ORIGINAL RESEARCH PAPER
VOLUME-6 | ISSUE-5 | MAY - 2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

Oxazoles from marine sponge *Mycale (Zygomycale) parishii*

Chemical Science

Srinu Meesala — Department of Biology, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pune-411008, INDIA.

Vijay R. Lakwal — Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, INDIA.

Dinesh S. Kharate — Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, INDIA.

Satish S. Mokashe — Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, INDIA.

Prerna. Raina — Cell and Translational Research Division, Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth University, Pune 411008, INDIA.

Ruchika Kaul-Ghanekar — Cell and Translational Research Division, Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth University, Pune 411008, INDIA.

Milind G Watve — Department of Biology, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pune-411008, INDIA.

ABSTRACT

Two new oxazole compounds (**1&2**) along with two known compounds (**3&4**) were isolated from the methanolic extract of *Mycale (Zygomycale) parishii*. Their structures were identified on the basis of IR, NMR, and HR-ESI-MS analysis. The oxazole compounds (**1&2**) showed broad-spectrum activity against bacteria and fungi at 100 µg/disk and exhibited very weak cytotoxic activity against HeLa, SiHa and MDA-MB-231 cell lines.

KEYWORDS:

Introduction:

Marine sponges are a rich source of metabolites reported in marine libraries [Faulkner. 2002; Blunt et al., 2009, 2015.]; among them, sponges of the genus *Mycale* are especially rich in metabolites, such as mycalamides [Perry et al., 1990; Fusetani et al., 1989], mycalolides [Fusetani, et al., 1989, Matsunaga et al., 1998, Phuwapraisirisan et al., 2002], pateamine [Northcote et al., 1991], peloruside A [West et al., 2000], azumamides A-E [Nakao et al., 2006], peloruside B [Singh et al., 2010] and 5-octadecylpyrrole-2-carboxaldehyde [Reddy et al., 2000]. In the genus *Mycale*, only three species are distributed worldwide i.e.: *M. topsent, M. angulosa, M. parishii* [Van Soest et al., 2002], Out of these species, *Mycale (Zygomycale) parishii* is commonly found in Indian Exclusive Economic Zone [Dendy, 1905]. There are no prior reports in the literature on the secondary metabolites from the species *M. parishii* was carried out. Isolation of compounds and their strucutre elucidation is reported in this paper.

Experimental Methods

General experimental details

Optical rotations were determined on a Rudolph Research Analytical (AUTOPOL V) Polarimeter at a wavelength of 589 nm (sodium D line) using a 1.0-decimeter cell with a total volume of 1.0mL. The UV spectra were measured on an Agilent UV-VIS spectrophotometer and infrared spectra on Bruker ALPHA. All solvents were of analytical grade. Column chromatography was performed on Merck silica gel (120-200 mesh) and Sephadex LH-20 (Sigma-Aldrich Chemie GmbH). Agilent 1260 infinity series HPLC system equipped with phenomenix semi-prep C_{18} column 10 X 150 mm, 5µ, flow rate 2 mL/min, have been used for the purification. Thin layer chromatography was carried out with silica gel GF254 plates, Merck, USA. The ^{1}H and ^{13}C, DEPT-135, COSY, TOCSY, HSQC, HMBC and 400 MHz (or 100 MHz for ^{13}C) at Bruker 400 MHz and JEOL, (Internal standard: TMS). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The positive ion HR-ESI-MS spectra were recorded on a Mass Q-TOF-LC-MS spectrometer (Waters synapt G2).

Collection of sponge

The sponge *Mycale (Zygomycale) parishii* was collected from Mirkarwada (N 18°19.092 E 072°57.343), Arabian sea, identified by Dr. Satish S.Mokashe, Associate Professor, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Maharashtra, INDIA. A voucher specimen (No. BAU 12) was deposited at Dr. Babasaheb Ambedkar Marathwada University, INDIA. The sponge sample was placed in plastic bags and kept cool on the ice during transportation

