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Biapillarmycin A: a novel alkaloid from Ghana with potent antitrypanosomal activity

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Full Research Paper

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Abstract

Microbe-derived alkaloid natural products containing the phenethylamine structural motif are gradually emerging as scaffolds capable of modulating several essential microbial metabolic processes. As such, these compounds possess potent biological activities that have gained interest in the drug discovery community. In this study, biapillarmycin A (**1**) was isolated from the Ghanaian bacterial strain *Streptomyces* sp. BNP-Pillar-1G-1/10B. The structure of compound **1** was elucidated using NMR, UV, FTIR, and HRESIMS/MS analysis, revealing an alkaloid featuring the rare structural motifs: ethane-1,2-diol, 2-phenoxyethan-1-ol, 2-(4-(2-

aminoethyl)phenoxy)ethan-1-ol, and *N*-(4-hydroxyphenethyl)benzamide. Compound **1** exhibited potent antitrypanosomal activity against *Trypanosoma brucei* subsp. *brucei* strain GUTat 3.1 with an IC_{50} of 0.7 ± 0.09 μg/mL, while the standard drug, diminazene aceturate, had an IC_{50} of 0.03 ± 0.01 μg/mL. Compound **1** is a new addition to the gradually growing list of microbe-derived alkaloids containing the phenethylamine moiety.

Introduction

During the last two decades, our research group has endeavoured to discover new drug scaffolds for the neglected tropical parasites *Trypanosoma brucei* (Human African Trypanosomiasis), *Leishmania donovani* (Leishmaniasis), and *Schistosoma mansoni* (Schistosomiasis). So far, our most potent antiparasitics have come from alkaloids biosynthesized from the aromatic amino acids (AAs) phenylalanine, tyrosine, tryptophan, and histidine [1-5]. Microbial alkaloids derived from modified AAs represent a structurally diverse and pharmacologically important class of natural products, including compounds bearing indoles, isoquinolines, imidazole-based alkaloids, phenethylamines, and related heterocyclic scaffolds [6]. Nevertheless, a detailed search through the literature of microbe-derived alkaloids biosynthesized from AAs show that structural motifs such as ethane-1,2-diol (**2**), 2-phenoxyethan-1-ol (**3**), 2-(4-(2-aminoethyl)phenoxy)ethan-1-ol (**4**), and *N*-(4-hydroxyphenethyl)benzamide (**5**) are rare (Figure 1). The antifungal agents PF1163A and PF1163B (**6**, **7**) isolated from the *Penicillium* sp. strain I are two examples of microbe-derived AA alkaloids containing the 2-(4-(2-aminoethyl)phenoxy)ethan-1-ol structural motif [7] (Figure 1). Interestingly, the 4-(2-aminoethyl)phenol (**8**), and *N*-(4-hydroxyphenethyl)formamide (**9**) derived directly from a dedicated tyrosine decarboxylase and carboxylic acid reductase, respectively, are ubiquitous in the chemical structures of many microbial dipeptides and polypeptides, with only a few examples as alkaloids [8-10].

In the current study, we isolated the Ghanaian *Streptomyces* sp. BNP-Pillar-1G-1/10B, GenBank number PZ239911 (Supporting Information File 1, Figure S2), from rhizosphere soils collected from the Bia National Park (6°30'0.00'' N and 3°0'0.00'' W), a highly biodiverse biosphere reserve located in the Western North region of Ghana (Supporting Information File 1, Figure S1). This strain was found to produce the novel compound, biapillarmycin A (**1**), which contains the four rare structural motifs, ethane-1,2-diol (**2**), 2-phenoxyethan-1-ol (**3**), 2-(4-(2-aminoethyl)phenoxy)ethan-1-ol (**4**), and *N*-(4-hydroxyphenethyl)benzamide (**5**). The name 'biapillarmycin' was coined because the producer strain was obtained from sediments collected from the Bia National Park (BNP), and the collection site was close to PILLAR 1G, one of the demarcation landmarks within the forest. We determined the structure of

