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MPC1001 and Its Analogues: New Antitumor Agents from the Fungus *Cladorrhinum* Species

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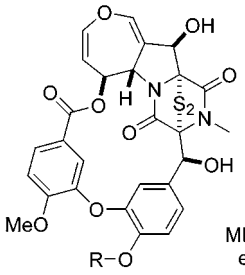
ABSTRACT



MPC1001 (**1**) : R = Me
emestrin (**9**) : R = H

Eight new compounds, MPC1001 and MPC1001B–H, were isolated from the fungus *Cladorrhinum* sp. KY4922. Multiple NMR experiments and CD data revealed MPC1001 to be an *O*-methyl derivative of emestrin, a 15-membered antifungal antibiotic containing a unique epidithiodioxopiperazine skeleton. Other compounds were elucidated to be structurally related novel analogues. MPC1001 and the analogues exerted potent antiproliferative activities against a human tumor cell line.

In the course of our screening program of microbial metabolites for new antitumor antibiotics, the fungus *Cladorrhinum* sp. KY4922 was found to produce a series of new compounds.[1] Eight new compounds, MPC1001 (**1**) and MPC1001B−H (**2**−**8**), were isolated mainly by column chromatographies from the fermentation mycelium. We described herein structure elucidations and antitumor activities of these new compounds.

The mycelium from 5 L of culture was extracted with MeOH, and the extract was purified by a combination of chromatographies over Diaion HP-20, YMC-GEL ODS-AQ, Sephadex LH-20, and Silica gel 60. Additional purifications by preparative HPLC using a YMC-PACK ODS-AM column, (20 i.d. × 250 mm, 0−40% aqueous MeCN, linear gradient) afforded MPC1001 (**1**, 260.2 mg), MPC1001B (**2**, 7.7 mg), MPC1001C (**3**, 1.7 mg), MPC1001D (**4**, 60.6 mg), MPC1001E (**5**, 58.9 mg), MPC1001F (**6**, 6.1 mg), MPC1001G (**7**, 12.0 mg), and MPC1001H (**8**, 14.3 mg).

The molecular formula of **1** was established as $C_{28}H_{24}N_2O_{10}S_2$ by HRFAB-MS analysis (*m*/*z* 611.0802, $[M - H]^-$, calcd for $C_{28}H_{23}N_2O_{10}S_2$, +0.8 mmu error). The positive FABMS (NBA matrix) ions at *m*/*z* 531 $[M - S_2 - H_2O + H]^+$ (base peak) and *m*/*z* 548 $[M - S_2]^+$ (second dominant) suggested the presence of a disulfide moiety in **1**. The ^{13}C NMR spectrum of **1** measured in $CDCl_3$ displayed 28 carbon signals consisting of three methyl, 13 methine (4 sp^3 + 9 olefinic), and 12 quaternary (2 sp^3 + 3 carbonyl + 7 olefinic) carbons as judged by the DEPT spectrum. ^{13}C NMR signals at δ_c 165.1, 166.7, and 163.4, in addition to IR absorptions at 1716, 1684, and 1668 cm^{-1}, indicated the presence of an

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(1) Tsumagari, N.; Nakai, R.; Onodera, H.; Hasegawa, A.; Rahayu, E. S.; Ando, K.; Yamashita *J. Antibiot.* **2004**, *57*, 532−534.

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ester and two amide groups. Moreover, two broad signals (δ_H 4.75 and 5.21) in the ^{1}H NMR spectrum measured in $CDCl_3$ and IR absorption at 3442 cm^{-1} implied two hydroxy groups. Two methoxy groups (δ_c 56.0, δ_H 3.94 and δ_c 56.2, δ_H 3.99) and an *N*-methyl group (δ_c 27.9, δ_H 3.41) were also readily assignable. ^{1}H−^{1}H spin systems and one-bond ^{1}H−^{13}C connectivities were analyzed by DQF−COSY and HSQC data, respectively. The following ^{1}H−^{1}H spin systems were assigned by the interpretation of DQF−COSY spectrum: H-8−H-7−H-6−H-5a−H-10, H-2′−H-6′−H-5′, and H-3″−H-4″−H-6″. In addition to these proton spin systems, $^{3-4}J_{H,C}$ HMBC correlations allowed the establishment of an unconjugated 5a,6-dihydrooxepine moiety and a 3′,4′-substituted benzoate moiety as well as the connection of these two moieties through the ester bond (Figure 1). Dereplication on