Extraction and isolation

The sponge was washed with distilled water to remove surface salts, sand, and epiphytes. The sponge was dabbed with tissue paper to remove excess water, cut into small pieces and placed in a lyophilizer to dry completely. The freeze-dried sponge (0.86g) was extracted sequentially with hexane and methanol. The extracts were concentrated under vacuum using Rotary evaporator at 40 C, and subjected to bioactivity studies in preliminary screening against bacteria and fungi. Repeated chromatography over silica gel (gradient elution, hexane-EtOAc), followed by Sephadex LH20 (MeOH: DCM, 1:1) resulted in 20 (F1-F20) fractions. Two fractions F11 and F 12 exhibited antimicrobial activity. These were mixed together and purified by C_{18} semi-prep column (10X150 mm, 5µ) for HPLC, using 0.01% aqueous TFA in water : MeOH, and resulting in the isolation of the four compounds. The pure compounds were eluted at different retention times (t_R), which are as follows: t_R 13 (6mg, compound 1), t_R 17 (15 mg, compound 3), t_R 19 (8 mg, compound 2), t_R 23 (6mg, compound 4) respectively as showed in Fig. S1.

Antibacterial and antifungal activity:

The antimicrobial activities were determined using the disc diffusion assay [Lippert et al., 2003] in Petri dishes. The crude sponge extract

and pure compounds 1 to 4 were tested for antimicrobial activity against *Escherichia coli* (NCIM 2065), *Salmonella typhimurium (*NCIM 2501), *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), and four fungi *Aspergillus niger* (NCIM 1207), *Penicillium chrysogenum* (NCIM 1315), *Alternaria sp* (NCIM 900), and *Fusarium sp* (NCIM 1372). The bacterial and fungal strains were supplied by National Collection of Industrial Microorganisms (NCIM), Pune, India. The crude extracts and pure compounds were dissolved in DMSO at a concentration of 2 mg/mL. The discs were loaded with different concentrations of pure compounds (10-100 µg/disc), which were added via a pipette onto a sterile antibiotic filter disc of 6 mm diameter, and dried, to find out the inhibitory potential growth of microbes.

Cytotoxic assay

Cytotoxicity assays were performed on HeLa, SiHa (cervical cancer cells) and MDA-MB-231 (Breast cancer cell line) line. Cell proliferation was followed by the colorimetric MTT test [Visconti et a.l, 1991].

Results and discussion

As part of our ongoing research program to discover natural products from sponges from the West coast of India. The methanolic extract of the sponge exhibited broad-spectrum activity against bacteria and fungi in preliminary studies. It was selected for detailed bioassay-guided purification. Repeated chromatography over silica gel, Sephadex LH_{20} (MeOH: DCM, 1:1), followed by semi-preparative HPLC led to the isolation of compounds 1 (6 mg), 2 (8 mg), 3 (15 mg), and 4 (6 mg) respectively as showed in Fig. S1 and named as 5-methoxy-5-(oxazol-2-yl)pentane-1,2,3,4-tetraol (1), 5-oxazol-2-yl - pentane-1,2,3,5-tetraol (2), 4-hydroxy-4-methylpentan-2-one (3), and 4-(3,3-dimethylbutyl) phenol (4), as shown respectively in Fig. 1. We report herein the isolation and structural elucidation of the compounds 1to 4, as well as their bioactivity against bacteria and fungal strains, and cytotoxicity against HeLa, SiHa and MDA-MB-231 cell lines.

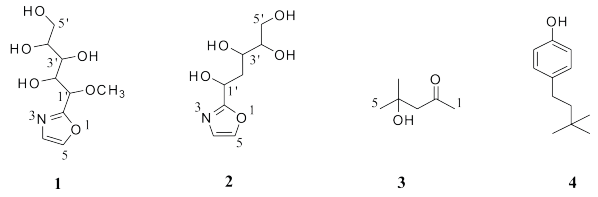

Figure 1. Chemical structures of isolated compounds **1-4** from *Mycale parishii*