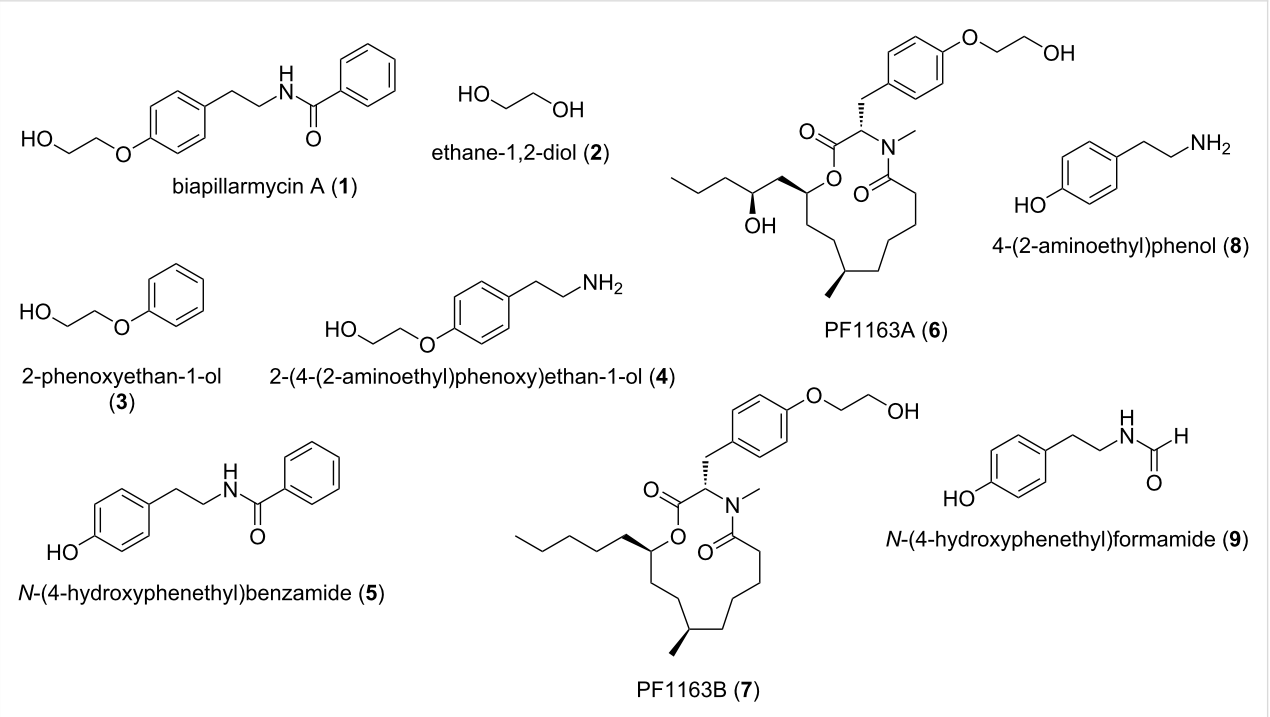


Figure 1: Structures of biapillarmycin A, PF1163A and PF1163B and the rare structural motifs.

this new compound, biapillarmycin A (**1**), using a combination of IR, UV, 1D, and 2D NMR spectroscopy and tandem mass spectrometry.

Subsequently, we tested the novel compound **1** against our in-house *Trypanosoma brucei* subsp. *brucei* strain GUTat 3.1 using diminazene aceturate as the reference drug and found it to exhibit potent antitrypanosomal bioactivity.

Results and Discussion

Taxonomy of the strain *Streptomyces* sp. BNP-Pillar-1G-1/10B (GenBank number PZ239911)

The organism exhibited a range of chemotaxonomic and phenotypic traits typical of *Streptomyces*. A neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationship of strain BNP-Pillar-1G-1/10B with closely related members of the genus *Streptomyces* is shown in Figure 2. The tree was constructed using the neighbour-joining method, and branch lengths are proportional to the number of nucleotide substitutions per site. Bootstrap values (>50%) based on 1000 replications are shown at the nodes. *Kitasatospora setae* KM-6054^T was used as an outgroup. Phylogenetic analysis based on nearly complete 16S rRNA gene sequences indicated that strain BNP-Pillar-1G-1/10B belongs to the genus *Streptomyces*. The strain showed the highest sequence similarity to *Streptomyces varsoviensis* NRRL ISP-5346^T and *Streptomyces luteireticuli* NBRC 13422^T (both 99.45%), followed by *Streptomyces zagrosensis* HM 1154^T (99.24%), *Streptomyces abikoensis* NBRC 13860^T (99.17%), *Streptomyces buecherae* AC541^T (98.90%), and *Streptomyces thioluteus* LMG 20253^T (98.89%). The strain showed lower sequence similarities (<98.8%) to other closely related *Streptomyces* species. The 16S rRNA gene sequence of strain BNP-Pillar-1G-1/10B was deposited in GenBank under the accession number PZ239911.