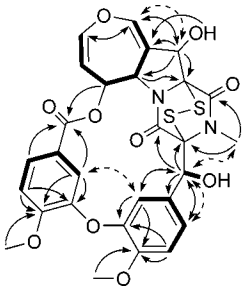
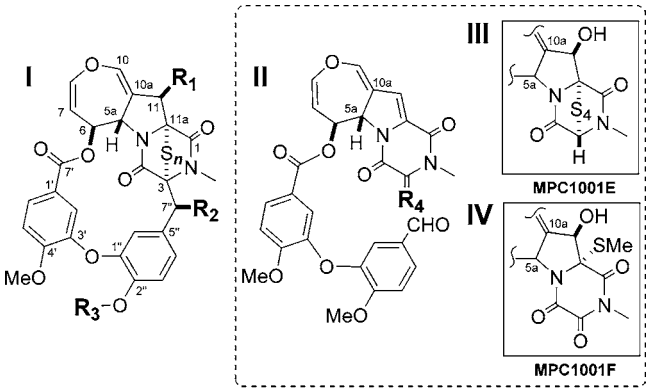


Figure 1. Structure of MPC1001 (**1**) with COSY-defined spin systems (bold lines) and selected HMBC correlations (solid arrows) in $CDCl_3$ and selected NOESY correlations (dashed arrows) in DMSO-d_6.

the basis of this partial structure revealed the structural similarity of **1** to emestrin (**9**), a 15-membered antifungal macrolide having a peculiar epidithiodioxopiperazine skeleton in the molecule.[2] The ^{1}H and ^{13}C chemical shifts of **1**, remeasured in DMSO-d_6, were in good agreement with literature values for dioxopiperazine and hydroxypyrrolidine rings except at the neighboring C-2″ position. The difference of molecular weights (14 mu) corresponded to a methyl group, and the chemical shift values of this methyl group (δ_c 56.0, δ_H 3.94) indicated a methoxy group. Because of apparent HMBC correlation between the methyl protons with C-2″, **1** was consequently determined to be a 2″-*O*-methyl derivative of **9**.

Determination of the stereochemistry of **1** was performed on the basis of NMR and CD data comparisons with **9**, whose structure has been definitively determined by X-ray crystallographic analysis.[3] Acceptable agreements of all proton coupling constants ($^{3-4}J_{H,H}$) of **1** with those of **9**, especially around a hydroxypyrrolidine ring, and observations of the similar NOE correlations of H-10 (δ_H 6.91) with H-11 (δ_H 4.92) and of H-7″ (δ_H 5.43) with *N*-CH_3 (δ_H 3.41) and with



	type	*n*	R_1	R_2	R_3	R_4
MPC1001 (**1**)	I	2	OH	OH	Me	-
MPC1001B (**2**)	I	2	H	OH	Me	-
MPC1001C (**3**)	I	2	OH	H	Me	-
MPC1001D (**4**)	I	3	OH	OH	Me	-
MPC1001E (**5**)	III	-	-	-	Me	-
MPC1001F (**6**)	IV	-	-	-	Me	-
MPC1001G (**7**)	II	-	-	-	Me	S
MPC1001H (**8**)	II	-	-	-	Me	O
Emestrin (**9**)	I	2	OH	OH	H	-

H-4″ (δ_H 7.10) in the NOESY spectrum suggested that the relative configurations were identical, including hydroxy groups at C-11 and C-7″ (Figure 1). The CD spectra of the compounds possessing epidithiodioxopiperazine chromophores are known to show maxima at 235, 270, 310, and 340 nm, and the sign of the cotton effect at 230−270 nm were reported to depend on the disulfide configuration both theoretically[4] and experimentally.[3] In the CD spectrum of **1**, cotton effects for the epidithiodioxopiperazine at 235.7 and 267.6 nm and for the benzoate at 301.6 nm were in good agreement with those of **9**. Thus, **1** must have the configuration 3*R*,5a*S*,6*S*,11*R*,11a*R*,7″*S*, identical to **9** as shown in Figure 1. The absolute stereochemistry of **1** was further supported by the similarity of the optical rotations of **1** ($[\alpha]^{20}_D$ +117° (*c* 0.3, MeOH)) and **9** ($[\alpha]^{15}_D$ +184°).[2,3]

1 was definitively detected in the fungal broth and the mycelium, while no trace of **9** appeared in the HPLC analysis. We, therefore, concluded that **1** is not the methylated artifact of **9** that was generated during extraction and purification processes using MeOH. Although some analogues of **9** have so far been reported,[5] this is the first time the methyl derivative has been detected.