Compound **1** was obtained as a white crystalline powder. The specific rotation was $[\delta]^{25}_{D}$ = 0.0 (c 1.0, CH_3OH), indicating optically inactive compound. The ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 256.083 $[M+Na]^+$ (calcd. 256.082) (Fig. S2), corresponding to the molecular formula of $C_9H_{15}NO_6Na$ and indicating three degrees of unsaturation. The strong UV absorption at δ_{max} 258 nm (Fig. S4) indicating a possibility of aromatic moiety in the compound. The IR spectra showed bands at 3313 (OH stretch), 2943 (CH stretch), 2830 (CH stretch), 1450 (C-OH stretch), 1115 & 1020 cm^{-1} (C=C stretch) (Fig. S3). The ^{1}H NMR spectrum (Fig. S5 & Table 1) exhibited two methine signals at δ_H 5.62 (1H, d, *J*= 7 Hz, H-4); δ_H 7.39 (1H, d, *J*= 7 Hz, H-5), clearly indicated mono-substituted five member ring, a characteristic feature of oxazoles [Grundmann et al., 2012]. Another non-olefinic methine signal at δ_H 4.62 (1H, d, J= 11Hz, H-1'), three oxy methine signals at δ_H 3.77 (1H, m, H-2'), 3.47 (1H, m, H-3'), 3.63 (1H, m, H-4'), oxy methylene signal at δ_H 3.70 (2H, m, H-5'), and a methoxy signal at δ_H 3.72 (3H, S, H-6') indicate the presence of methoxy tetraol in the compound 1.

The ^{13}C and DEPT NMR spectrum (Fig. S6 & S7) of 1 showed nine resolved peaks, which were classified into one methoxy carbon at δ_C 53.8, oxy methane carbon at δ_C61.4, three oxy methine carbons at δ_C 67.2, δ_C73.0, δ_C71.2, three olefinic carbons at δ_C101.6, 100.3, 142.4 and a quaternary carbon at $_C$166.2. The connectivity of proton and carbon atoms was confirmed by ^{1}H-^{13}C HSQC (Fig. S8). Comparison of UV, IR, and NMR spectral data of compound 1 with reported literature values suggested that compound 1 was an oxazole derivative. Further interpretation of the COSY, HSQC and HMBC data allowed assignment of all signals in both the ^{1}H and ^{13}C NMR spectra (Table 1and Fig. 2).

Table 1 NMR data of compound 1& 2 (400 MHZ, CD_3OD)

Position	Compound **1** δ_H	δ_C	HMBC	Compound **2** δ_H	δ_C	HMBC
1	-	-	-	-	-	-
2	-	166.2, C	5	-	163.6, C	4,5
3	-	-	-	-	-	-
4	5.62 (1H,	100.3,CH	5	5.71(1H,	102.4, CH	2,5
5	d,	142.4, CH	1',2,4	d= 7.7Hz)	142.4, CH	1',2,4
1'	J=7.7Hz)	101.6,CH	2',6'	8.01(1H,	86.5, CH	5
2'	7.39(1H,	71.2, CH	1',3',6'	d= 7.7	41.3, CH	1'
3'	d,	73.0, CH	1',3',6'	Hz)	72.2, CH	1',5'
4'	J=7.7Hz)	67.2, CH	-	6.28(1H,	88.9, CH	3',5'
5'	4.62 (1H,	61.4, CH_2	-	t,	62.7, CH_2	-
6'	d, J=	53.8,	1'	J=13.40H	-	-
	11.0Hz)	OCH_3		z)		
	3.77 (1H,			2.22(1H,		
	m)			m)		
	3.47 (1H,			4.40(1H,		
	m)			m)		
	3.63 (1H,			3.93(1H,		
	m)			m)		
	3.70 (2H,			3.75(2H,		
	m)			d, J=		
	3.36 (3H,			4.0Hz)		
	S)			-		