Structure elucidation of biapillarmycin A (**1**)

Compound **1** was isolated by reversed-phase HPLC at retention time of 29 minutes (Supporting Information File 1, Figure S3) and obtained as a pale white substance, colourless when in solution of methanol and/or dichloromethane (Supporting Information File 1, Figures S4 and S5).

The molecular formula of **1** was determined to be $C_{17}H_{19}NO_3$ based on the high-resolution electrospray ionization mass spectrometry (HRESIMS/MS) at *m*/*z* 286.1438 (Supporting Information File 1, Figure S6) corresponding to the $[M + H]^+$ peak and molecular formula $C_{17}H_{20}NO_3^+$ (Δ = 0 ppm and 9 degrees

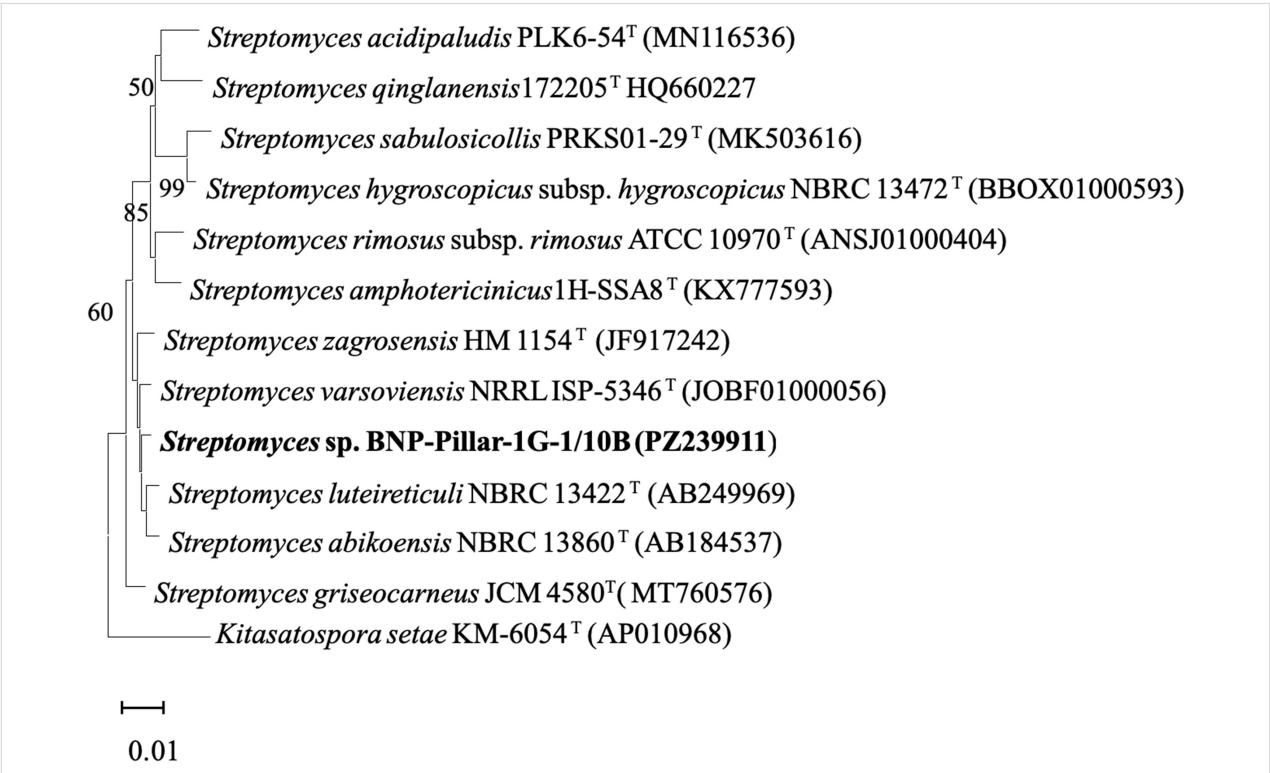


Figure 2: Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relationship of strain BNP-Pillar-1G-1/10B with closely related members of the genus *Streptomyces*.

of unsaturation). The complete structure of the molecule in this study was determined by analysis of the ^{1}H,^{13}C, multiplicity edited pulsed field gradient heteronuclear single quantum coherence (gHSQCAD) and several pulsed field gradient ^{1}H,^{13}C heteronuclear multiple bond correlations (gHMBCAD) spectra of **1**, suggesting the presence of 4 quaternary, 9 methines, and 4 methylenes (Figure 3, Table 1).

