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Syntheses of the marine alkaloids 6-oxofascaplysin, fascaplysin and their derivatives

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Abstract: A simple approach towards the pyrido[1,2-*a*:3,4-*b'*]diindole system *via* the reaction of indigo with methylene active compounds was used for the syntheses of the marine alkaloids 6-oxofascaplysin, fascaplysin, and their derivatives. It was also demonstrated that the reaction with ketones led to indigo decomposition and the formation of isatin derivatives. The derivative of fascaplysin with a phenyl substituent at C-7 demonstrated 2-3 times greater inhibitory activity against selected cancer cell lines than fascaplysin.

Dedicated to the memory of Dmitriy A. Maiboroda.

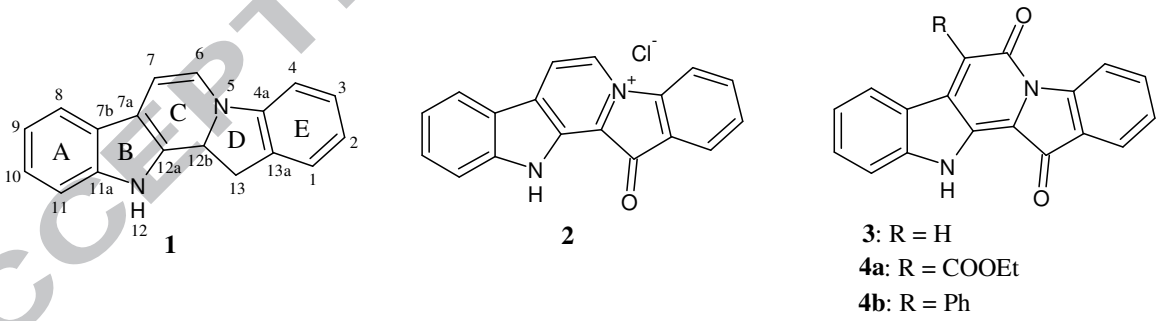
Keywords: Fascaplysin; Indigo; 6-Oxofascaplysin, Total synthesis; Isatin derivatives

Introduction

The 12*H*-pyrido[1,2-*a*:3,4-*b*']diindole ring system (**1**, Fig. 1) forms the framework of several marine alkaloids, such as fascaplysin, homofascaplysins A-C, and their brominated analogues.[1] The red pigment fascaplysin (**2**), isolated in 1988 from the sponge *Fascaplysinopsis* sp., is the most investigated representative alkaloid.[2] This compound exhibits a broad range of bioactivities including antibacterial, antifungal, antiviral, HIV-1-RT, p56 tyrosine kinase, and antimalarial properties, as well as suppressing the proliferation of numerous cancer cell lines.[3] In addition, fascaplysin inhibits the growth of S180 cell-implanted tumors and

possesses anti-angiogenesis properties.[4, 5] The mechanisms of action include the selective inhibition of cyclin-dependent kinase 4, which regulates the G_0–G_1/S checkpoint of the cell cycle, the intercalation of DNA, and the induction of apoptosis, in part, as a result of the activation of the TRAIL signaling pathway by upregulating DR5 expression.[6–8] Recent research has shown that selective CDK4/6 inhibitors not only induce tumour cell cycle arrest, but also promote anti-tumour immunity.[9] Besides its antitumor properties, fascaplysin triggers cell shrinkage and phospholipid scrambling of the erythrocyte cell membrane, an effect at least in part due to Ca^{2+} entry, oxidative stress and ceramide abundance.[10] It also could be used as a P-gp inhibitor for the development of anti-Alzheimer agents and as a "balanced agonist" of the opioid receptor with a signaling profile that resembles endorphins.[11, 12]

These facts demonstrate the significant potential of fascaplysin and related compounds for therapeutic assays. Recently, a novel representative of these alkaloids named 6-oxofascaplysin (**3**) was isolated from the Australian marine sponge *Hyrtios* sp.[13] The absence of some correlations in the HMBC spectra led the authors to utilise DFT NMR calculations to support their structural assignment. Herein, we report the development of a simple approach for the first synthesis of 6-oxofascaplysin and selected derivatives. A one-step transformation of 6-oxofascaplysin and its derivatives into fascaplysin and its derivatives which are inaccessible by other methods was also demonstrated.