The oxazole moiety in compound **1** was elucidated by COSY and HMBC correlations showed in Fig. 2. The ^{1}H-^{1}H COSY data revealed the coupling between H-4/ H-5 and HMBC correlations of H-5 to C-4 (δ_C100.3) and H-4 to C-5 (δ_C142.4); presence of only two field protons in ^{1}H NMR spectrum confirmed the monosubstitution at C-2 position in five member ring in compound **1** [Grundmann et al., 2012]. COSY correlations of H-1'-H-2'-H-3'-H-4'-H-5' (Fig. S9) along with HMBC cross peaks of H-1' to C-6' (δ_C 53.8), C-2' (δ_C 71.2) and H-2'/H-3' to C-1' (δ_C 101.6), C-3 '(δ_C73.0) and C-6' (δ_C53.8) (Fig. S10); and the upfield shift at δ_H 4.62/δ_C 101.6 (C-1') which was due to the substitutions of methoxy group (δ_H 3.36, 3H, s, 6'); confirmed the presence of 5-methoxy pentane 2', 3', 4', 5' tetraol. Further HMBC correlations H-6' to C-1', H-5 to C-2 (δ_C 166.2) and C-1' (δ_C 101.6), established that the 1'-methoxy pentane 2', 3', 4', 5' tetraol moiety was linked to the quaternary carbon at C-2 position (δ_C 166.2). A similar substitution pattern has been observed in the reported oxazole derivatives such as labradorin 1, 2, 3 [Grundmann et al, 2012]. Based on the above-mentioned spectral data, the structure of compound 1 was determined as 1'-methoxy-1'-(oxazol-2-yl) pentane-2', 3', 4', 5' tetraol. This secondary metabolite, which has been reported for the first time as a natural compound from sponge *M. parishii*, was designated as mycaoxazole A, whose complete ^{1}H and ^{13}C NMR assignments were based on COSY, HSQC, and HMBC spectra as described in Table 1 and in Fig. 2.

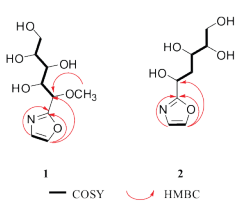

Figure 2. Key COSY and HMBC correlations of compound 1 & 2

Compound **2** was obtained as a white crystalline powder, with

specific rotation $[\alpha]^{25}_{D}$ = 0.0 (c 1.0, CH_3OH), indicating optically inactive compound. The ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 242.043 $[M+K]^+$ (calc. 243.04) (Fig. S12), corresponding to the molecular formula of $C_8H_{13}NO_5K$ and indicating three degrees of unsaturation. The UV & IR (Fig. S13, S14), and ^1H, ^{13}C NMR spectra (Fig. S15, 16, 17 & Table 1) showed similarity with compound 1; the difference being the presence of upfield shift and observed splitting pattern at δ_H 6.28 (1H, t, J= 13.4Hz, H-1')/ δ_C 86.5, which asserted the location of hydroxyl group at C-1' position. In addition, presence of low field signal at δ_H 2.22 (2H, m, H-2')/ δ_C 41.3 indicated the absence of functional groups at C-2' position; and the presence of two oxymethines at δ_H 4.40 (1H, m, H-3'), δ_H 3.93 (1H, m, H-4') and one oxy methylene signal at δ_H 3.75 (2H, d, J= 4.0Hz, H-5') respectively confirmed the presence of pentane 1', 3', 4', 5' tetraol moiety in the compound **2**. The COSY and HMBC correlations (Fig. 2) established that the pentane 1', 3', 4', 5' tetraol moiety was linked to quaternary carbon at C-2 (δ_C 163.6) [16]. Based on these spectral data, the structure of compound 2 was established as 2-oxazol-2-yl - pentane-1', 3', 4', 5' tetraol. This is another secondary metabolite to be reported from the sponge *M. parishii* and was designated as mycaoxazole B, whose complete ^1H and ^{13}C NMR assignments were based on HSQC, COSY, and HMBC spectra as depicted in Fig. S 18, 19, 20.

Compound **3** was obtained as a white crystalline powder. The ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 117.167 $[M+H]^+$ (calcd. 117.16) (Fig. S 22), corresponding to the molecular formula of $C_6H_{12}O_2$. Examination of physical and spectral data like ^1H, ^{13}C, TOCSY, HMBC (Fig. S 22-26), compound 3 was identified as 4-hydroxy-4-methyl pentan-2-one and as a known compound. This compound was commercially available in Sigma with CAS Number 123-42-2. Literature reports suggest its role in mediating oviposition behavior in *Delia platura* females. [Gouinguené et al, 2006].