Four spin systems could be easily identified by combining the coupling constant information in the ^{1}H NMR and a detailed analysis of the ^{1}H–^{1}H homonuclear correlation spectroscopy (gCOSY), and ^{1}H–^{1}H total correlation spectroscopy (2D-ghsqc-TOCSY) data. The first spin system was represented by the ^{1}H NMR chemical shifts δ_H 7.22 (2H, overlapping, H-3/H-7), 7.29 (2H, overlapping, H-4/H-6), and 7.29 (^{1}H, overlapping,

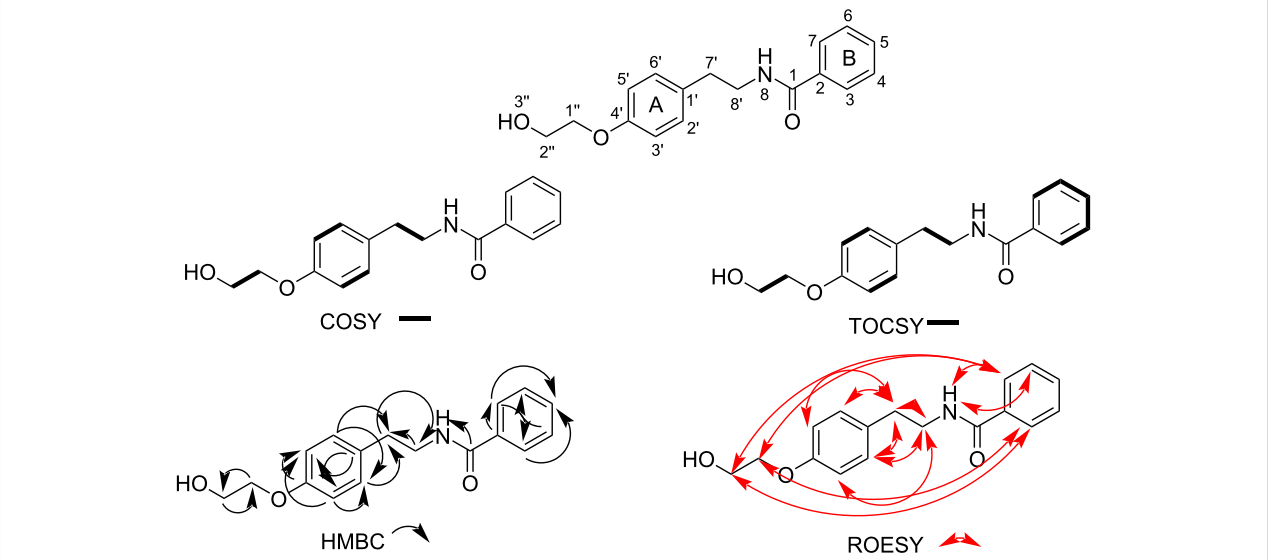


Figure 3: Complete primary structure of compound **1** showing all the labeled carbon atoms, key COSY and TOCSY correlations confirming all spin systems, and HMBC and NOESY correlations confirming the full structure.

Table 1: ^{1}H NMR and ^{13}C NMR spectroscopic data for compound **1** (biapillarmycin A) in CD_3OD.

