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Discovery of \( p \)-Terphenyl Metabolites as Potential Phosphodiesterase PDE4D Inhibitors from the Coral-Associated Fungus \( \textit{Aspergillus} \) sp. ITBBc1

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Abstract: Chemical investigation of the fermentation extract of the coral-associated fungus \( \textit{Aspergillus} \) sp. ITBBc1 led to the discovery of five unreported \( p \)-terphenyl derivatives, sanshamycins A–E (1–5), together with five previously described analogues, terphenyllin (6), 3-hydroxyterphenyllin (7), candidusin A (8), 4,5-dimethoxycandidusin A (9), and candidusin C (10). Their structures were elucidated by HRESIMS data and NMR spectroscopic analysis. Compound 1 represents the first example of \( p \)-terphenyls with an aldehyde substitution on the benzene ring. Compounds 2–4 feature varying methoxyl and isopentenyl substitutions, while compound 5 features a five-membered lactone linked to a biphenyl. These findings expand the chemical diversity of the family of \( p \)-terphenyl natural products. Compounds 1–6 and 9 were evaluated for their inhibitory activity against type 4 phosphodiesterase (PDE4), which is a fascinating drug target for treatment of inflammatory, respiratory, and neurological diseases. Compound 3 was the most potent and exhibited PDE4D inhibitory activity with an IC\( _{50} \) value of 5.543 \( \mu \)M.

Keywords: coral-associated fungus; \( \textit{Aspergillus} \) sp.; natural products; \( p \)-terphenyls; phosphodiesterase PDE4D inhibitor

1. Introduction

Phosphodiesterases (PDEs), the only enzymes that degrade the important secondary messenger 3',5'-cyclic nucleotides, regulate a myriad of physiological processes in human health and disease [1]. A total of 11 biochemical and pharmacological enzyme families of PDEs (PDE1–PDE11) have been characterized by differences in structure, substrate specificity, inhibitor sensitivities, and tissue distribution [1,2]. Currently they are being explored as important therapeutic targets for treatment of several diseases such as those affecting the respiratory system, nervous system, cardiovascular system, immune system, fertility, and cancer [1]. Type 4 phosphodiesterase (PDE4), a PDE enzyme family that exclusively catalyzes the hydrolysis of the secondary messenger cyclic adenosine 3',5'-monophosphate (cAMP) in numerous cell types, comprises four subtypes (PDE4A, PDE4B, PDE4C, and PDE4D) with a high degree of sequence identity within the catalytic domains. These
enzymes have been reported to be involved in many physiological processes and development of inflammatory, respiratory, autoimmune, neurological diseases, and cancers [1–3].
PDE4 has been demonstrated to be a promising drug target for the treatment of chronic obstructive pulmonary disease (COPD), asthma, rheumatoid arthritis, lupus, atopic dermatitis, psoriasis, and neurological disorders [2,3]. Although a number of marketed PDE4 inhibitor drugs have been developed over the last few decades, their side effects, such as nausea, diarrhea, weight loss, and headaches, cannot be neglected [2].

Since natural products remain a reliable resource for novel drug leads, the search for new natural PDE4 inhibitors is considered to be an attractive project. Recently, naturally occurring PDE4 inhibitors have been reported from several species of plants or marine corals [4–7]. p-Terphenyl natural products are a group of aromatic compounds produced by Aspergillus [8–12], Streptomyces [13], and Burkholderia species [14]. This group of metabolites features a chain of three benzenes with varying substitutions, including hydroxyl, methoxyl and isoprenyl substituents, and has diverse biological activities including antitumor, antimicrobial, antioxidant, neuraminidase, and phosphodiesterase inhibitory activities [8–16]. As part of an ongoing project to identify structurally unique and pharmacologically significant natural products from microbes that are isolated from unexplored or underexplored ecological niches [17–21], our group has obtained a wide variety of structurally diverse and biologically active natural products from marine-derived fungi from the South China Sea [22–29]. During our continuing discovery of novel bioactive secondary metabolites from underexplored fungi from marine resources, five unreported p-terphenyl derivatives, namely sanshamycins A–E (1–5), were isolated along with five previously described analogues (6–10) from the coral-derived fungus Aspergillus sp. ITBBc1 (Figure 1). Structurally, sanshamycin A (1) represents the first example of p-terphenyls with an aldehyde substitution on the benzene ring and sanshamycins B–D (2–4) share the same p-terphenyl framework with varying methoxyl and isopentenyl substitutions, while sanshamycin E (5) features a five-membered lactone linked to a biphenyl. The structures of all the compounds were unambiguously determined by HRESIMS and NMR data. Compounds 1–6 and 9 were screened for PDE4D inhibition. Here, we reported the isolation, structure elucidation, and the PDE4D inhibitory activities of these p-terphenyls.