1 **2**

3: R = H
4a: R = COOEt
4b: R = Ph

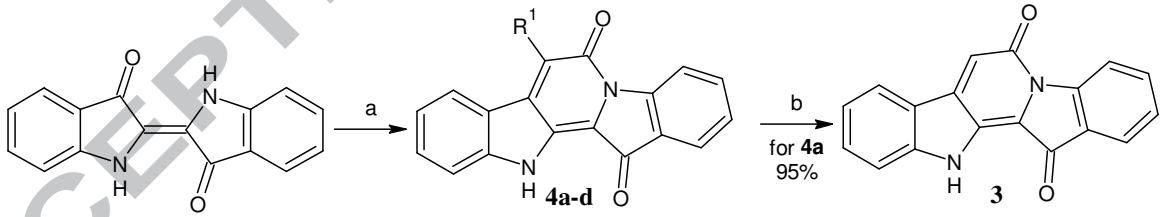
Figure 1. Structures of 12*H*-pyrido[1,2-*a*:3,4-*b'*]diindole (**1**), fascaplysin (**2**), 6-oxofascaplysin (**3**), and its known derivatives.

Fascaplysin and its naturally occurring analogues have been synthesized by several groups and more than ten syntheses have been reported to date.[14] However, none can be considered effective for the synthesis of 6-oxofascaplysin. At the same time, condensation of the well-known dye indigo with a malonic ester in the

presence of base followed by subsequent hydrolysis and decarboxylation of the obtained product **4a** would represent a simple approach towards 6-oxofascaplysin **3**. The obtainment of mixtures of highly functionalized pyrazino[1,2-*a*:4,3-*a*`]diindole, pyrido[1,2-*a*:3,4-*b*`]diindole and benzo[*b*]indolo[1,2-*h*]naphthyridine heterocyclic systems *via* the base-induced propargylation of indigo was previously reported.[15] Compound **4a** was previously obtained in 1923-1924 along with the product of condensation with ethyl phenylacetate in order to confirm the structure of indigo (Fig. 1).[16, 17] Recently, compound **4b** and series of related compounds were obtained by the condensation of selected arylacetic chlorides with *N,N*-diacetylindigo, a more soluble derivative of indigo.[18]

Results and Discussion

Our work began with the optimization of the preparation of compound **4a** using unsubstituted indigo as a starting material (Scheme 1). After preliminary experiments, sodium hydroxide was replaced by sodium hydride and DMF was used instead of nitrobenzene. For subsequent hydrolysis and decarboxylation reactions, compound **4a** was heated at reflux in 40% hydrobromic acid for two hours. A single compound was isolated with spectral characteristics which were identical to those of natural 6-oxofascaplysin. The overall yield of the target compound was 70%.

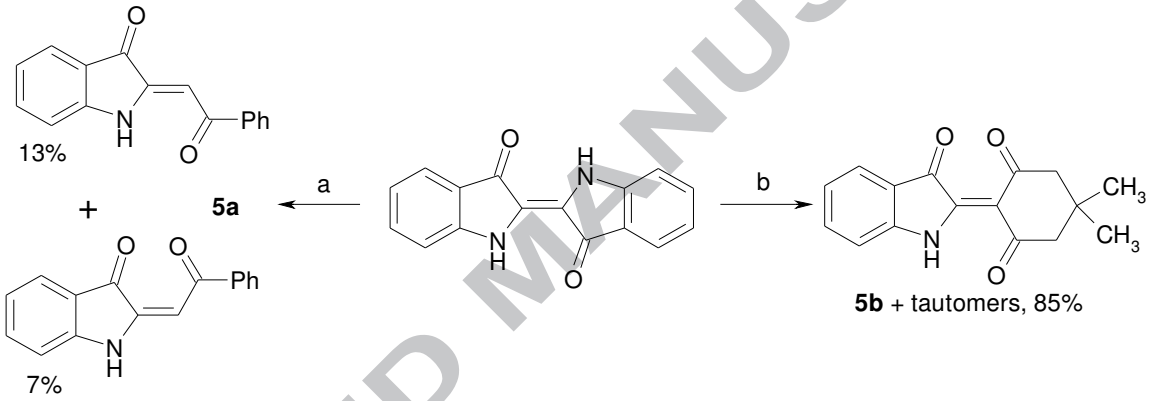


a: R^1 = COOEt 75%
a: R^1 = Ph 55%
c: R^1 = $CO\text{-}CH_3$ 88%
d: R^1 = CN 50%

Scheme 1. Syntheses of 6-oxofascaplysin (**3**) and its derivatives (**4a-d**) Reagents and conditions: (a) R-CH_2-COOEt (8 equiv.), NaH (4 equiv.), DMF, Δ, 0.5 h; (b) 40% HBr (excess), Δ, 2 h