HRFAB-MS analyses exhibited that **2** and **3** had the same molecular formula $C_{28}H_{24}N_2O_9S_2$. Since the polarities followed the order **1** > **2** > **3** as judged by chromatographic behaviors and RP-HPLC analyses, **2** and **3** were deduced to be the deoxy derivatives of **1**. The proton signal at δ_H 3.02

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(5) (a) Seya, H.; Nozawa, K.; Udagawa, S.; Nakajima, S.; Kawai, K. *Chem. Pharm. Bull.* **1986**, *34*, 2411−2416. (b) Kawai, K.; Nozawa, K.; Seya, H.; Kawahara, N.; Udagawa, S.; Nakajima, S. *Heterocycles* **1987**, *26*, 475−479. (c) Nozawa, K.; Udagawa, S.; Nakajima, S.; Kawai, K. *Chem. Pharm. Bull.* **1987**, *35*, 3460−3463. (d) Ooike, M.; Nozawa, K.; Kawai, K. *Pytochemistry* **1997**, *46*, 123−126.

having the ddd coupling pattern (18.1, 2.2, and 2.0 Hz) was newly observed in **2**. Compared to **1**, ^{13}C NMR chemical shifts of **2** over the pyrrolidine and dihydrooxepine rings varied, especially the chemical shift of C-11 (δ_C 34.9), which apparently shifted upfield (Δ −41.7 ppm). The two-dimensional NMR spectra revealed that the newly generated proton was located geminally to H-11 (δ_H 4.16) and bound to C-11, indicating that **2** had the similar structure except for the 11-hydroxy group. The proton of δ_H 3.02 was determined to be H-11α by the NOE correlation with H-6 (δ_H 4.99), and further stereochemistry of **2** was identical to that of **1** by interpretations of coupling constants and NOESY spectra.

Likewise, **3** was suggested as the 7″-deoxy derivative of **1** because the geminal proton signals of δ_H 3.45 (d, J = 13.3 Hz) and δ_H 3.83 (d, J = 13.3 Hz), both bound to C7″ at δ_C 36.2, were observed. The NOESY experiment elucidated the proton of δ_H 3.45 as α (NOE with H-6″) and the other of δ_H 3.83 as β (NOEs with H-4″ and *N*-CH_3) configurations, respectively.

The one-dimensional NMR data of **4** showed considerable similarity to those of **1**. Only carbon signals for C-11a, C-4″, and C-6″ slightly shifted downfield (Δ +9.6, +4.5, and +5.8 ppm, respectively). HRFAB-MS analysis (*m*/*z* 643.0516, [M − H]$^-$, +0.2 mmu error) suggested that the molecular formula of **4** was $C_{28}H_{24}N_2O_{10}S_3$, which indicated the existence of trisulfide moiety compared to the formula of **1**. The trisulfide moiety was further supported by the positive fragment ion peaks of *m*/*z* 531 [M − S_3 − H_2O + H]$^-$ and *m*/*z* 548 [M − S_3]$^-$ in the same manner observed for the disulfide in **1**. Consequently, **4** was deduced to be the trisulfide analogue of **1**. As for **9**, the corresponding trisulfide analogue, emestrin B, was also reported. All the NMR data for **4** were in good agreement with the literature values for emestrin B.[5c]