Figure 1. The structures of 1–10.

2. Results
2.1. Structure Elucidation of New Compounds 1–5

Sanshamycin A (1) was isolated as a yellow powder. The molecular formula was established as C21H18O6 (13 degrees of unsaturations) on the basis of its HRESIMS (Figure S8) and the 1H and 13C NMR data (Tables 1 and 2). The initial analysis of the 1H (Figure S1), 13C (Figure S2), DEPT135 (Figure S3), and HSQC (Figure S4) spectra in acetone-d6 revealed that
was very similar to terphenyllin (6) [8], but with an aldehyde group (3-CHO) displaying characteristic proton and carbon signals at $\delta_H$ 10.06 (s) and $\delta_C$ 197.3. Further observation of the key HMBC correlations (Figure 2 and Figure S5) from $\delta_H$ 10.06 proton signal to C-2 ($\delta_C$ 136.2), C-3 ($\delta_C$ 120.6) and C-4 ($\delta_C$ 160.1), from H-2 ($\delta_H$ 7.83, d, $J = 2.2$ Hz) to $\delta_C$ 197.3 carbon signal, and the NOESY correlation (Figure 2 and Figure S7) from $\delta_H$ 10.06 proton signal to H-2 located the aldehyde group at C-3. Characteristic HMBC correlations from 4-OH ($\delta_H$ 11.02, s) to C-3, C-4, and C-5 ($\delta_C$ 116.1), 2′-OH ($\delta_H$ 7.90, s) to C-1′ ($\delta_C$ 114.8), C-2′ ($\delta_C$ 148.3), and C-3′ ($\delta_C$ 139.3), 4′-OH ($\delta_H$ 8.55, s) to C-3′′ ($\delta_C$ 115.2), C-4′′ ($\delta_C$ 157.1), and C-5′′ ($\delta_C$ 115.2), 3′-OCH$_3$ ($\delta_H$ 3.40, s) to C-3′, 6′-OCH$_3$ ($\delta_H$ 3.78, s) to C-6′ ($\delta_C$ 153.5) were observed. Meanwhile, the ROESY spectrum showed correlations from 4-OH to H-5 ($\delta_H$ 7.02, d, $J = 8.6$ Hz), 3′-OCH$_3$ to 2′-OH, H-2′′ ($\delta_H$ 7.55, d, $J = 8.6$ Hz) and H-6′′ ($\delta_H$ 7.55, d, $J = 8.6$ Hz), and 4′′-OH to H-3′′ ($\delta_H$ 6.96, d, $J = 8.6$ Hz) and H-5′′ ($\delta_H$ 6.96, d, $J = 8.6$ Hz).

Further comprehensive interpretation of the HSQC, HMBC, $^1$H-$^1$H COSY (Figure S6), and ROESY data allowed for the full assignment of the structure as shown in Figure 1.

