M. Amigó, M.C. Terencio, M. Mitova, C. Iodice, M. Payá and S. De Rosa, *J. Nat. Prod.*, **67**, 1459 (2004).
9. M. Amigó, M. Payá, A. Braza-Boils, S. De Rosa and M.C. Terencio, *Life Sci.*, **82**, 256 (2008).
10. P.F. Lizzul, A. Aphale, R. Malaviya, Y. Sun, S. Masud, V. Dombrovskiy and A.B. Gottlieb, *J. Invest. Dermatol.*, **124**, 1275 (2005).
11. M. Peslyak, Model of Pathogenesis of Psoriasis, Systemic Psoriatic Process, MYPE, Moscow, Part 1, pp. 84 (2012).
12. E.W. Rosenberg, P.W. Noah and R.B. Skinner Jr., *J. Natl. Med. Assoc.*, **86**, 305 (1994).
13. R. Parslew and P.S. Friedmann, *J. Dermatol.*, **141**, 469 (1999).
14. W.E.G. Müller, A. Maidhof, R.K. Zahn, H.C. Schröder, M.J. Gašić, D. Heidemann, A. Bernd, B. Kurelec, E. Eich and G. Seibert, *Cancer Res.*, **45**, 4822 (1985).
15. W.E.G. Müller, A. Sarin, Y. Kuchino, A. Dorn, G. Hess, K.-H. Meyer zum Büschenfelde, M. Rottmann and H.C. Schröder, in eds.: W. Vettermann and M. Schauzu, AIDS, Bundesministeriums für Forschung und Technologie, Bonn, p. 354 (1987).
16. M. Karaman, N. Mimica-Dukic, P. Knezevic, Z. Svircev and M. Matavuly, *Int. J. Med. Mushrooms*, **11**, 269 (2009).