Position	δ_C (mult.)	δ_H (mult., *J* in Hz)	HMBC (H→C)	COSY (^{1}H–^{1}H)	TOCSY (H→C)	ROESY (^{1}H–^{1}H)
1	170.8, C	–	–	–	–	–
2	137.6, C	–	–	–	–	–
5	130.4, CH	7.29, overlapping	2, 3, 7,	–	3, 7	8
4/6	129.8, 2CH	7.29, overlapping	2, 3, 7, 5	–	3, 7	8
3/7	128.2, 2CH	7.22, overlapping	5	–	4, 6, 5	1'', 2'', 3', 5'
8	–	8.48, s	1	–	–	5
1'	128.7, C	–	–	–	–	–
2'/6'	130.7, 2CH	7.04, d (8.3)	2', 6', 3', 5', 4', 7'	3', 5'	3', 5'	7', 8', 3', 5'
3'/5'	116.7, 2CH	6.74, d (8.5)	1', 3', 5', 4'	2', 6'	2', 6'	1'', 2'', 2', 6', 3, 7
4'	157.5, C	–	–	–	–	–
4'	–	–	–	–	–	–
7'	33.8, CH_2	2.83, t (7.8)	2', 6', 8'	8'	8'	8', 2', 6',
8'	42.1, CH_2	3.05, t (7.8)	1', 7'	7'	7'	7', 2', 6',
1''	73.2, CH_2	3.54, t (5)	2''	2''	2''	3, 7, 3', 5'
2''	61.8, CH_2	3.66, t (4.8)	1''	1''	1''	3, 7, 3', 5'
3''	–	–	–	–	–	–

H-5) supported by the 2D-ghsqcTOCSY correlations H-5 (δ_H 7.29)/C3/C7 (δ_C 130.4), H-5 (δ_H 7.29)/C4/C6 (δ_C 129.8), H-3/H-7 (δ_H 7.22)/C5 (δ_C 128.2), and H-4/H-6 (δ_H 7.29)/C5 (δ_C 128.2). The second spin system in this compound was represented by the ^{1}H NMR chemical shifts δ_H 2.83 (2H, t, J = 7.8 Hz, H7'), and 3.05 (2H, t, J = 7.8 Hz, H8'). This spin system was supported by the gCOSY correlations H-7' (δ_H 2.83)/H-8' (δ_H 3.05), H-8' (δ_H 3.05)/H-7' (δ_H 2.83), and 2D-ghsqcTOCSY correlations H-8' (δ_H 3.05)/C7' (δ_C 33.8), and H-7' (δ_H 2.83)/C8' (δ_C 42.1). The third spin system was represented by a symmetrical di-substituted benzene ring with protons d_H 7.04 (2H, d, J = 8.3 Hz, H-2'/H-6'), and 6.74 (2H, d, J = 8.5 Hz, H-3'/H-5'). Apart from the extracted ^{1}H NMR coupling constant, which shows that these two protons are coupled, we also collected gCOSY correlation H-2'/H-6' (δ_H 7.04)/H-3'/H-5' (δ_H 6.74) and 2D-ghsqcTOCSY correlations H-3'/H-5' (δ_H 6.74)/C2'/C6' (δ_C 130.7) and H-2'/H-6' (δ_H 7.04)/C3'/C5' (δ_C 116.7). The fourth spin system was that of an ethane-1,2-diol side chain. This was represented by δ_H 3.54 (2H, t, J = 5.0 Hz, H-1''), and 3.66 (2H, t, J = 4.8 Hz, H-2''). Further proof of their direct connection was obtained from the gCOSY correlation H-1'' (δ_H 3.54)/H-2'' (δ_H 3.66) and the 2D-ghsqcTOCSY correlation H-2'' (δ_H 3.66)/C1'' (δ_C 73.2). Subsequently, we relied on crucial gHMBCAD correlations such as H-2'/H-6' (δ_H 7.04)/C4' (δ_C 157.5), H-3'/H-5' (δ_H 6.74)/C4' (δ_C 157.5), H-3'/H-5' (δ_H 6.74)/C1' (δ_C 128.7), H-8' (δ_H 3.05)/C1' (δ_C 128.7), H-3/H-7 (δ_H 7.22)/C2 (δ_C 137.6), and H-4/H-6 (δ_H 7.29)/C2 (δ_C 137.6) to complete the structure elucidation of biapillarmycin A (**1**).

All 2D NMR data correlations are shown in Figure 3 and in Table 1; all the 1D and 2D NMR data for compound **1** as measured in CD_3OD are provided (Supporting Information File 1, Figures S7–S19).

