Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1361–1364

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

S1319: A NOVEL β_2-ADRENOCEPTOR AGONIST FROM A MARINE SPONGE *DYSIDEA SP.*

Hidefumi Suzuki, Kazutoshi Shindo, Akihiro Ueno, Toru Miura, Masao Takei, Masayuki Sakakibara, Hiromi Fukamachi, Junichi Tanaka† and Tatsuo Higa†

Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., 3 Miyahara, Takasaki, Gunma 370-1295, Japan
†College of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

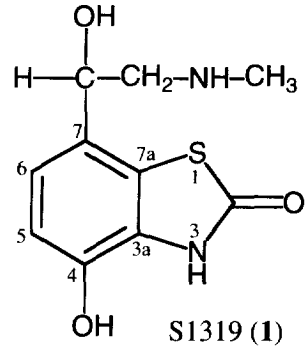
Received 2 February 1999; accepted 1 April 1999

Abstract: In the course of screening of potential leads for β_2-receptor agonists, we found a novel β_2-adrenoceptor selective agonist, S1319, from a marine sponge *Dysidea sp.* The active compound was isolated and structurally characterized as 4-hydroxy-7-[1-(1-hydroxy-2-methylamino)ethyl]-1,3-benzothiazole-2(3*H*)-one, a new member of the β_2-adrenoceptor agonist. This is the first example of a sponge-derived β_2-adrenoceptor agonist. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Natural products; Marine metabolites; Agonists; Receptors

Introduction

β_2-Adrenoceptor agonists are widely used as anti-asthmatic drugs in the treatment of reversible airway obstruction. Isoproterenol has been the most popular β-adrenoceptor stimulant but it has some disadvantages such as causing tachycardia due to its low selectivity towards the airway. Therefore, many bronchodilators such as trimetoquinol,[1,2] salbutamol,[3] procaterol,[4] formoterol [5] and salmeterol,[6] which are selective for bronchial smooth muscle, have been developed. These stimulants are either rationally designed epinephrine mimetics. Our continued interest to find novel bronchodilating natural products led to the discovery of S1319 (4-hydroxy-7-[1-(1-hydroxy-2-methylamino)ethyl]-1,3-benzothiazole-2(3*H*)-one), which is a novel benzothiazol-2-one analogue, from a marine sponge *Dysidea sp.* We investigated the tracheal relaxing effect in guinea pig to characterize its property. As a result, S1319 exhibited much more potent tracheal relaxing activity than isoproterenol, and this activity seemed to be mediated through β_2-adrenoceptors.



S1319 (**1**)

Isolation

A sample of *Dysidea sp.*[7] (8 kg wet wt.) collected in Okinawa was freeze-dried (935g) and extracted three

0960-894X/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(99)00205-X

times with CH_2Cl_2-MeOH (1:1) overnight. The extract was concentrated and partitioned between EtOAc and H_2O. The aqueous phase was concentrated to dryness and subjected to silica gel chromatography using stepwise elution: CH_2Cl_2-MeOH (1:1), CH_2Cl_2-MeOH-H_2O (3:1:0.1), and CH_2Cl_2-MeOH-H_2O (1:1:0.1). The active fraction eluted with CH_2Cl_2-MeOH-H_2O was applied onto an activated carbon column and eluted with 70% acetone (0.4% TFA). Finally, the active fractions were further purified by HPLC on ODS (YMC, SH-343-7) using MeOH-phosphate buffer (pH 7.0) to afford S1319 (**1**) (4.8 mg).

Structure Elucidation

The molecular formula of **1** was determined to be $C_{10}H_{12}O_3N_2S$ by HRFABMS [calcd for $(M+H)^+$ 241.0636; obs, 241.0641]. Analyses of ^1H and ^{13}C NMR, ^1H-^1H COSY and ^1H-^{13}C COSY spectra disclosed the presence of $CH(O)CH_2$ (δ_H 5.03 and 3.26), NCH_3 (2.71), 1,2,3,4-tetrasubstituted benzene ring (6.80 and 7.00) and one carbonyl carbon (165.5), which accounted for five out of six degrees of unsaturation. Thus, one more ring must be present in the molecule. The ^1H and ^{13}C NMR data are summarized in Table 1.