The molecular formula of **5** was established as $C_{28}H_{24}N_2O_{10}S_4$ by HRFAB-MS analysis (*m*/*z* 676.0339, [M]$^-$, calcd for $C_{28}H_{24}N_2O_{10}S_4$, +2.6 mmu error). The positive fragment ion at *m*/*z* 548 [M − S_4]$^+$ suggested the presence of a tetrasulfide moiety in **5**. On the NMR data, new signals at δ_C 190.8 and δ_H 9.81, characteristic to an aldehyde group, appeared, in addition to the signals for dihydrooxepine, benzoate, and benzene moieties. The benzaldehyde structure was proposed on account of correlations of these aldehyde signals to C-4″−C-6″ detected in the HMBC spectrum. Another distinct signal at δ_H 4.97 (singlet) was deduced to be H-3 located between a carbonyl carbon and an amide nitrogen by its chemical shift[6] and HMBC correlation to C-1, C-4, and *N*-CH_3. The above results suggested that **5** was a retro-aldol-type seco-aldehyde cleaved between a dioxopiperazine ring and a benzene ring as depicted in Figure 2. The NMR data as well as the physicochemical properties, including a unique UV absorption [λ_{max} MeOH nm (ϵ): 228 (35,800), 263.5 (22,000)], agreed well with those of the corresponding seco-aldehyde analogue of **9**, known as secoemestrin C (**10**).[5d]

The molecular formula of **6** was established to be $C_{29}H_{26}N_2O_{11}S$ by HRFAB-MS analysis (*m*/*z* 611.1334, [M + H]$^+$, calcd for $C_{29}H_{27}N_2O_{11}S$, −0.8 mmu error), and the structure of **6** was supposed to include 4 methyls, 13 methines, and 12 quaternary carbons by ^{13}C-DEPT estimation. The negative FABMS fragment ion at *m*/*z* 563 [M − SCH_3 − H]$^-$ and the chemical shifts, δ_C 13.0 and δ_H 2.06, suggested that **6** had an *S*-methyl group. The presence of oxepin and benzoate moieties was supported by comparing the NMR data of **6** to that of **1**, while the greater shifts observed for quaternary C-3 (δ_C 156.6), corresponding to a carbonyl carbon, oxymethine at C-7″, and benzaldehyde signals (δ_C 190.7, δ_H 9.81), indicated that **6** had an open circular structure as observed in **5**. Because a signal at δ_C 76.3, which has a small long-range C−H coupling[7] to H-11 (δ_H 4.89) in its LSPD (long-range selective proton decoupling) spectrum, was concomitantly found to have HMBC correlation to *S*-methyl (δ_H 2.06), the binding of *S*-methyl to C-11a was supported. The newly observed carbonyl carbon at δ_C 156.6 exhibited HMBC correlation with N_1-methyl (δ_H 3.30), while the other carbonyl at δ_C 152.5 had LSPD correlation with H-5a (δ_H 5.39). Furthermore, chemical shifts of three carbonyl carbons in piperazine of **6** were consistent with those in the known structures (data not shown).[8] These results proved that **6** had a particularly characteristic structure, including a trioxopiperazine ring with methyl sulfide moiety as shown in Figure 2.

Purified **7** and **8** appeared dark orange and pale yellow in color, respectively. Bathochromic shifts in UV spectra of both **7** (λ_{max}^{MeOH} nm (ϵ) 225 sh, 263.5 (23 300), 341 sh (7100), 415.5 (6400)) and **8** (226 sh, 261.5 (26 200), 364.5

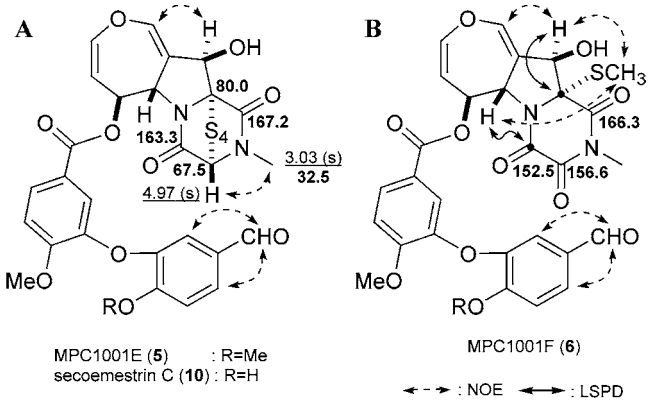

Figure 2. Structure elucidation of **5** (A) and **6** (B) by interpretation of NMR data in $CDCl_3$. Bold and underlined values indicate carbon (δ_C) and proton (δ_H) chemical shifts in ppm, respectively.