After the successful synthesis of 6-oxofascaplysin **3**, the condensation reaction was applied to acetoacetic, cyanoacetic, phenylacetic and nitroacetic esters. In most cases, the condensation was successful with yields ranging from 50-88% (**4b-d**).[19] However, when nitroacetic ester was used the reaction did not occur.

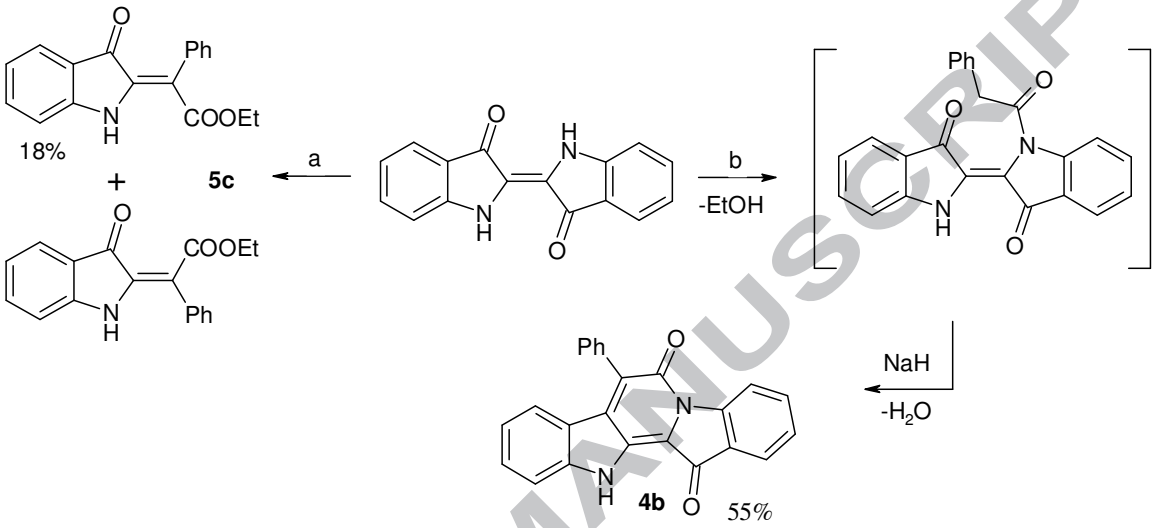
Unfortunately, the poor solubility of 6-oxofascaplysin and its derivatives **4a-d** in both water and organic solvents such as DMSO and DMF prevented an investigation of their bioactivities. At the same time, it can be expected that the reaction of indigo and a wide range of ketones should allow the preparation of fascaplysin derivatives with substitution at the C-6 and C-7 positions (Fig. 1). Due to that fact, the reaction of indigo with acetophenone as a model compound was investigated. According to the spectral data, this resulted in a mixture of *Z*-, and *E*-isomers of compound **5a** (Scheme 2). The *E*-isomer was unstable and spontaneously converted into the *Z*-isomer. The reaction of more reactive dimedone resulted in the formation of a mixture of indigo decomposition product **5b** and its tautomers with 85% total yield (Scheme 2, see also the ESI).



Scheme 2. Reactions of indigo with acetophenone (a) and dimedone (b). Reagents and conditions: (a) acetophenone (1.5 equiv.), NaH (5 equiv.), DMF, Δ, 0.5 h; (b) dimedone (5 equiv.), NaH (7 equiv.), DMF, Δ, 0.5 h.

