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Novel alkaloids of the aaptamine class from an Indonesian marine sponge of the genus *Xestospongia*

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Abstract—Four novel alkaloids of the aaptamine class have been isolated in addition to the known aaptamine, isoaaptamine, demethyl(oxy)aaptamine and its dimethylketal from an unidentified species of Indonesian marine sponge of the genus *Xestospongia*. Their structures were elucidated on the basis of detailed 1D and 2D NMR spectroscopic data. Their antimicrobial activity was tested towards Gram (+) (*S. aureus*), Gram (−) (*E. coli*, *V. anguillarum*) bacterial strains, a fungus (*C. tropicalis*); their cytotoxic activity was evaluated against KB cells.

1. Introduction

Marine sponges of the genus *Xestospongia* (phylum Porifera, class Desmospongia, order Haplosclerida, family Petrosiidae) have been proven to be a rich source of diverse secondary metabolites including alkaloids,[1] polycyclic quinones and hydroquinones[2] polyacetylenic derivatives,[3] aminoalcools,[4] heterocyclic compounds,[5] original sterols.[6,7] Some of these compounds displayed significant cytotoxic, antimicrobial or vasodilatory activity. Recent studies indicate that marine sponges of the genus *Xestospongia* continue to be an excellent source of unusual alkaloids such as hachijodines A–G,[8] xestosin A,[9] amphimedine and its related derivatives, which are topoisomerase II inhibitors that catenate DNA.[10,11]

In our search for pharmacologically active substances from marine organisms, we examined the MeOH extract of the Indonesian marine sponge *Xestospongia* sp. collected off Jakarta. On the basis of a preliminary pharmacological screening, the MeOH crude extract was selected for its promising antimicrobial activity against *S. aureus*, *E. coli*, *V. anguillarum*, and *C. tropicalis* (ϕ inhibition at 1 mg/disc of 22, 8, 10 and 14 mm respectively). A bioassay-guided fractionation led us to isolate five new alkaloids **4**–**8** related to the aaptamines, in addition to the known aaptamine **1**,[12] isoaaptamine **2**,[13,14] and demethyl(oxy)aaptamine **3**[15] previously isolated from the marine sponge *Aaptos aaptos*. Compounds (**1**–**8**) were tested towards Gram (+) (*S. aureus*), Gram (−) (*E. coli*, *V. anguillarum*), a fungus (*C. tropicalis*) and also on KB cells.

2. Results and discussion

Successive chromatographies of the MeOH crude extract on silica gel, Sephadex LH20 and reverse-phase columns, guided by antimicrobial bioassays, yielded eight compounds: the known benzo[*de*] [1,6]naphthyridine type compounds **1**–**3** and the new metabolites **4**–**8**, structurally related to the aaptamines (See Fig. 1). The structures and complete ^{1}H and ^{13}C NMR spectral assignments for the new compounds **4**–**8**, as well as those for aaptamine **1**, isoaaptamine **2** and demethyl(oxy)aaptamine **3**, were determined based on extensive 1D and 2D NMR studies.

Compound **1**, the major component of the MeOH crude extract, was obtained as bright yellow crystals, mp 110–112°C. HR-FABMS established the molecular formula as $C_{13}H_{12}N_2O_2$ with a protonated molecular ion $[M+H]^+$ at *m/z* 229.0971 (Δ −0.6 mmu). Detailed examination of spectroscopic data (UV, IR, 1D and 2D NMR) readily established identity with aaptamine **1** previously described from the Okinawan marine sponge *Aaptos aaptos*, by comparison with the spectral data reported in the literature.[11]

