# New Cytotoxic Indolic Metabolites from a Marine *Streptomyces*

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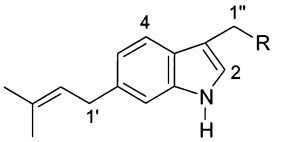
Three new cytotoxic 3,6-disubstituted indoles (**1**−**3**) were isolated from the mycelium of a strain identified as *Streptomyces* sp. (BL-49-58-005), which was separated from a Mexican marine invertebrate, and their structures established by analysis of NMR and mass spectral data. $GI_{50}$ values for **1** and **2** in cytotoxic bioassays against a panel of 14 different tumor cell lines were estimated at micromolar range, while compound **3** showed no activity in the same assays.

Marine microorganisms have proven to be a rich source of compounds that might be useful for the development of new pharmaceutical agents.[1] In the course of our screening program for new cytotoxic compounds produced by marine microorganisms, the actinomycete strain BL-49-58-005, isolated from an unidentified marine invertebrate collected in Mexico, was analyzed and three new indolic derivatives were isolated by bioassay-guided fractionation. The strain BL-49-58-005 was identified as *Streptomyces* sp.[2] and deposited in the "Colección Española de Cultivos Tipo", Spain, with the accession No. CECT 3364. In this paper we describe the isolation and structure elucidation of compounds **1**−**3**. Previously, several closely related structures were reported from a red alga[3] and various species and genera of myxobacteria,[4] fungi,[5] sponges,[6] and actinomycetes.[7]




**1** R= -$CH_2$OH

**2** R= -CH=NOH

**3** R= -C≡N

Compound **1** (6-prenyltryptophol) has a molecular formula of $C_{15}H_{19}ON$ established by HRFABMS ($M^+$ at *m/z* 229.1466), $^{13}C$ NMR, and DEPT data. NMR data revealed the presence of a heteroaromatic and two small aliphatic moieties. The $^{13}C$ NMR spectrum displayed eight aromatic carbon signals, four methines (110.7, 118.9, 120.9, and 122.3 ppm), and four quaternary carbons (112.3, 125.8, 136.4, and 137.1 ppm), and the pattern of the aromatic region in the $^1H$ NMR spectrum showed the presence of two coupled protons (7.55 and 6.98 ppm), two singlet protons (7.18 and 7.02 ppm), and a broad singlet at 7.94 ppm corresponding to a NH signal. All these data suggested the presence of a 3,6-disubstituted indolic nucleus. Carbon 6 is substituted by an isoprene unit, as confirmed by the presence of signals due to a 3,3-dimethylallyl group in its NMR spectrum and the HMBC correlation of the methylene signal at 3.45 ppm ($H_2$-1′) with the quaternary carbon signal at 136.4 ppm (C-6). HMBC correlations of both methylene signals at 3.02 and 3.90 ppm ($H_2$-1″ and $H_2$-2″, respectively) with the quaternary carbon signal at 112.3 ppm (C-3), along with NMR and IR spectra (3399 $cm^{-1}$), suggested that carbon 3 bears a 2-hydroxyethyl unit. The complete assignments of $^1H$ and $^{13}C$ NMR spectra of compound **1** were finally established by 2D NMR experiments (COSY, HMQC, and HMBC), and its spectroscopic data are shown in Table 1.

**Table 1.** $^{13}C$ and $^1H$ NMR Spectral Data for Compound **1** [$\delta$ (ppm), $J_{HH}$ (Hz); $CDCl_3$]

| position | $^{13}C$ ($\delta$) | $^1H$ ($\delta$) | HMBC |
|---|---|---|---|
| 1-NH | | 7.94 (br s) | |
| 2-CH | 122.3 | 7.02 (d, 2.0) | 137.1, 125.8, 112.3 |
| 3-C | 112.3 | | |
| 3a−C | 125.8 | | |
| 4-CH | 118.9 | 7.55 (d, 8.1) | 137.1, 136.4, 125.8, 112.3 |
| 5-CH | 120.9 | 6.98 (dd, 8.1, 1.4) | 125.8, 110.7, 34.8 |
| 6-C | 136.4 | | |
| 7-CH | 110.7 | 7.18 (s) | 125.8, 120.9, 34.8 |
| 7a−C | 137.1 | | |
| 1′-$CH_2$ | 34.8 | 3.45 (d, 7.4) | 136.4, 132.3, 124.2, 120.9, 110.7 |
| 2′-CH | 124.2 | 5.40 (tm, 7.4) | 34.8, 26.0, 18.1 |
| 3′-C | 132.3 | | |
| Me-3′ | 26.0 | 1.80 (s) | 132.3, 124.2, 26.0, 18.1 |
| Me-3′ | 18.1 | 1.80 (s) | 132.3, 124.2, 26.0, 18.1 |
| 1″-$CH_2$ | 29.1 | 3.02 (t, 6.2) | 125.8, 122.3, 112.3, 62.8 |
| 2″-$CH_2$ | 62.8 | 3.90 (t, 6.2) | 112.3, 29.1 |