Table 1. $^1$H NMR (500 MHz) spectroscopic data for 1–5.

| Position | 1 | 2 | 3 | 4 | 5 |
|----------|---|---|---|---|---|
| 2        | 7.83, d (2.2) | 7.17, d (2.1) | 7.09, d (2.1) | 3.08, dd (6.4, 4.5) | 3.96, dd (6.4, 4.5) |
| 3        | 10.06, s | 11.02, s | | | |
| 3-CHO    | 7.02, d (8.6) | 6.87, d (8.7) | 6.85, d (8.2) | 3.98, s | 1.94, s |
| 4-OH     | 7.68, dd (8.6, 2.2) | 7.17, overlapping | 7.22, dd (8.2, 2.1) | 7.56, s | |
| 4-OCH$_3$| 7.90, s | 3.66, s | 3.44, s | 3.71, s | 3.66, s |
| 5        | 6.55, s | 6.69, s | 6.48, s | 6.68, s | 6.43, s |
| 6′-OCH$_3$| 3.78, s | 3.74, s | 3.75, s | 4.03, s | 3.84, s |
| 2′″      | 7.55, d (8.6) | 7.59, d (7.5) | 7.64, m | 7.25, s | 7.41, d (8.5) |
| 3′″      | 6.96, d (8.6) | 7.45, t (7.5) | 7.46, t (7.5) | 6.90, d (8.5) | |
| 4′″      | 7.37, t (7.5) | 7.37, t (7.5) | | | |
| 4′′-OH   | 8.55, s | 6.96, d (8.6) | 7.45, t (7.5) | 7.46, t (7.5) | 6.86, d (8.3) | 6.90, d (8.5) |
| 5′″      | 7.55, d (8.6) | 7.59, d (7.5) | 7.64, m | 7.38, d (8.3) | 7.41, d (8.5) |
| 1′‴      | 3.40, d (7.2) | 6.35, d (9.7) | 6.40, d (9.7) | | |
| 2′‴      | 5.40, dq (7.2, 1.4) | 5.60, d (9.7) | 5.66, d (9.7) | | |
| 4′‴      | 1.78, d (1.4) | 1.47, s | 1.49, s | | |
| 5′‴      | 1.79, br s | 1.47, s | 1.49, s | | |

a Spectra were recorded in acetone-$d_6$; b Spectra were recorded in CDCl$_3$. $\delta$ in ppm.

Table 2. $^{13}$C NMR (125 MHz) spectroscopic data for 1–5.

| Position | 1 | 2 | 3 | 4 | 5 |
|----------|---|---|---|---|---|
| 1        | 126.1, C | 126.1, C | 125.0, C | 115.3, C | 173.9, C |
| 2        | 136.2, CH | 132.1, CH | 128.7, CH | 150.6, C | 34.0, CH$_2$ |
| 3        | 120.6, C | 125.9, C | 120.8, C | 95.4, CH | 45.7, CH |
| 3-CHO    | 197.3, CH | 161.0, C | 153.5, C | 152.3, C | 149.1, C | 118.3, C |
| 4        | 116.1, CH | 115.2, CH | 116.0, C | 146.2, C | 23.9, CH$_3$ |
| 4-OCH$_3$| 140.0, CH | 130.0, CH | 131.5, CH | 104.3, CH | |
| 5        | 114.8, C | 124.7, C | 116.7, C | 114.8, C | 114.6, C |
| 6        | 148.3, C | 152.0, C | 147.2, C | 149.3, C | 149.9, C |
| 3′″      | 60.8, CH$_3$ | 139.3, C | 144.8, C | 138.9, C | 136.7, C | 136.0, C |
Table 2. Cont.