The obtained data can be easily explained within the framework of the HSAB principle. However, when indigo reacts with ethyl phenylacetate both variants of this reaction can be realized depending on the conditions utilized (Scheme 3). Thus, upon the slow addition of ethyl phenylacetate to indigo and excess sodium hydride, the product of indigo decomposition **5c** was obtained. As indicated above for compound **5a**, a mixture of geometric isomers was obtained, consisting of an unstable *E*-isomer that spontaneously transformed into the *Z*-isomer. On the other hand when indigo and ethyl phenylacetate were mixed in advance then heated in the presence of NaH, the main reaction product was compound **4b**. Thereby it can be assumed that the reaction of indigo with active methylene compounds is determined by their methylene activities that result in the prevalence of one of two different processes: i) Michael reaction between the

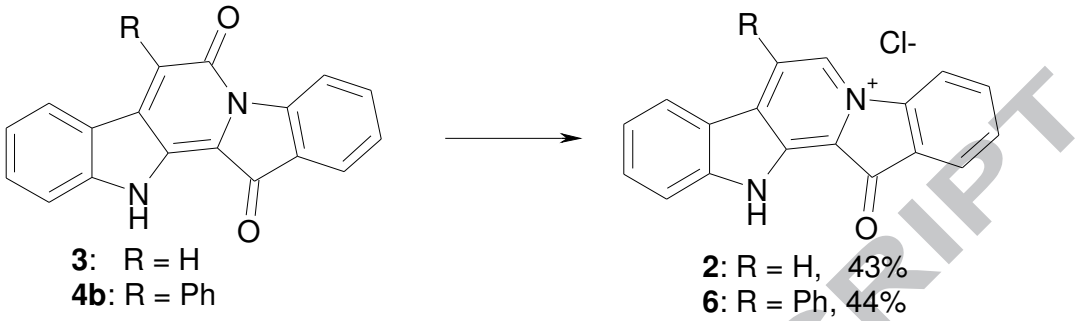
deprotonated methylene active compound and the activated double bond of indigo followed by the decomposition of indigo and formation of products **5**; ii) acylation of the nitrogen atom of indigo followed by intramolecular nucleophilic attack on the "opposite" carbonyl group with formation of the heterocyclic 12*H*-pyrido[1,2-*a*:3,4-*b*']diindole system.



Scheme 3. Reactions of indigo with ethyl phenylacetate under different conditions**.** Reagents and conditions: (a) NaH (4 equiv.), then slowly add $PhCH_2COOEt$ (10 equiv.), DMF, r.t., 30 min, then Δ, 30 min; (b) Ph-CH_2-COOEt (8 equiv.), then NaH (4 equiv.), Δ, 30 min.

Since the anticipated fascaplysin derivatives with substitution at the C-6 and C-7 positions were not obtained, it was decided to investigate the potential application of compounds **4** as starting materials for the preparation of fascaplysin derivatives. For the development of this conversion, 6-oxofascaplysin was chosen as a model compound and the formation of fascaplysin was explored (Scheme 4). Initially lithium aluminum hydride was examined for the reduction of the 2-pyridone fragment. The reaction of 6-oxofascaplysin with $LiAlH_4$ was carried out in THF for 24 hours at room temperature and followed by air oxidation, resulting in a complex mixture of compounds containing trace amounts of fascaplysin. Numerous attempts to optimize the reaction conditions were unsuccessful. Replacement with the more selective BH_3·THF complex in THF at reflux for two hours followed by hydrolysis and air oxidation gave fascaplysin in 43% isolated yield (see the ESI for a comparison of the $^{1}H/^{13}C$ NMR data from natural and synthetic fascaplysin). The product of the condensation of indigo and ethyl

phenylacetate (**4b**) under the developed conditions afforded compound **6** in 44% yield.



Scheme 4. Syntheses of fascaplysin and its derivative **6**. Reagents and conditions: $BH_3 \cdot THF$ (24 equiv.), THF, Ar, Δ, 2 h, then H_2O/H^+, air, Δ, 2 h.