Compound **4** showed a light yellow color. ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 178.94 $[M]^+$ (calcd. 178.14) (Fig. S 27), corresponding to the molecular formula of $C_{12}H_{18}O$. Examination of physical and spectral data like ^1H, ^{13}C, COSY, and HMBC data (Fig. S 28-31) compound 3 was identified as identified as 4-(3, 3-dimethyl butyl) phenol, and commercially available with CAS Registry Number 101005-18-9. According to literature, these phenol derivatives act as pest repellents [Yoji, 1985].

The class of oxazole compounds reported from sponges [Ichiba et al.,1991], *Trichoderma sp* [Lee et al., 1995, Takahashi et al, 1996], *Streptomyces* [Joshi et al., 1963], *Streptoverticillium* [Koyama et al., 1981; Umehara et al., 1984], *Pseudomonas* [Gouinguené et al., 2006] exhibit anticancer, antiviral, antifungal, antibacterial, and antiproliferative activities [Swellmeen et al., 2016]. The isolated compounds 1 & 2 also exhibited broad spectrum activity against *B. subtilis*, *E. coli*, *S. aureus*, *A. niger*, and *P. chrysogenum* at 100 µg/disk, as shown in Table S1. Compounds 3 & 4 did not show any bioactivity. None of the compounds exhibited any cytotoxic activity up to 500 µg/mL, against HeLa and SiHa (cervical cancer cells), and MDA-MB-231(Breast cancer cell line) lines, as shown in Fig. S 32.

In conclusion, four metabolites have been isolated from *Mycale parishii* for the first time, and their structures elucidated by NMR and mass spectroscopic analysis, as shown in Fig. 1. Among these substances, compounds 1 & 2 have been reported as new oxazoles, and 3 & 4 are known compounds, isolated for the first time from the sponge *Mycale parishii*.

Compound 1 was a white crystalline powder, the specific rotation was $[\alpha]^{25}_{D}$ = 0.0 (c 1.0, CH_3OH), UV (MeOH) λ_{max} 258 nm, FTIR (MeOH): 3313, 2943, 2830, 1450, 1115& 1020 cm^{-1}. ^1H and ^{13}C, 2D NMR data is shown in Table 1. HRESI-TOF-MS positive mode m/z 256.083 $[M+Na]^+$ (Calcd. for $C_9H_{15}NO_6Na$, 256.082).

Compound 2 was a white crystalline powder, the specific rotation was $[\alpha]^{25}_{D}$ = 0.0 (c 1.0, CH_3OH), UV (MeOH) λ_{max} 262 nm, FTIR (MeOH): 3332, 2948, 2835, 1451, 1451 and 1113 cm^{-1}. ^1H and ^{13}C NMR data is shown in Table 1. HRESI-TOF-MS positive mode m/z 242.043 $[M+K]^+$ (Calcd.for $C_8H_{13}NO_5K$, 243.04)

Compound 3 was a white crystalline powder, ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 117.167 $[M+H]^+$ corresponding to the molecular formula of $C_6H_{12}O_2$.

Compound 4 was light yellow color, ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 178.94 $[M]^+$ corresponding to the molecular formula of $C_{12}H_{18}O$.

Acknowledgments

This work was financially supported (RGSTC/File-2007/DPP-054/CR-28) by the Rajiv Gandhi Science and Technology Commission, Maharashtra, India.We are thankful to NMR Research Centre and Mass facility at IISER Pune. My special thanks to NMR technicians Chinmay Lowalekar and Deepali Jadhav, Sandeep C.Kanade for NMR and mass services. I am grateful to Avantika for her help in the manuscript preparation.

Supplementary information

Supplementary data associated with this article can be found in the online version.

The authors declare no competing financial interests.