Compound **2**, the second abundant component of the MeOH

Keywords: *Xestospongia* sp; sponge metabolites; alkaloids; aaptamine; benzo[*de*] [1,6]naphthyridine; triaza-pyrene; triaza-cyclopenta[*cd*]-phenalene; antibiotic; cytotoxic activity.
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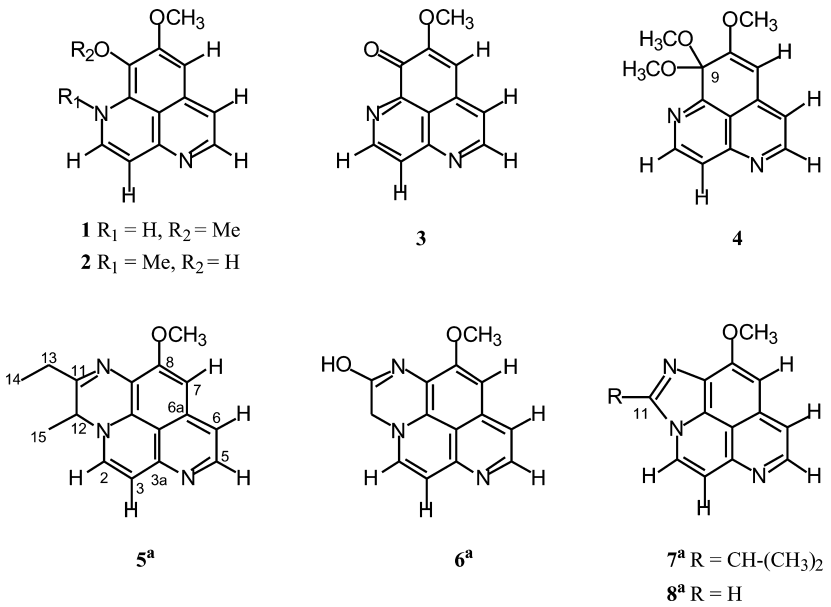

Figure 1. Structures of compounds **1**–**8** isolated from the marine sponge *Xestospongia* sp. [a]For a better comparison of δ values, we have kept for compounds **5**–**8** the numbering of the benzo[*de*] [1,6]naphthyridine skeleton as in aaptamine **1**.

crude extract, had the same molecular formula as that of aaptamine **1**. ^{1}H NMR data (See Table 1) were similar to those of **1** and the ^{13}C NMR spectrum (See Table 2) differed only by the presence of an additional N–CH_3 group (δ ^{13}C 46.0) and the presence of only one O–CH_3 group (δ ^{13}C 55.6). Detailed analyses of 2D NMR data including ^{1}H–^{1}H COSY, HSQC and HMBC spectra easily identified compound **2**, as being isoaaptamine, also possessing a benzo[*de*] [1,6]naphthyridine skeleton previously reported from two sponges of the Suberitidae family, a *Suberites* sp. collected in the Indian Ocean[12] and a Red Sea sponge *Aaptos aaptos*.[13]

Compound **3** had the molecular formula $C_{12}H_8N_2O_2$. The ^{1}H NMR spectrum was similar to that of aaptamine **1** except the loss of one methoxyl group. Furthermore, the IR spectrum indicated the presence of a carbonyl group (1661 cm^{-1}). These spectral data, and analysis of the complete 2D NMR study, suggested that compound **3** was demethyl(oxy)aaptamine, previously isolated from the Okinawan marine sponge *Aaptos aaptos*.[14] Our study permitted to inverse previous assignments of carbone C-8 and C-3a, which were erroneous.

Compound **4** was isolated as a brown gum with a molecular formula of $C_{14}H_{14}N_2O_3$, as determined by HR-FABMS analysis (*m/z* 259.1080 $[M+H]^+$), implying the presence of nine unsaturations in the molecule. Compound **4** exhibited spectral data very similar to those of demethyl(oxy)aaptamine **3**. The occurrence of two sets of coupled protons at δ 8.97 (d, *J*=4.5 Hz) and 7.40 (d, *J*=4.5 Hz), 8.88 (d, *J*=5.7 Hz) and 7.90 (d, *J*=5.7 Hz), one isolated singlet at δ 6.48 and one methoxyl group at δ 3.89 in the ^{1}H NMR spectrum, recorded in DMSO-d_6, suggested the benzo-[*de*][1,6]naphthyridine skeleton. HMBC correlations confirmed this hypothesis. In addition, two further methoxyl groups at δ 3.06 (δ ^{13}C 51.1) were observed. HMBC correlations between the methoxyl groups at δ 3.06 and the quaternary carbon at δ 96.0 identified their geminal position on carbon C-9. NOESY correlations from the methoxyl group at δ 3.89 with the aromatic proton at δ 6.48 and from this aromatic proton with the aromatic proton at δ 7.40 gave