Compound **2** was assigned the molecular formula $C_{15}H_{18}ON_2$ on the basis of HRFABMS ($M^+$ at *m/z* 242.1416). In the $^{13}C$ NMR spectrum of **2**, however, the total number of carbons seemed to be more than that indicated by HRFABMS spectral information. This discrepancy may be ascribed to the presence of two isomeric aldoximes. All attempts to separate them by normal- or reversed-phase flash chromatography were unsuccessful. Fortunately, the concomitant signals were limited and most of the signals were easily assignable.

NMR spectra of **2** were almost the same as those of **1**, except for signals due to the 3-substituent moiety. Spectral data suggested the presence of a $-CH_2-CH=NOH$ group in **2**, instead of the $-CH_2-CH_2-OH$ in **1**. A literature search[4,8] allowed us to assign the signals at 7.60/151.2 and 3.65/26.1 ppm to the $-CH_2-CH=N-$ of the *anti* isomer and 6.94/152.0 and 3.86/21.9 ppm to the $-CH_2-CH=N-$ of the *syn* isomer. The ratio of *syn* and *anti* isomers was almost 1:1 on the basis of the $^1H$ NMR integration. HPLC analysis, using MeOH/$H_2O$ (70:30) as mobile phase, showed two different peaks corresponding to both geometrical isomers ($t_R$ = 16.43 min for *anti* and $t_R$ = 18.04 min for *syn*), in a ratio of 54:43 in favor of *syn* geometry. Thus, the

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structure of **2** was concluded as shown in the previous figure, and two-dimensional NMR experiments supported this conclusion. As an additional proof of the presence of the aldoxime mixture, we performed the dehydration of both isomers, by treating **2** with $Ac_2O$ under reflux to yield the corresponding nitrile **3**.[9] This nitrile derivative also appeared as a minor metabolite in the initial extract, but we first failed to detect it because of its lack of cytotoxic activity.

These three compounds (**1**−**3**) possibly represent the products of different steps along the same biosynthetic pathway, starting from tryptophan or tryptamine as presumed precursors. Water elimination from aldoximes **2** would afford nitrile **3**, which could be easily hydrolyzed to the corresponding acid, and subsequent reduction would afford alcohol **1**.

Cytotoxic assays for these compounds were performed against a panel of 14 different tumor cell lines. Compound **1** showed the best activity against K-562 (leukemia), with a $GI_{50}$ value of 8.46 $\mu$M. Aldoxime mixture **2** showed activity with $GI_{50}$ values within micromolar range against LN-caP (prostate cancer), HMEC1 (endothelial cancer), K-562 (leukemia), PANC1 (pancreas cancer), and LOVO and LOVO-DOX (colon cancer) and slightly higher values against the rest of the tumor cell lines, without any particular specificity. Bioassays performed on nitrile **3** showed no activity.

## Experimental Section

**General Experimental Procedures.** IR spectra were determined using a Perkin-Elmer 600. $^1$H NMR and $^{13}$C NMR data were obtained on a Varian "Mercury 400" spectrometer at 400 and 100 MHz, respectively. HMQC and HMBC experiments were carried out using an inverse resonance probe. Chemical shifts are reported in ppm relative to solvent ($CDCl_3$ $\delta_H$ 7.24, $\delta_C$ 77.0). FABMS experiments employed a *m*-NBA (*meta*-nitrobenzyl alcohol) matrix and were performed on a VG ZAB-SE (LRFAB) and 70-VSE (HRFAB). HPLC separations were performed on a Hewlett-Packard 1100 liquid chromatograph with a photodiode-array detector.