References:

1. Faulkner, D.J. 2002.Nat. Prod. Rep.19, 1-49.
2. Blunt, J.W.; Copp, B.R.; Ping Hu, W.; Munro, M.H.G., Northcote, P.T.; Prinsep, M. R. 2009. Nat. Prod. Rep.26, 170-244.
3. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munroa, M.H.G.; Prinsep, M.R.2015. Nat. Prod. Rep. 32, 116-211.
4. Perry, N.B.; Blunt, J.W.; Munaro, M.H.G.; Pannel, L.K.; J. Am. Chem. Soc.1988. 110, 4850-4851.
5. Perry, N.B.; Blunt, J.W.; Munro, M.H.G.; Thompson, A.M.1990. J. Org. Chem. 55, 223-227.
6. Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hashimoto, K. 1989. Tetrahedron Lett. 30, 2809-2812.
7. Matsunaga, S.; Sugawara, T.; Fusetani, N. 1998. J. Nat. Prod. 61, 1164-1167.
8. Phuwapraisirisan, P.; Matsunaga, S.; Van Soest, R.W.M.; Fusetani, N. 2002. J. Nat. Prod. 65, 942-943.
9. Northcote, P.T.; Blunt, J.W.; Munro, M.H.G. 1991, Tetrahedron Lett. 32, 6411-6414.
10. West, L.M.; Northcote, P.T.; Battershill, C.N. 2000. J. Org. Chem. 65, 445-449.
11. Nakao, Y.; Yoshida, S.; Matsunaga, S.; Shindoh, N.; Terada, Y.; Nagai, K.; Yamashita, J.K.; Ganesan, A.; Van Soest, R. W. M.; Fusetani, N. 2006. Angew. Chem. Int. Ed. 45, 7553-7557.
12. Singh, A.J.; Xiao Xu, C.; Xu, X.; West, L.M.; Wilmes, A.; Chan, A.; Hamel, E.; Miller, J.H.; Northcote, P.T.; Ghosh, A.K. 2010. J. Org. Chem. 75, 2–10.
13. Reddy, G.B.S.; Dhananjaya, N. 2000. Bio-org. Med. Chem. 8, 27-36.
14. Van Soest, R.W.M.; Hajdu, E. 2002.Family Mycalidae Lundbeck, 1905. Pp. 669-690. In J. N. A. Hooper, & R. W. M. Van Soest, (ed.) Systema Porifera. A guide to the classification of sponges. 1 (Kluwer Academic/ Plenum Publishers: New York, Boston, Dordrecht, London, Moscow).
15. Dendy, A. 1905. Report on the sponges collected by Professor Herdman, at Ceylon, in 1902. Pp.57-246, pls I-XVI. In: Herdman, W.A. (Ed.), Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar. 3(Supplement 18). (Royal Society: London).
16. Lippert, H.; Brinkmeyer, R.; Mulhaupt, T.; Iken, K. 2003.Polar Biol. 26, 591-600.
17. Visconti, A.; Minervini, F.; Lucivero, G.; Gambatesa, V. 1991. Mycopathologia. 113, 181-186.
18. Grundmann, F.; Dill, V.; Dowling A.; Thanwisai, A.; Bode, E.; Chantratita, N.; ffrench, R.C.; Bode, H.B. 2012. Beilstein J. Org. Chem. 8, 749–752.
19. Gouinguené, S.P.; Städler, E. 2006. J. Chem. Ecol., 32, 1399-1413.
20. Yoji, T.; Masato, K. 1985patent, JP 60231601.
21. Ichiba, T.; Yoshida, W.Y.; Scheuer, P.J. 1991. J. Am. Chem. Soc. 113, 3174-3175.
22. Lee, C.; Chung, M.; Lee, H.; Kho, Y. 1995. J. Nat Prod. 58, 1605-1 607.
23. Takahashi, S.; Hashimoto, R.; Hamano, K.; Suzuki, T.; Nakagawa, A. 1996. J. Antibiot. 49, 513-518.
24. Joshi, B.S.; Taylor, W.I.; Bhate, D.S.; Karmarkar, S.S. 1963, Tetrahedron. 19, 1437–1439.
25. Koyama, Y.; Yokose, K.; Dolby, L.J. 1981. J. Agric. Biol. Chem. 45, 1285–1287.
26. Umehara, K.; Yoshida, K.; Okamoto, M.; Iwami, M.; Tanaka, H.; Kohsaka, M.; Imanaka, H. 1984. J. Antibiot. 37, 1153–1160.
27. Swellmeen, L. 2016. Der PharmaChemica. 2016. 8, 269-286.