Table 1. ^{1}H NMR data of compounds **1**–**4** and **6**–**8** recorded in DMSO-d_6 [δ_H (multiplicity, *J* in Hz)]

No.	1	2	3	4	6	7	8
2	7.80 (d, 7.0)	7.73 (d, 7.4)	9.11 (d, 5.5)	8.88 (d, 5.7)	8.72 (d, 7.7)	8.74 (d, 7.7)	8.74 (d, 7.7)
3	6.49 (d, 7.0)	6.26 (d, 7.4)	8.23 (d, 5.5)	7.90 (d, 5.7)	7.34 (d, 7.7)	7.28 (d, 7.7)	7.35 (d, 7.7)
5	7.30 (d, 7.1)	7.23 (d, 7.1)	9.15 (d, 4.4)	8.97 (d, 4.5)	8.67 (d, 5.8)	8.63 (d, 5.8)	8.69 (d, 5.8)
6	6.78 (d, 7.1)	6.79 (d, 7.1)	7.77 (d, 4.4)	7.40 (d, 4.5)	7.78 (d, 5.8)	7.75 (d, 5.8)	7.79 (d, 5.8)
7	7.02 (s)	7.15 (s)	7.18 (s)	6.48 (s)	7.26 (s)	7.23 (s)	7.28 (s)
11							8.98 (s)
12					5.13 (s)	3.80 (m)	
13						1.47 (d, 6.8)	
14						1.47 (d, 6.8)	
8-OMe	3.93 (s)	3.96 (s)	3.91 (s)	3.89 (s)	4.18 (s)	4.16 (s)	4.19 (s)
9-OMe	3.77 (s)			3.06 (s)[a]			
1-NH	12.80 (brs)						
1-NMe		4.06 (s)					
9-OH		9.45 (brs)					

[a] This peak integrates 6 protons.

Table 2. ^{13}C NMR data of compounds **1**–**4** and **6**–**8** in DMSO-d_6 (δ_C)

No.	1	2	3	4	6	7	8
2	141.8	148.9	148.8	147.1	127.8	127.2	128.1
3	97.9	97.4	126.5	122.1	114.9	114.8	115.3
3a	149.6	149.3	148.1	148.7	146.9	146.1	146.6
5	129.6	127.8	157.1	156.5	145.8	145.3	145.9
6	112.6	113.2	122.1	118.1	116.3	115.9	115.9
6a	132.6	129.4	136.3	138.6	134.3	133.9	135.0
7	100.7	101.5	108.9	99.9	98.8	97.3	97.6
8	156.8	153.6	155.8	161.2	156.0	155.6	155.7
9	131.3	132.2	177.1	96.0	125.4	125.1	126.1
9a	133.6	129.4	147.8	155.2	130.0	128.9	128.2
9b	116.2	118.1	117.8	115.9	112.1	111.7	111.8
11					146.8	152.0	135.0
12					56.9	26.2	
13						21.4	
14						21.4	
8-OMe	56.5	55.6	56.1	55.9	56.6	56.0	56.3
9-OMe	60.4			51.1			
1-NMe		46.0					

evidence that the structure of compound **4** was the dimethylketal derivative of demethyl(oxy)aaptamine **3**.