| Position | 1 | 2 | 3 | 4 | 5 |
|----------|---|---|---|---|---|
| 3'-OCH₃  | 59.8, CH₃ | 60.9, CH₃ | 60.9, CH₃ | 61.1, CH₃ | 60.7, CH₃ |
| 4'       | 133.4, C | 134.7, C | 132.7, C | 131.1, C | 136.4, C |
| 5'       | 103.3, CH | 108.1, CH | 104.0, CH | 105.6, CH | 106.1, CH |
| 6'       | 153.5, C | 153.2, C | 153.5, C | 150.0, C | 151.5, C |
| 6'-OCH₃  | 55.3, CH₃ | 56.1, CH₃ | 56.0, CH₃ | 55.9, CH₃ | 56.0, CH₃ |
| 1''      | 129.4, C | 138.5, C | 138.1, C | 131.0, C | 130.5, C |
| 2''      | 129.9, CH | 129.2, CH | 128.8, CH | 127.4, CH | 130.5, CH |
| 3''      | 115.2, CH | 128.2, CH | 128.5, CH | 121.0, C | 115.1, CH |
| 4''      | 157.1, C | 127.3, CH | 127.5, CH | 152.3, C | 155.1, C |
| 5''      | 115.2, CH | 128.2, CH | 128.5, CH | 116.1, CH | 115.1, CH |
| 6''      | 129.9, CH | 129.2, CH | 128.8, CH | 130.2, CH | 130.5, CH |
| 1'''     | 29.9, CH₂ | 122.4, CH | 122.4, CH |            |            |
| 2'''     | 122.0, CH | 130.3, CH | 130.8, CH |            |            |
| 3'''     | 134.7, C | 76.4, C  | 76.5, C  |            |            |
| 4'''     | 125.8, CH₂| 28.4, CH₃| 28.2, CH₃|            |            |
| 5'''     | 17.9, CH₃ | 28.4, CH₃| 28.2, CH₃|            |            |

a Spectra were recorded in acetone-d₆; b Spectra were recorded in CDCl₃. δ in ppm.

Figure 2. Key HMBC, ¹H-¹H COSY and ROESY correlations of 1–5.

Sanshamycin B (2) was isolated as a yellowish powder, and had the molecular formula C₂₆H₂₈O₄ on the basis of the HRESIMS (Figure S18) and 1D NMR data (Tables 1 and 2). Analysis of the ¹H (Figure S11), ¹³C (Figure S12), DEPT135 (Figure S13) and HSQC (Figure S14) NMR data of 2 showed the presence of three methoxyl groups, two methyl groups, one methylene, and 20 sp² carbons (ten of which were protonated). Analysis of the ¹³C NMR data, the coupling constants of the aromatic protons in the ¹H NMR spectrum and also the correlations observed in the COSY spectrum revealed the presence of a pentasubstituted, a 1,3,4-trisubstituted, and a monosubstituted benzene rings, indicating a p-terphenyl framework structure similar to 1. The presence of an isopentyl group could be deduced from HMBC correlations (Figure S15) from H₂-1‴‴ (δ_H 3.40, d, J = 7.2 Hz), H₃-4‴‴ (δ_H 1.78, d, J = 1.4 Hz) and H₃-5‴‴ (δ_H 1.79, br s) to C-3‴‴ (δ_C 134.7), H₃-4‴‴ and
H$_3$S’’’ to C-2’’’ ($\delta_C$ 122.0), $^1$H-$^1$H COSY correlations (Figure S16) from H$_2$-1’’’ to H-2’’’ ($\delta_H$ 5.40, dq, $J = 7.2, 1.4$ Hz), and NOESY correlations (Figure S17) from H$_2$-1’’’ to H$_3$S’’’ and H-2’’’ to H-3’’’.

That the isopentyl moiety was located at C-3 was evidenced from HMBC correlations from H$_2$-1’’’ to C-2 ($\delta_C$ 132.1), C-3 ($\delta_C$ 125.9), and C-4 ($\delta_C$ 153.5), and NOESY correlation from H$_2$-1’’’ to H-2 ($\delta_H$ 7.17, d, $J = 2.1$ Hz). Characteristic HMBC correlations from the methoxyl group at $\delta_H$ 3.66 to C-2’ ($\delta_C$ 152.0), the methoxyl group at $\delta_H$ 3.61 to C-3’ ($\delta_C$ 144.8) and the methoxyl group at $\delta_H$ 3.74 to C-6’ ($\delta_C$ 153.2) and NOESY correlations between 2’-OCH$_3$ and H-6 ($\delta_H$ 7.17, overlap), between 3’-OCH$_3$ and H-2’’’ (H-6’’’), ($\delta_H$ 7.59, d, $J = 7.5$ Hz), and between 6’-OCH$_3$ and H-5’ ($\delta_H$ 6.69, s), confirmed the positions of these methoxyl groups.