Preliminary *in vitro* bioassays for compound **6** showed its activity against HeLa (cervix cancer) and THP-1 (acute monocytic leukemia) cell lines with IC_{50} values of 290 and 320 nM, respectively, compared to 550 and 890 nM, respectively, for fascaplysin. To explain the obtained data, the molecular docking of fascaplysin and its derivative **6** with a model of cyclin-dependent kinase 4 was carried out. The results of this experiment in the program Autodock 4 indicated the larger binding energy for compound **6** in comparison with fascaplysin (-9.55 and -8.67 kcal/mol, respectively). Based on the analysis of the theoretical complex (see ESI), the large substituent does not violate the structure of intermolecular contacts specific for fascaplysin, but allows the formation of additional interactions that increases the affinity of this compound to the therapeutic target.[20]

Conclusion

In conclusion, a simple one-step approach was developed for the synthesis of the heterocyclic 12*H*-pyrido[1,2-*a*:3,4-*b'*]diindole system from indigo. This method was used for the first synthesis of the marine alkaloid 6-oxofascaplysin and the confirmation of its proposed structure. Preliminary *in vitro* tests of the fascaplysin C-7 derivative demonstrated the potential of related compounds for therapeutic assays and will necessitate further investigations of their syntheses and detailed studies of the structure activity relationships among these potentially bioactive substances.

Acknowledgments

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References

1. Bharate, S. B.; Manda, S.; Mupparapu, N.; Battini, N.; Vishwakarma, R. A. *MiniRev. Med. Chem.* **2012**, *12*, 650–664.
2. Roll, D. M.; Ireland, C. M.; Lu, H. S.; Clardy, J. *J. Org. Chem.***1988**, *53*, 3276–3278.
3. For example, see: (a) Jimenez, C.; Quinoa, E.; Adamczeski, M.; Hunter, L. M.; Crews, P. *J. Org. Chem.* **1991**, *56*, 3403–3410; (b) Schmidt, E. W.; Faulkner, D. J. *Tetrahedron Lett.* **1996**, *37*, 3951–3954; (c) Kirsch, G.; Konig, G. M.; Wright, A.D.; Kaminsky, R. *J. Nat. Prod.* **2000**, *63*, 825–829; (d) Charan, R. D.; McKee, T. C.; Gustafson, K. R.; Pannell, L. K.; Boyd, M. R. *Tetrahedron Lett.* **2002**, *43*, 5201–5204; (e) Popov, A. M.; Stonik, V. A. *Antibiot. Khimioter.* **1991**, *36*, 12–14; (f) Hamilton, G. *Mar. Drugs.* **2014**, *12*, 1377-1389.
4. Yan, X.; Chen, H.; Lu, X.; Wang, F.; Xu, W.; Jin, H.; Zhu, P. Eur. *J. Pharm. Sci.* **2011**, *43*, 251–259.
5. Zheng, Y.; Lu, X.; Lin, J.; Chen, H.; Yan, X.; Wang, F.; Xu, W. *Biomed. Pharmacother.* **2010**, *64*, 527–533.
6. Soni, R.; Muller, L.; Furet, P.; Schoepfer, J.; Stephan, C.; Zunstein-Mecker, S.; Fretz, H.; Chaudhuri, B. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 877–884.
7. Hormann, A.; Chaudhuri, B.; Fretz, H. *Bioorg. Med. Chem.* **2001**, *9*, 917–921.
8. (a) Lu, X.; Zheng, Y.; Chen, H.; Yan, X.; Wang, F.; Xu, W. *Yao XueXueBao* **2009**, *44*, 980–986; (b) Wang, F.; Chen, H.; Yan X.; Zheng Y. *Chin. J. Oceanol. Limnol.* **2013**, *31*, 560-569.
9. Goel, S.; DeCristo M. J.; Watt A. C.; Jones H. B.; Sceneay J.; Li B. B.; Khan N.; Ubellacker J. M.; Xie S.; Metzger-Filho O.; Hoog J.; Ellis M. J.; Ma C. X.; Ramm S.; Krop I. E.; Winer E. P.; Roberts T. M.; Kim H.-J.; McAllister S. S.; Zhao J. J. *Nature* **2017**, *548*, 471–475.