Compound **5** was isolated as an orange gum. Based on HR-FABMS that exhibited a protonated molecular ion at *m/z* 280.1449 $[M+H]^+$, the molecular formula was determined to be $C_{17}H_{17}N_3O$. The ^1H NMR spectrum of compound **5** recorded in $CDCl_3$ showed better information than in DMSO-d_6. ^1H NMR spectrum in $CDCl_3$ showed two sets of coupled protons at δ 8.69 (d, J=5.9 Hz) and 7.65 (d, J=5.9 Hz), 8.15 (d, J=7.7 Hz) and 7.32 (d, J=7.7 Hz) and one isolated singlet at δ 7.04, which are similar to those of aaptamine **1**, suggesting the presence of a benzo[*de*][1,6]-naphthyridine skeleton. However, only one signal due to a methoxyl group was observed at δ 4.20 (δ ^{13}C 56.8). In addition, one methine proton at δ 3.39, two protons for one methylene group at δ 2.10 and 1.90, one methyl group as a doublet at δ 1.58 (d, J=6.9 Hz) and one methyl group as a triplet at δ 0.94, were observed. Furthermore, the ^1H NMR spectrum of **5** recorded in DMSO-d_6 did not show any exchangeable proton. Taking into account this information and the eleven degrees of unsaturation in the molecule, COSY correlations and HMBC experiments (See Table 3) provided evidence for structure **5**. In particular, decisive information came from HMBC correlations observed from the methine proton at δ 3.39, the methylene protons at δ 2.10 and 1.90 and the methyl protons at δ 1.58 to the carbon resonating at δ 151.0. Further support in favour of this conclusion was obtained from the NOESY spectrum, which exhibited NOESY correlations between the methyl protons

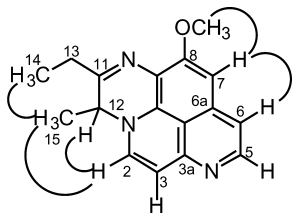

Figure 2. Selected NOESY (∩) correlations used to establish the structure of compound **5**.

at δ 1.58 and the methyl protons at δ 0.94 and the aromatic proton at δ 8.15 and between this aromatic proton and the methine proton at δ 3.39 (See Fig. 2). Based on these data, the structure of compound **5** could be unambiguously assigned as a novel compound having a triaza-pyrene skeleton as shown in Figure 2.

Compound **6** was isolated as a brown gum. Its molecular formula $C_{14}H_{11}N_3O_2$ was obtained from HR-FABMS of the protonated molecular ion $[M+H^+]$ at *m/z* 254.0933, indicating the presence of eleven unsaturations in the molecule. Inspection of the ^1H NMR spectrum (See Table 1) also indicated that compound **6** possesses the benzo[*de*][1,6]naphthyridine skeleton and only one methoxyl group (δ ^{13}C 56.6 ppm). An additional signal for a methylene group was observed at δ 5.13 ppm as a singlet, which might be located adjacent to an heteroatom. Furthermore, the IR spectrum revealed the presence of an absorption band at 3318 cm^{-1}, characteristic of an hydroxyl function. Careful analysis of the HMBC spectrum confirmed the presence of the benzo[*de*] [1,6]naphthyridine skeleton (See Fig. 3). However, taking into account the presence of eleven unsaturations in the molecule, HMBC correlations between the methylene protons at δ 5.13 and the quaternary carbon at δ 146.8, allowed to propose structure **6** possessing a triaza-pyren-ol skeleton for the novel compound. Crucial NOESY correlations observed between the methylene protons at δ 5.13 and the aromatic proton at δ 8.72 and between the methoxyl protons at δ 4.18 and the aromatic proton at δ 7.26, unambiguously confirmed structure **6** as presented in Figure 3.

The molecular formula of compound **7**, isolated as a brown gum, was determined to be $C_{16}H_{15}N_3O_2$ based on HR-FABMS analysis that exhibited a protonated molecular ion at *m/z* 266.1299 $[M+H]^+$, indicating the presence of eleven unsaturations. In the ^1H NMR spectrum the presence of two sets of coupled protons at δ 8.74 (d, J=7.7 Hz) and 7.28 (d, J=7.7 Hz), 8.63 (d, J=5.8 Hz) and 7.75 (d, J=5.8 Hz) and one isolated singlet at δ 7.23, suggested