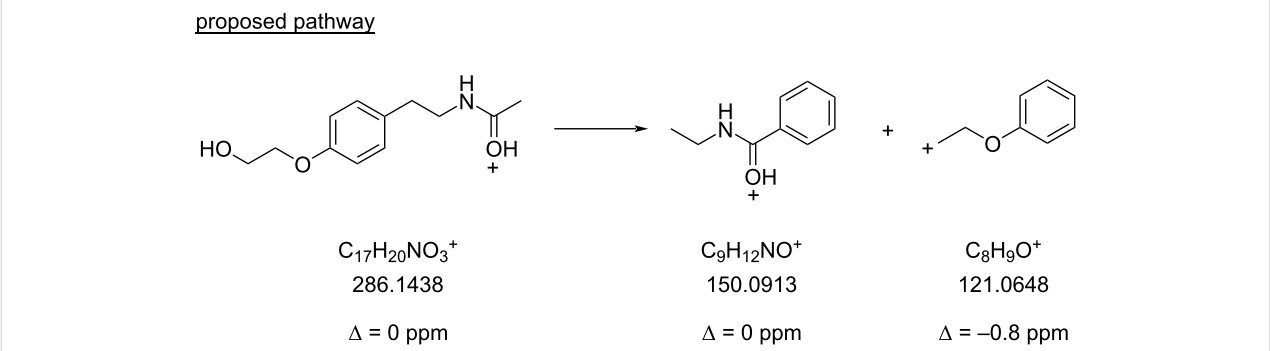
Confirmation of structure: MS data interpretation of biapillarmycin A (**1**)

Compound **1** has low molecular weight and contains some of the most labile functional groups in mass spectrometry, including an alkane, alcohol, ether, amide, carbonyl, and substituted aromatics. Therefore, compound **1** appears to fragment profusely in the ESI mass spectrometer, yielding possible fragments that are undetected and/or below the detection limit of the instrument. Nevertheless, the HRESIMS/MS measured mass of m/z 286.1438 is in exact agreement with the calculated mass of 286.1438, with Δ = 0 ppm corresponding to the molecular formula of $C_{17}H_{19}NO_3$. From this $[M + H]^+$ ion, two other ions are prominent and confirmatory of the major substructures within the compound **1** scaffold. The first occurs at m/z 150.0913, Δ = 0 ppm with formula $C_9H_{12}NO^+$, and the second at m/z 121.0648, Δ = −0.8 ppm, with formula $C_8H_9O^+$ as shown in Scheme 1 and Supporting Information File 1, Figure S3.

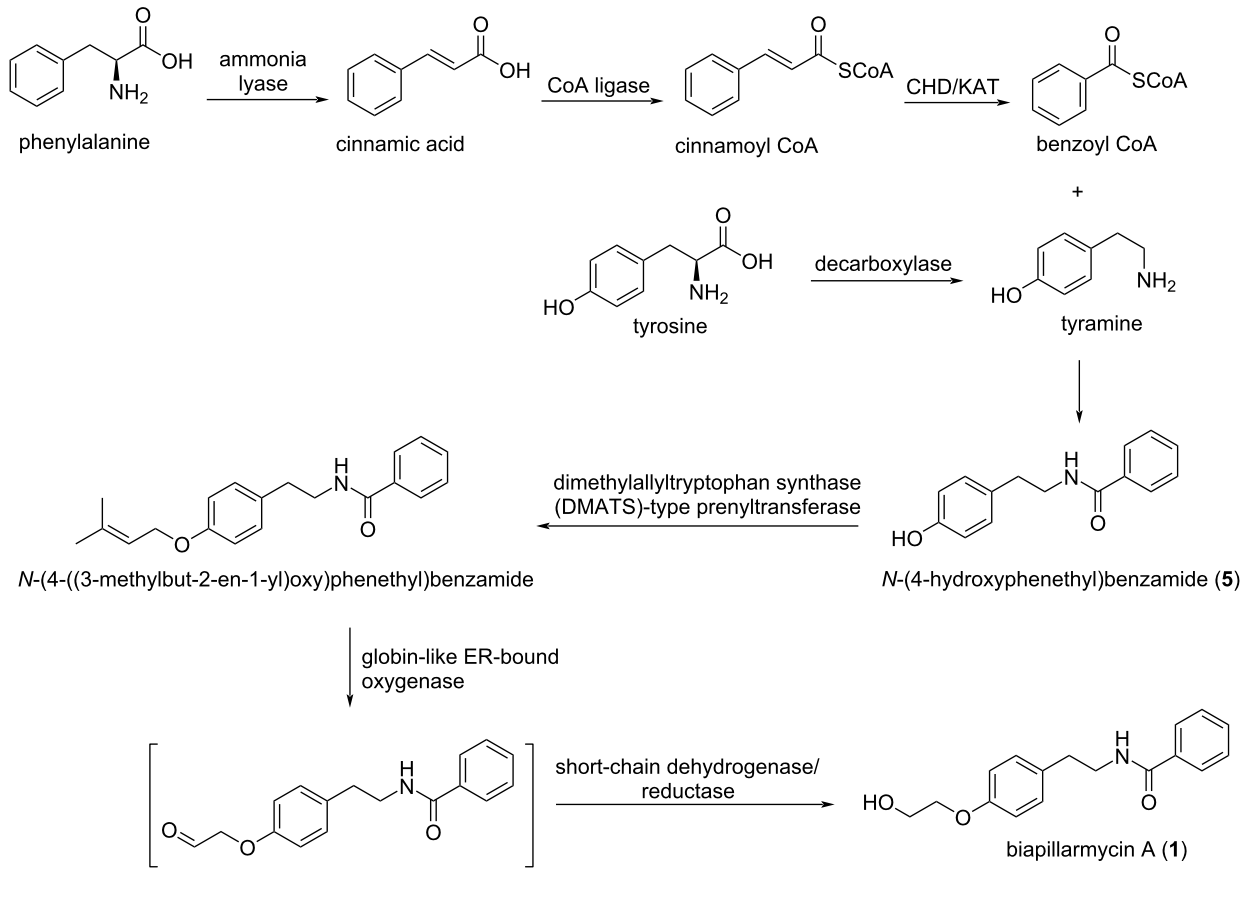
Plausible biosynthesis of biapillarmycin A (**1**)