HMBC experiment on **1** proved the connectivities of the partial structures as shown in Figure 1. Treatment of **1** in MeOH with ethereal diazomethane gave a dimethyl derivative **2** [NCH_3 (δ_H 3.72, δ_C 34.3) and OCH_3 (δ_H 3.92, δ_C 57.7)]. HMBC experiments on **2** showed a long range coupling from OCH_3 to δ_C 137.9 (C-4). NOE was observed between OCH_3 and H-5. Thus a phenolic OH function could be placed at C-4. HMBC correlation between NCH_3 (δ_H 3.72) and C-3a (δ_C 128.5) and a carbonyl carbon (δ_C 172.4) confirmed the connectivity from C-2 to C-3a via a nitrogen atom (Figure 2). To complete the structure a sulfur atom was placed between C-2 and C-7a, forming a 2-thiazolidinone ring. Thus the planar structure of S1319 (**1**) represents a benzothiazol-2-one having an ethanolamine branch. It is the first isolation of a bronchodilator from a marine sponge.

Table 1. NMR data of S1319 in D_2O

Position	δ_c	δ_H ($J_{H,H}$ in Hz)	HMBC
1	-	-	-
2	165.5	-	-
3	-	-	-
3a	128.2	-	H5
4	144.7	-	H6
5	115.6	6.83 (d, 8.5)	-
6	124.2	7.00 (d, 8.5)	CH
7	127.6	-	CH, H5
7a	124.8	-	CH, H6
CH	70.4	5.03 (dd, 4.3, 8.5)	CH_2, H6
CH_2	55.8	3.26 (m)	NCH_3, CH
NH	-	-	-
NCH_3	35.6	2.71 (s)	CH_2

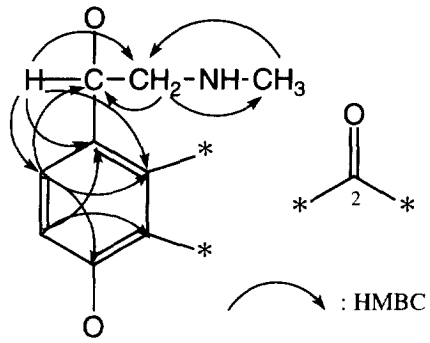


Figure 1. Partial structures of **1** connected by HMBC correlation.

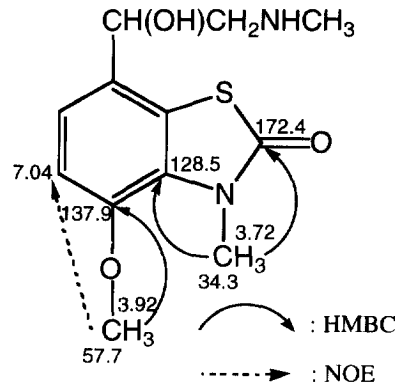


Figure 2. HMBC and NOE correlations for **2**.

Biological Activity

Potency and selectivity of S1319 were determined by measurement of displacement of [^{3}H]-CGP-12177 binding to human β_1- and β_2-adrenoceptors.[8] S1319 showed selective inhibition of β_2-adrenoceptor binding relative to β_1-adrenoceptor binding. S1319 exhibited Kd values of 120 nM and 51 nM for β_1- and β_2-adrenoceptor membrane preparations, respectively. The antilog delta -pK_d value of 2.5 (delta-pK_d β_1-versus β_2-binding=0.39) confirms that S1319 is an order of magnitude more selective for β_2-adrenoceptors than isoproterenol[9] at the molecular level. To determine whether S1319 is an agonist or antagonist, we measured the ability of the relaxation of the tracheal muscle in the tissue bath following administration of S1319.[10] S1319 concentration-dependently relaxed the histamine-induced contraction of isolated trachea at 1 x 10^{-11} M or less (Figure 3). The pD_2 value of S1319 was 10.60 ± 0.06 (n=4). Based on the pD_2 value, the relaxation effect of S1319 was 3300 times more potent than that of isoproterenol (pD_2 value of 7.08 ± 0.07, n=4) and the same potency as that of formoterol, an extremely potent and highly selective synthetic β_2-adrenoceptor agonist.[11]

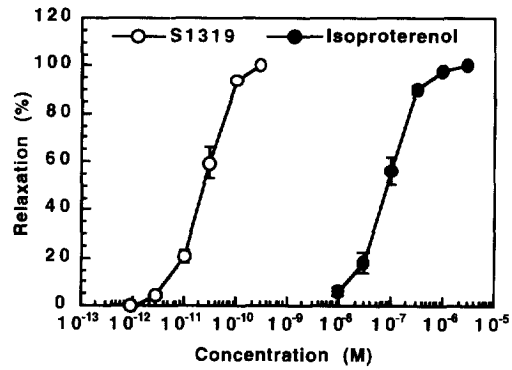


Figure 3. Relaxation in the isolated guinea pig trachea. Each point is the mean ± S.E. of the responses from four runs. ○; S1319, ●; Isoproterenol.