Table 3. ^1H and ^{13}C NMR data of compound **5** recorded in $CDCl_3$

No.	δ_C	δ_H (m, J in Hz)	HMBC (H→C)	No.	δ_C	δ_H (m, J in Hz)	HMBC (H→C)
2	125.1	8.15 (d, 7.7)	3, 3a, 9a	9	126.2		
3	116.1	7.32 (d, 7.7)	2, 9b	9a	129.1		
3a	147.3			9b	112.8		
5	145.6	8.69 (d, 5.9)	3a, 6, 6a	11	151.0		
6	116.2	7.65 (d, 5.9)	5, 6a, 9b	12	34.5	3.39 (q)	11, 13, 14, 15
6a	133.4			13	29.2	2.10 (m)–1.90 (m)	11, 12, 14, 15
7	96.5	7.04 (s)	6, 8, 9, 9b	14	12.1	0.94 (t)	12, 13
8	156.2			15	19.2	1.58 (d, 6.9)	11, 12, 13
				8-OMe	56.8	4.20 (s)	8

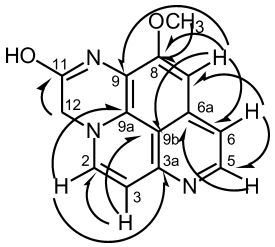

Figure 3. Selected HMBC correlations used to determine the structure of compound **6**.

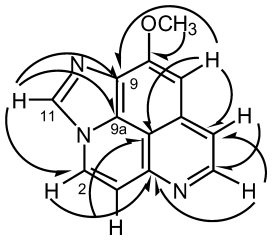

Figure 5. Selected HMBC correlations used to determine the structure of compound **8**.

that compound **7** has the same skeleton as aaptamine **1**. The most significant differences observed in the ^{1}H NMR spectrum were the presence of only one methoxyl group at δ 4.16 (δ ^{13}C 56.0), the addition of one methine proton at δ 3.80 and six methyl protons as a doublet at δ 1.47 (d, J=6.8 Hz). Furthermore, ^{1}H–^{1}H COSY correlations between the methine proton at δ 3.80 and methyl groups at δ 1.47 established the presence of an isopropyl group. Significant HMBC correlations between the methyl protons at δ 1.47 and the quaternary carbon at δ 152.0, suggested the presence of an imidazole skeleton substituted by an isopropyl group (See Fig. 4). Crucial NOESY correlations between the methyl protons at δ 1.47 and the aromatic proton at δ 8.74 and between the methoxyl protons at δ 4.16 and the aromatic proton at δ 7.23, confirmed structure **7** as shown in Figure 4.

Compound **8** was isolated as a brown gum. Its molecular formula was determined to be $C_{13}H_9N_3O$ based on HR-FABMS analysis that exhibited a protonated molecular ion at m/z 224.0818 $[M+H]^+$, indicating the presence of eleven unsaturations. The ^{1}H spectrum of **8** was similar to that of **7** with two sets of coupling protons at δ 8.74 (d, J=7.7 Hz) and 7.35 (d, J=7.7 Hz), 8.69 (d, J=5.8 Hz) and 7.79 (d, J=5.8 Hz) and one isolated singlet at δ 7.28. However, we noticed in the spectrum the presence of an additional proton at δ 8.98 and the absence of protons for an isopropyl group. Extensive analysis of the NMR data provided support for the complete assignments of the protons and carbons (See Tables 1 and 2). Key HMBC correlations between the methine proton at δ 8.98 and carbons C-2 at δ 128.1, C-9 at δ 126.1 and C-9a at δ 128.2, suggested that compound **8** possesses the triaza-cyclopenta[*cd*]phenalene skeleton as in compound **7** (See Fig. 5). A NOESY correlation between the methine at δ 8.98 and the aromatic proton at δ 8.74, confirmed the proposed structure of the novel compound **8**.

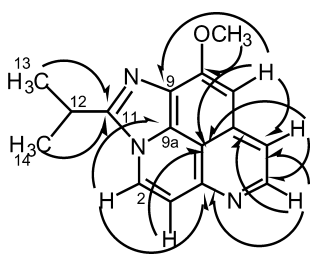

Figure 4. Selected HMBC correlations used to determine the structure of compound **7**.