Detailed inspection of the structure of biapillarmycin A (**1**) led to the speculation that this compound could be biosynthesized using the AAs phenylalanine and tyrosine (Scheme 2). Phenylalanine is first converted to cinnamic acid, which is subsequently transformed into benzoyl-CoA. Meanwhile, tyrosine undergoes decarboxylation to produce tyramine, which then condenses with benzoyl-CoA to form the intermediate *N*-(4-hydroxyphenethyl)benzamide (**5**), which serves as the scaffold for subsequent enzymatic modifications.

The aromatic precursor then undergoes O-prenylation, catalysed by a DMATS-type prenyltransferase, which introduces a dimethylallyl group onto the phenolic oxygen. Subsequently, a globin-like ER-bound oxygenase oxidatively cleaves the prenyl substituent, as observed in mycophenolic acid and PF1163A biosynthesis [11-13], generating an aldehyde intermediate. A



Scheme 1: Proposed HRESIMS/MS fragmentation pathway of compound **1** showing major product ions detected above m/z 100 during collision-induced dissociation (CID). The observed fragment ions support the presence of the major substructures in compound **1**.



Scheme 2: Proposed biosynthetic pathway for biapillarmycin A (**1**).

short-chain dehydrogenase/reductase (SDR) then reduces this intermediate to the corresponding alcohol, yielding the hydroxyethoxy moiety in biapillarmycin A (**1**). This hydroxyethoxy substituent was also reported in the chemical structures of PF1163A and PF1163B, which are fungal natural products whose biosynthetic pathway was elucidated through identification of the *pfa* biosynthetic gene cluster and heterologous expression experiments in *Aspergillus oryzae* [11-13].

Biological activity of biapillarmycin A (**1**)

Antitrypanosomal bioactivity

Phenethylamine-derived natural products have previously been reported to possess significant antitrypanosomal bioactivity due to their structurally diverse nitrogen-containing heterocyclic frameworks and their ability to interfere with essential microbial metabolic processes [4,14]. In the present study, the isolated alkaloid **1** was tested against our in-house *Trypanosoma brucei* subsp. *brucei* strain GUTat 3.1. Biapillarmycin A (**1**) exhibited strong inhibitory activity with an IC_{50} of 0.70 ± 0.09 µg/mL. In comparison, the standard reference drug, diminazene aceturate, produced an IC_{50} of 0.03 ± 0.01 µg/mL (Figure 4). This means that the novel compound biapillarmycin A (**1**) was only 23-fold less active than the prescribed drug diminazene aceturate. Compound **1**, therefore, represents a good scaffold for the development of potent antitrypanosomal drugs.