It was shown that in the catechol series, agonists more potent than epinephrine at β_2-adrenoceptors were obtained simply be replacing its *N*-methyl group by large non-polar substituents like isoproterenol.[12] Though S1319 and adrenaline have the same *N*-alkyl substitutent group, S1319 is seemed to be more potent than

epinephrine[13] and isoproterenol (Figure 3) on the relaxation of tracheabronchial muscle. Furthermore, S1319 is similar to salbutamol in β_2-adrenoceptor selectivity[13] and is about 1000 times more active (unpublished observation). These results suggest that benzothiazole-2-one derivatives having an ethanolamine branch are potent selective β_2-adrenoceptor agonists.

In conclusion, the potentiality of S1319 as a potent bronchodilator from a marine sponge is evident.

Acknowledgements

We would like to thank Dr. K. Ishizaka for helpful discussion, and Ms. M. Nakajima and Ms. C. Kitazume for technical assistance.

References and Notes

1. Iwasawa, Y.; Kiyomoto, A. *Japan. J. Pharmacol.* **1967**, *17*, 143.
2. Sato, M.; Ikezawa, K.; Nagao, T.; Kiyomoto, A. *Pharmacometrics* **1980**, *19*, 269.
3. Cullum, V.A.; Farmer, J.B.; Jack, D.; Levy, G.P. *Br. J. Pharmacol.* **1969**, *35*, 141.
4. Yabuuchi, Y. *Br. J. Pharmacol.* **1977**, *61*, 513.
5. Ida, H. *Arzneimittelforschung* **1976**, *26*, 839.
6. Bradshaw, J.; Brittain, R.T.; Coleman, R.A.; Jack, D.; Kennedy, I.; Lunts, L.H.C.; Skidmore, I.F. *Br. J. Pharmacol.* **1987**, *92*, 590P.
7. The sponge was collected in Yonaguni Island, Okinawa in March, 1995 and identified by Dr. John N. A. Hooper, Queensland Museum, South Brisbane, Queensland, Australia. A voucher specimen (QMG312706) has been deposited at the museum.
8. Human recombinant β_1- or β_2-adrenoceptor (Biosignal Inc., Canada) were incubated for 60 min at 27 °C with 0.16 nM [^{3}H]CGP-12177 (Amersham, England) in Tris buffer (75 mM Tris, 12.5 mM $MgCl_2$, 2 mM EDTA, pH 7.4) . The reaction was stopped by adding cold Tris buffer and rapid vacuum filtration through a GF/C glass fiber filter plate (Millipore) presoaked with 0.3% polyethylenimine. The filters were washed nine times with ice-cold Tris buffer. The radioactivity in each well was determined by a scintillation counter (Topcount Packard).
9. Anderson, G.P. *Life Sci.* **1993**, *52*, 2145.
10. Male hartley guinea pigs (250-700 g, Charlesriver, Japan) were killed by exsanguination with a blow on the head. The trachea was dissected. Tracheal strips were prepared and mounted in a 10 ml organ bath filled with Tyrode's solution (137.0 mM NaCl, 11.9 mM $NaHCO_3$, 2.68 mM KCl, 1.89 mM $CaCl_2$, 1.09 mM $MgCl_2$, 0.24 mM NaH_2PO_4 and 5.6 mM glucose), which was continuously gassed with 95% O_2-5% CO_2 and maintained at 37 °C. Test compounds were added cumulatively in the tonic phase of the contraction. Tension changes of the preparation were recorded isometrically with a strain gauge transducer (TB-611T, Nihon Kohden) on an ink-writing recorder (AP-621G, Nihon Kohden). The preparation was stretched to a resting tension of 0.5 g and allowed to equilibrate for 1 h. Agonistic activities of the compounds were estimated by pD_2 (negative log molar concentration that produced 50% relaxation) obtained from each concentration-response curve and by their intrinsic activity.
11. Ida, H. *Arzneimittelforchung* **1976**, *26*, 839.
12. Brittain, R.T.; Dean, C.M.; Jack, D. *Pharmacol. and Ther. Bull.* **1976**, *2*, 423.
13. Jack, D. *Br. J. Clin.Pharmacol.* **1991**, *31*, 501.