In order to evaluate their antimicrobial activity, all the compounds described were tested towards Gram (+) (*S. aureus*), Gram (−) (*E. coli*, *V. anguillarum*) bacterial strains and a fungus (*C. tropicalis*). Their cytotoxic activity was also evaluated towards the human buccal carcinoma KB. Results are summarized in Table 4. Aaptamine **1**, isoaaptamine **2**, demethyl(oxy)aaptamine **3** and compound **8** showed moderate antibacterial activity, activity on the ichthyopathogenic strain *V. anguillarum* being the most significative. Only aaptamine **1** and isoaaptamine **2** exhibited antifungal activity towards *C. tropicalis*. A significant cytotoxic activity on KB cells was confirmed for aaptamine **1**, isoaaptamine **2**, demethyl(oxy)aaptamine **3**, according to the previous studies;[16] the new dimethylketal **4** also displayed interesting cytotoxic activity.

Previously, aaptamine **1** showed α-adrenoreceptor blocking and cardiotonic activity,[17] activity in a cell adherence assay related to PKC inhibition[18] and more recently antiviral activity towards HSV-1.[19] Minor compounds **5**–**8** possess a novel skeleton, derived from the benzo[*de*][1,6]naphthyridine moiety. Synthesis of these novel compounds is in progress in order to determine their biological potential.

In a previous paper, aaptamine was presented as a taxonomic marker for sponges of the order Hadromerida, within the sub-class Tetractinomorpha.[20] Isolation of aaptamine in large quantity from a sponge of the order Haplosclerida, which is included in the sub-class Ceractinomorpha led to the conclusion that aaptamine is not a faithful chemotaxonomic marker.

Table 4. Effect of compounds **1**–**8** on growth of microbial strains (MIC in μg/ml) and of KB tumor cells (ID_{50} in μg/ml)

Compound	MIC[a]				ID_{50}[b]
	S. aureus	*E. coli*	*V. anguillarum*	*C. tropicalis*	KB cells
1	25	>100	12	25	3.7
2	6	>100	12	12	0.5
3	25	100	100	>100	1.8
4	>100	>100	>100	>100	3.5
5	>100	>100	>100	>100	>10
6	>100	>100	>100	>100	>10
7	>100	>100	>100	>100	>10
8	>100	>100	100	>100	>10
Penicillin G	0.01	25	12	>100	–
Adriamycin	–	–	–	–	0.015

[a] Compounds were tested twice.
[b] Compounds were tested twice in triplicate.

3. Experimental

3.1. General experimental procedures

Silica gel column chromatographies were carried out using Kieselgel 60 (230–400 mesh, E. Merck), gel filtrations were carried out using LH20 (Sephadex LH20 17-0090-01 Pharmacia Biotech). Fractions were monitored by TLC using aluminium-backed sheets (Si gel 60 F254, 0.25 mm thick) with visualization under UV (254 and 366 nm) and vanilline spray reagent. All analytical reversed-phase HPLC (Kromasil RP18 column K2185, 4.6×250 mm, MeOH/H_2O 8/2) were performed with a L-6200A pump (Merck-Hitachi) equipped with a UV–vis detector (λ=366 nm) L-4250C (Merck-Hitachi) and a chromato-integrator D-2500 (Merck-Hitachi).

UV spectra were obtained in EtOH, using a Kontron-type Uvikon 930 spectrophotometer, and IR spectra were recorded on a Nicolet AVATAR 360 FT-IR spectrophotometer.

Mass spectra were recorded on an API Q-STAR PULSAR I of Applied Biosystem and on a JEOL MS 700BE for low and high-resolution, respectively.

^{13}C NMR spectra were obtained on a Bruker AC300 at 75.47 MHz, ^{1}H NMR spectra 1D and 2D (COSY, HSQC, HMBC, NOESY) were obtained on a Bruker AVANCE 400. HMQC and HMBC experiments were acquired at 400.13 MHz using a $^{1}H-^{13}C$ Dual probehead. The delay preceding the ^{13}C pulse for the creation of multiple quanta coherences through several bounds in the HMBC was set to 70 ms.