**Isolation.** Fermentation broth (6 L) of BL-49-58-005 was filtered through Celite and the mycelial cake extracted twice with 1.8 L of a mixture of $CHCl_3$/MeOH (2:1). The resultant suspension was filtered and partitioned between $CHCl_3$ and water. The organic layer was taken to dryness, and the crude extract (3.6 g) was fractionated by VFC (vacuum flash chromatography) on silica gel, eluting with a stepwise gradient of hexane/EtOAc. Active fractions containing alcohol **1** (eluted with hexane/EtOAc, 6:4) were finally purified by $C_{18}$ reversed-phase column chromatography (MeOH/$H_2O$, 75:25), affording 32 mg of compound **1**. Fractions containing the mixture of aldoximes **2** (eluted with hexane/EtOAc, 65:35) were purified following the same protocol, using MeOH/$H_2O$ (80:20) to give 26 mg of compounds **2**.

**6-Prenyltryptophol (1):** UV (MeOH) $\lambda_{max}$ 226 and 282 nm; IR (KBr) $\nu_{max}$ 3399 (br s), 2930, 1625, 1451, 1340, 1214, 1043, and 757 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) and $^{13}$C NMR (100 MHz, $CDCl_3$), see Table 1; HRMS(FAB$^+$) *m*/*z* 229.1466 (calcd for $C_{15}H_{19}ON$ 229.1467).

**Compound 2:** UV (MeOH) $\lambda_{max}$ 226 and 280 nm; IR (KBr) $\nu_{max}$ 3390, 1622, 1449, 1411, 1338, 1089, 1042, 925, and 808 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.96 (1H, bs, NH), 7.60 (2H, t, $J$ = 6.2, H-2″ *anti* isomer), 7.52 (1H, d, $J$ = 8.1, H-4), 7.19 (1H, s, H-7), 6.98 (1H, d, $J$ = 8.1, H-5), 6.98 (1H, s, H-2), 6.94 (2H, t, $J$ = 5.3, H-2″ *syn* isomer), 5.38 (1H, tm, $J$ = 6.8, H-2′), 3.86 (2H, d, $J$ = 5.3, H-1″ *syn* isomer), 3.65 (2H, d, $J$ = 6.2, H-1″ *anti* isomer), 3.42 (2H, d, $J$ = 6.8, H-1′), 1.79 (6H, s, 2Me C-3′); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 152.0 (d, C-2″ *syn* isomer), 151.2 (d, C-2″ *anti* isomer), 137.0 (s, C-7a), 136.2 (s, C-6), 132.0 (s, C-3′), 125.6 (s, C-3a), 124.1 (d, C-2′), 122.0 (d, C-2), 121.0 (d, C-5), 118.3 (d, C-4), 111.0 (s, C-3), 110.8 (d, C-7), 34.8 (t, C-1′), 26.1 (t, C-1″ *anti* isomer), 26.0 (q, Me C-3′), 21.9 (t, C-1″ *syn* isomer), 18.0 (q, Me C-3′); HRMS(FAB$^+$) *m*/*z* 242.1416 (calcd for $C_{15}H_{18}ON_2$ 242.1419).

**Compound 3:** UV (MeOH) $\lambda_{max}$ 226 and 278 nm; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.05 (1H, bs, NH), 7.50 (1H, d, $J$ = 8.3, H-4), 7.19 (1H, s, H-7), 7.16 (1H, m, H-2), 7.03 (1H, dd, $J$ = 8.3; 1.4, H-5), 5.38 (1H, tm, $J$ = 7.3, H-2′), 3.82 (2H, s, H-1″), 3.43 (2H, d, $J$ = 7.3, H-1′), 1.77 (6H, s, 2Me C-3′); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 137.5 (s, C-6), 137.0 (s, C-7a), 132.8 (s, C-3′), 124.3 (s, C-3a), 124.0 (d, C-2′), 122.5 (d, C-2), 121.0 (d, C-5), 118.5 (s, C-3), 118.0 (d, C-4), 111.0 (d, C-7), 104.8 (s, C-2″), 34.6 (t, C-1′), 26.1 (q, Me C-3′), 18.2 (q, Me C-3′), 14.7 (t, C-1″); API-ES-negative *m*/*z* 223 (M − H$^-$, 100).

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## References and Notes

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