Conclusion

In conclusion, a new Ghanaian *Streptomyces* sp. BNP-Pillar-1G-1/10B strain was isolated from rhizosphere soils collected from the Bia National Park, a highly biodiverse biosphere reserve located in the Western North region of Ghana. The 16S rRNA gene sequence obtained from this organism was deposited in GenBank under accession number PZ239911. The novel compound biapillarmycin A (**1**) was isolated from the new strain, and its complete structure was determined from IR, UV, 1D, and 2D NMR data, in combination with hyphenated mass spectrometry. Compound **1** showed potent antitrypanosomal activity in our in-house *Trypanosoma brucei* subsp. *brucei* strain GUTat 3.1 bioassay, making it a promising scaffold for the development of new antitrypanosomal drugs.

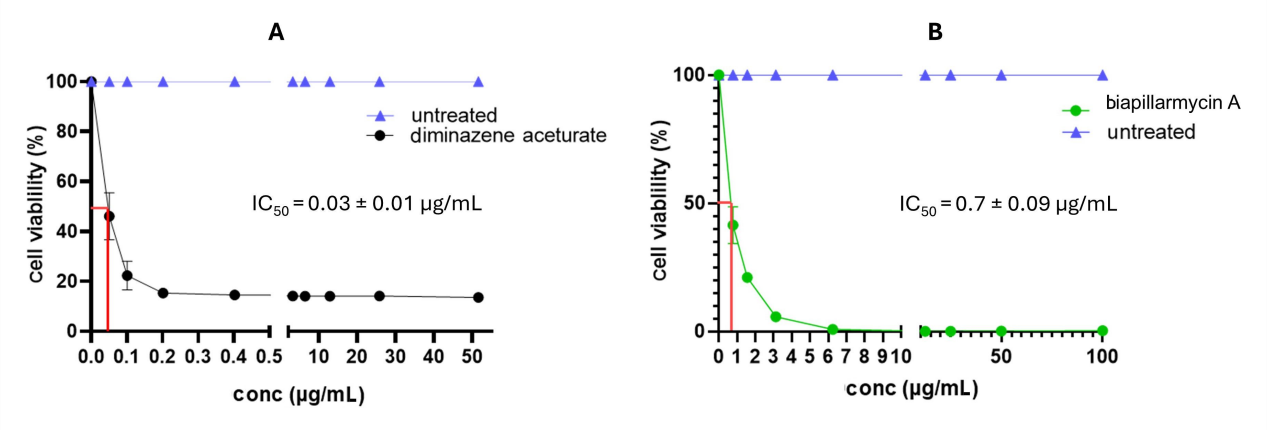


Figure 4: Dose-response curves of (A) diminazene aceturate and (B) biapillarmycin A (**1**) on bloodstream forms of *Trypanosoma brucei brucei*. The value of the IC_{50} is expressed as mean ± SD of the replicates (n = 3).

Supporting Information

Supporting Information File 1

Experimental protocols, phylogenetic data, compound isolation and characterization data (HPLCs, 1D, 2D NMR, IR, UV), tables, and figures.
[https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-22-145-S1.pdf]

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Author Contributions

Thomas Mensah: conceptualization; formal analysis; investigation; methodology; project administration; writing – original draft; writing – review & editing. Evans Gyapong: investigation; methodology; writing – original draft. Abigail Aning: investigation; methodology; writing – original draft. Theresa Manful Gwira: conceptualization; formal analysis; investigation; methodology; resources; writing – original draft; writing – review & editing. Victor Yao Atsu Barku: conceptualization; supervision; writing – original draft; writing – review & editing. Anil Sazak Camas: conceptualization; formal analysis; investigation; methodology; writing – original draft; writing – review & editing. Jioji Tabudravu: conceptualization; formal analysis; investigation; methodology; writing – original draft; writing – review & editing. Hai Deng: conceptualization; funding acquisition; investigation; methodology; writing – review & editing. Marcel Jaspars: conceptualization; funding acquisition; methodology; project administration; resources; writing – original draft; writing – review & editing. Taye Beyene Demissie: conceptualization; formal analysis; funding acquisition; investigation; methodology; resources; software; writing – original draft; writing – review & editing. Minly Kaye Baguio: formal analysis; investigation; methodology; writing – original draft. Fleurdeliz Maglangit: conceptualization; formal analysis; investigation; methodology; resources; writing – original draft; writing – review & editing. Kwaku Kyeremeh: conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review & editing.

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Data Availability Statement

All data that supports the findings of this study is available in the published article and/or the supporting information of this article.

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