3.2. Animal material

Specimens of *Xestospongia* sp. were collected off Jakarta at a depth of 5 m in December 2001 and identified by Jean Vacelet (Station Marine d'Endoume). A voucher specimen is available from the Muséum National d'Histoire Naturelle in Marseilles as collection number MHNM 2003 Im5.

3.3. Extraction and isolation

Samples of fresh sponge (2 kg) were air-dried (dry weight 500 g), then directly extracted with methanol at room temperature for 2 days, concentrated under reduced pressure to obtain 20 g of methanolic crude extract. The extract was chromatographed on a silicagel (Merck silica gel 70–230 mesh) column using dichloromethane with increasing amounts of methanol as eluent. Bioassay-guided fractionation retained a maximum of activity in fractions eluted with 10, 20 and 25% MeOH.

The fraction eluted with 20% MeOH (1.58 g) was subjected to a further silicagel (Merck silica gel 230–400 mesh) column eluted with dichloromethane with increasing amounts of methanol to yield in large amount aaptamine **1** (260 mg).

In the same manner, the fraction eluted with 25% MeOH (2.17 g) was subjected on a silicagel column eluted with dichloromethane with increasing amounts of methanol to yield isoaaptamine **2** (30 mg).

The fraction eluted with 10% MeOH (1.15 g) was separated on a silicagel (Merck silica gel 230–400 mesh) column eluted with dichloromethane with increasing amounts of acetone. The subfraction eluted with 20% of acetone (40 mg) was subjected to reversed-phase HPLC using MeOH/H_2O 8/2 as eluent (flow rate: 1 ml/min, wavelength: 366 nm) to yield compound **5** (0.8 mg). The subfraction eluted with 30% of acetone (7.5 mg) was first separated by preparative TLC (CH_2Cl_2/acetone 1/1) then by reverse-phase HPLC as above using MeOH/H_2O 8/2 as eluent to yield compound **4** (1.4 mg), compound **3** (1.6 mg) and compound **7** (0.7 mg). The subfraction eluted with 40% of acetone (57 mg) was successively separated by preparative TLC (CH_2Cl_2/MeOH 9/1) then by reverse-phase HPLC using MeOH/H_2O 8/2 as eluent (flow rate: 0.5 ml/min, detection wavelength at 366 nm) to yield compound **8** (0.7 mg) and compound **6** (0.9 mg).

3.3.1. Compound 1. Aaptamine, 8,9-dimethoxy-1*H*-benzo[*de*][1,6]naphthyridine. (0.260 g, 0.052% dry weight) yellow crystals; mp 110–112°C; R_f (CH_2Cl_2/MeOH 8:2) 0.19; UV (EtOH) λ_{max} nm (ε): 209 (18407), 276 (7575), 291 (7575), 368 (1992); IR (ATR) ν cm^{-1}: 1647, 1621, 1601, 1460, 1328, 1243, 1106; ^{1}H and ^{13}C NMR data recorded in DMSO-d_6, see Tables 1 and 2; HR-FABMS $[M+H]^+$ found at *m/z* 229.0971 (Δ −0.6 mmu), calc. 229.0977, for $C_{13}H_{13}N_2O_2$.

3.3.2. Compound 2. Isoaaptamine, 8-methoxy-1-methyl-1*H*-benzo[*de*][1,6]naphthyridin-9-ol. (0.030 g, 0,006% dry weight) yellow gum; R_f (CH_2Cl_2/MeOH 8:2) 0.08; UV (EtOH) λ_{max} nm (ε): 206 (864), 234 (620), 253 (539), 368 (254); IR (ATR) ν cm^{-1}: 3359, 1643, 1594, 1301, 1203, 1105; ^{1}H and ^{13}C NMR data recorded in DMSO-d_6, see Tables 1 and 2; HR-MS $[M]^+$ found at *m/z* 228.0892 (Δ −0.6 mmu), calc. 228.0898, for $C_{13}H_{12}N_2O_2$.