(6) Chemical shifts of the dioxopiperazine ring in **5** were compared to similar structures such as (a) dithiosivation (Kawahara, N.; Nozawa, K.; Nakajima, S.; Kawai, K. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2099−2101) and (b) a synthetic intermediate of gliotoxin (Fukuyama, T.; Nakatsuka, S.; Kishi, Y. *Tetrahedron* **1981**, *37*, 2045−2078).

(7) Molecular modeling study of **6** using MM2 deduced a dihedral angle between C-4 and H-5a of about 90°, which resulted in the small coupling.

(8) Chemical shifts of the trioxopiperazine ring in **6** were compared to similar structures such as (a) neoechinuline (Cardillo, R.; Fuganti, C.; Gatti, G.; Ghiringhelli, D.; Grasselli, P. *Tetrahedron Lett.* **1974**, *36*, 3163−3166) and (b) a compound 1 (Mulliez, M.; Royer, J. *Tetrahedron* **1984**, *40*, 5143−5151).

(8800)) compared to **1** (205 (38 000), 263 (14 000), 283 (8000)) were observed, indicating the extension of conjugated systems. The molecular formulas of **7** ($C_{28}H_{22}N_2O_9S$) and **8** ($C_{28}H_{22}N_2O_{10}$) were established by HRFAB-MS analyses, and the preserved oxepin, benzoate, and benzene rings in both **7** and **8** were clarified by NMR data. Moreover, distinctive signals for benzaldehyde (δ_C 190.7, δ_H 9.8) suggested that both **7** and **8** were also ring-opening seco forms. Signals for C-11 and C-11a widely shifted relative to **1**, and olefinic signals (**7** δ_C 122.0, δ_H 6.83; **8** δ_C 122.1, δ_H 6.85) were newly observed, indicating the elimination of hydroxyl groups and the formation of double bonds at C-11 positions. Finally, **8** was concluded to have the trioxopiperazine skeleton, composed of three specific carbonyl carbons such as **6**, whereas the structure of **7**, containing a thione moiety in the piperazine ring, was led by the considerable downfield shift of C-3 (δ_C 186.9) and its molecular formula.

1 and the analogues containing both macrocyclic skeletons and polysulfide bridges over dioxopiperadine rings exerted antiproliferative activities against DU145 human prostate cancer cell line with IC_{50} values as follows: 9.3 nM for **1**, 39 nM for **2**, 12 nM for **3**, and 16 nM for **4**. The decrease of the antiproliferative activities in **5** (IC_{50} 83 nM), **7** (350 nM), and **8** (450 nM) indicated that the macrocyclic ring and polysulfide structures were critical for the antitumor activity. The details of biological activities of **1** will be reported elsewhere.

Several natural products containing polythiodioxopiperazine moieties have so far been reported, with their broad range of biological activities, typically antibiotic and antifungal, depending on their structures.[9] Among them, aranotin and apoaranotin[10] and its diester, emethallicins,[11] are especially of the same sort of structures as **1** and are considered to have common biosynthetic pathways,[12] in which phenylalanine would be enzymatically oxidized to its benzene oxide first and the valence tautomer oxepin is further oxidized to oxepin oxide. Subsequently, a nucleophilic ring contraction might take place by enzymatic catalysis. According to this biogenetic hypothesis, **1** is probably derived from the combination of one molecule of benzoic acid and two molecules of phenylalanine. **2** and **3** might be biosynthetic intermediates of **1**, while **4** and **5** are likely derived from **1** by sulfide bridge extension via intermolecular exchange. Finally, **7** and **8** are presumed to be degradatives by retro-aldol-type ring opening following dehydration.

In this study, we achieved structure elucidations and evaluations of biological activities of eight new natural compounds found from a fungus. MPC1001 (**1**) was revealed to be a new methyl derivative of emestrin, an antifungal 15-membered macrolide that has a unique epidithiodioxopiperazine moiety. Other compounds were also elucidated spectroscopically to be its deoxy [MPC1001B (**2**) and MPC1001C (**3**)] and trisulfide [MPC1001D (**4**)] analogues as well as retro-aldol-type seco-aldehydes [MPC1001E (**5**)−H (**8**)]. MPC1001 and the analogues, which had the polysulfide bridge in their molecule, showed antiproliferative activities against a human prostate cancer cell line (DU145).

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Supporting Information Available: Experimental procedures and CD data for **1** and NMR spectra for compounds **2**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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