10. Mischitelli, M.; Jemaà, M.; Almasry, M.; Faggio, C.; Lang, F. *Cell Physiol Biochem.* **2016**, *39*, 1638-1647.
11. Manda, S.; Sharma, S.; Wani, A.; Joshi, P.; Kumar, V.; Guru, S. K.; Bharate, S. S.; Bhushan, S.; Vishwakarma, R. A.; Kumar, A.; Bharate, S. B. *Eur J Med Chem.* **2016**, *107*, 1-11.
12. Johnson, T. A.; Milan-Lobo, L.; Che, T.; Ferwerda, M.; Lambo, E.; McIntosh, N. L.; Li, F.; He, L.; Lorig-Roach, N.; Crews, P.; Whistler, J. L. *ACS Chem Neurosci.* **2017**, *8*, 473-485.
13. Khokhar, S.; Feng, Y.; Campitelli, M. R.; Ekins, M. G.; Hooper, J. N. A.; Beattie, K. D.; Sadowski, M. C.; Nelson, C. C.; Davis, R. A. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3329–3332.
14. (a) Gribble, G. W.; Pelcman, B. *J. Org. Chem.* **1992**, *57*, 3636–3642; (b) Rocca, P.; Marsais, F.; Godart, A.; Quéguiner, G. *Tetrahedron Lett.* **1993**, *34*, 7917–7918; (c) Molina, P.; Fresneda, M.; Garciazafra, S.; Almendros, P. *Tetrahedron Lett.* **1994**, *35*, 8851–8854; (d) Radchenko, O. S.; Novikov, V. L.; Elyakov, G. B. *Tetrahedron Lett.* **1997**, *38*, 5339–5342; (e) Dubovitskii, S. V. *Tetrahedron Lett.* **1997**, *37*, 5207–5208; (f) Zhidkov, M. E.; Baranova, O. V.; Balaneva, N. N.; Fedorov, S. N.; Radchenko, O. S.; Dubovitskii, S. V. *Tetrahedron Lett.* **2007**, *48*, 7998–8000; (g) Waldmann, H.; Eberhardt, L.; Wittstein, K.; Kumar, K. *Chem. Commun.* **2010**, *46*, 4622–4624; (h) Zhidkov, M. E.; Baranova, O. V.; Kravchenko, N. S.; Dubovitskii, S. V. *Tetrahedron Lett.* **2010**, *51*, 6498–6499; (i) Bharate, S. B.; Manda, S.; Joshi, P.; Singh, B.; Vishwakarma, R. A. *Med. Chem. Commun.* **2012**, *3*, 1098-1103; (j) Zhidkov, M. E.; Kaminskii, V. A. *Tetrahedron Lett.* **2013**, *54*, 3530-3532; (k) Zhu, Y. P.; Liu, M.; Cai, Q.; Jia, F. C., Wu, A. X. *Chem. Eur. J.* **2013**, *19*, 10132 – 10137.
15. Shakoori, A.; Bremner, J. B.; Willis, A. C.; Haritakun, R.; Keller, P. A. *J. Org. Chem.* **2013**, *78*, 7639−7647.
16. Posner, T.; Pyl, G. *Ber.* **1923**, *56*, 31-44.
17. Posner, T.; Kemper, W. *Ber.* **1924**, *57*, 1311-1315.
18. Kolaczkowski, M. A.; He, B.; Liu, Y. *Org Lett.* **2016**, *18*, 5224-5227.
19. **General procedure for the condensation of indigo with methylene active compounds.** Sodium hydride (75 mg, 60% dispersion in mineral oil) was added to a suspension of powdered indigo (131 mg, 0.5 mmol) in dry DMF (10 mL). When the evolution of hydrogen was finished, the methylene active compound (4 mmol) was added gradually. The reaction mixture was heated at reflux and stirred for 30 min, then cooled, diluted with H_2O (50 mL), acidified with hydrochloric acid and filtered. The solid residue was washed with Et_2O (2 x 3 mL) and dried. The crude product was purified by

MPLC using chloroform as eluent. Compound samples for recording NMR and mass spectra were repurified by a Shimadzu HPLC system.

20. Morris, G.; Huey, R.; Lindstrom, W.; Sanner, M.; Belew, R.; Goodsell, D.; Olson, A. *J. Comput. Chem* **2009**, *30*, 2785-2791.

The alkaloid 6-oxofascaplysin and its derivatives were synthesized from indigo.

6-Oxofascaplysin was converted into the marine alkaloid fascaplysin in one step.

Reaction of indigo with ketones led to the formation of some of isatin derivatives.

Introduction of phenyl at C-7 of fascaplysin increase its activity up to 2-3 times.

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