3.3.3. Compound 3. Demethyl(oxy)aaptamine, 8-methoxy-benzo[*de*][1,6]naphthyridin-9-one. (0.0016 g, 3.2×10^{-4}% dry weight) yellow solid; mp 198–200°C; R_f (CH_2Cl_2/acetone 1:1) 0.29; UV (EtOH) λ_{max} nm (ε): 209 (7058), 240 (5980), 317 (1078), 368 (1372); IR (ATR) ν cm^{-1}: 2920, 2852, 1661, 1618, 1582, 1270, 1098; ^{1}H and ^{13}C NMR data recorded in DMSO-d_6, see Tables 1 and 2; HR-FABMS $[M+H]^+$ found at *m/z* 213.0669 (Δ +0.5 mmu), calc. 213.0664 for $C_{12}H_9N_2O_2$.

3.3.4. Compound 4. 8,9,9-Trimethoxy-9H-benzo[*de*][1,6]-naphthyridine. (0.0014 g, 2.8×10^{-4}% dry weight) brown gum; R_f (CH_2Cl_2/acetone 1:1) 0.30; UV (EtOH) λ_{max} nm (ε): 205 (7045), 229 (11331), 350 (4562); IR (ATR) ν cm^{-1}: 1664, 1593, 1575, 1561,1271, 1201; ^{1}H and ^{13}C NMR data recorded in DMSO-d_6, see Tables 1 and 2; HR-FABMS $[M+H]^+$found at *m/z* 259.1080 (Δ −0.3 mmu), calc. 259.1083 for $C_{14}H_{15}N_2O_3$.

3.3.5. Compound 5. (0.0008 g, 1.6×10^{-4}% dry weight) orange gum; R_f (CH_2Cl_2/acetone 1:1) 0.32; UV (EtOH) λ_{max} nm (ε): 208 (7399), 225 (6594), 337 (7390), 251 (5555), 261 (4400), 272 (3472), 360 (1967); IR (ATR) ν

cm^{-1}: 1565, 1439, 1416, 1365, 1090; ^1H and ^{13}C NMR data recorded in $CDCl_3$, see Table 3; HR-FABMS $[M+H]^+$ found at *m/z* 280.1449 (Δ −0.1 mmu), calc. 280.1450 for $C_{17}H_{18}N_3O$.

3.3.6. Compound 6. (0.0009 g, 1.8×10^{-4}% dry weight) brown gum; R_f (CH_2Cl_2/acetone 1:1) 0.07; UV (EtOH) λ_{max} nm (ε): 210 (5418), 222 (4795), 238 (4663), 260 (2937), 271 (2597), 357 (1332), 371 (1066); IR (ATR) ν cm^{-1}: 3318, 1564, 1442, 1421, 1370, 1073; ^1H and ^{13}C NMR data recorded in DMSO-d_6, see Tables 1 and 2; HR-FABMS $[M+H]^+$ found at *m/z* 254.0933 (Δ +0.3 mmu), calc. 254.0930 for $C_{14}H_{12}N_3O_2$.

3.3.7. Compound 7. (0.0007 g, 1.4×10^{-4}% dry weight) brown gum; R_f (CH_2Cl_2/acetone 1:1) 0.28; IR (ATR) ν cm^{-1}: 1581, 1430, 1354, 1061; ^1H and ^{13}C NMR data recorded in DMSO-d_6, see Tables 1 and 2; HR-FABMS $[M+H]^+$ found at *m/z* 266.1299 (Δ +0.6 mmu), calc. 266.1293 for $C_{16}H_{16}N_3O$.

3.3.8. Compound 8. (0.0007 g, 1.4×10^{-4}% dry weight) brown gum; R_f (CH_2Cl_2/acetone 1:1) 0.11; UV (EtOH) λ_{max} nm (ε): 209 (5845), 237 (5251), 258 (2985), 269 (2769), 355 (2014); IR (ATR) ν cm^{-1}: 1564, 1464, 1360; ^1H and ^{13}C NMR data recorded in DMSO-d_6, see Tables 1 and 2; HR-FABMS $[M+H]^+$ found at *m/z* 224.0818 (Δ −0.6 mmu), calc. 224.0824 for $C_{13}H_{10}N_3O$.

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