The key HSQC, HMBC, $^1$H-$^1$H COSY and ROESY of 2 was shown in Figure 2.

Sanshamycin C (3), obtained as a yellow powder, gave a molecular formula of C$_{25}$H$_{34}$O$_5$, as determined by HRESIMS (Figure S28), and its $^1$H and $^{13}$C NMR data (Tables 1 and 2), resembled those for sanshamycin B (2). The major differences observed in the $^1$H NMR spectrum (Figure S21) for 3 relative to those of 2 indicated the presence of an cis-disubstituted double bond due to the coupling constants of protons at $\delta_H$ 6.35 ($d, J = 9.7$ Hz, H-1’’’), and 5.60 ($d, J = 9.7$ Hz, H-2’’’), instead of a methylene signal at $\delta_H$ 3.40 and an olefinic methine signal at $\delta_H$ 5.40, and the absence of a methoxyl substituent in 3. Comprehensive consideration of the HRESIMS and NMR data suggested 3 could be derived from the cyclization between the hydroxyl group 4-OH and C-3’’’ of the isopentyl group of 2. This suggestion was confirmed by HMBC correlations (Figure S25) from H-1’’’ to C-4 ($\delta_C$ 152.3) and C-3’’’ ($\delta_C$ 76.4), H-2’’’ to C-3 ($\delta_C$ 120.8), H-4’’’ ($\delta_H$ 1.47, s) and H-5’’’ ($\delta_H$ 1.47, s) to C-2’’’ ($\delta_C$ 130.3) and C-3’’’ ($\delta_C$ 122.4), and NOESY correlation (Figure S27) from H-1’’’ to H-2. Correlations from the methoxyl group at $\delta_H$ 3.44 to C-3’ ($\delta_C$ 138.9) and the methoxyl group at $\delta_H$ 3.75 to C-6’ ($\delta_C$ 153.5) in the HMBC spectrum and NOESY correlations from 3’-OCH$_3$ to H-2’’’ (H-6’’’), ($\delta_H$ 7.64), and from 6’-OCH$_3$ to H-5’ ($\delta_H$ 6.48, s) were observed. Overall analysis of the 1D and 2D NMR data permitted the structural assignment for 3 as shown in Figure 1.

Sanshamycin D (4) was isolated as a white powder whose molecular formula of C$_{27}$H$_{32}$O$_6$ was obtained by analysis of HRESIMS (Figure S38) and NMR data (Figures S31–S37). The $^1$H and $^{13}$C NMR data of 4 were very similar to those of 4,5-dimethoxycedisudin A (9) [11], except for the presence of a prenyl and the absence of OH-4’’. The prenyl moiety was elucidated by HMBC correlations from H-4’’’/H-5’’’ ($\delta_H$ 1.49, s) to C-2’’’ ($\delta_C$ 130.8) and C-3’’’ ($\delta_C$ 76.5), and $^1$H-$^1$H COSY correlations from H-1’’’ ($\delta_H$ 6.40, d, $J = 9.7$ Hz) to H-2’’’ ($\delta_H$ 5.66, d, $J = 9.7$ Hz). That the prenyl group was at C-3’’’ was evidenced from the HMBC correlations from H-1’’’ to C-2’’’ ($\delta_C$ 127.4) and C-4’’’ ($\delta_C$ 152.3), H-2’’’ to C-3’’’ ($\delta_C$ 121.0), and NOESY correlation from H-1’’’ to H-2’’’ ($\delta_H$ 7.25, s). The HMBC spectrum displayed correlations from the methoxyl signals at $\delta_H$ 3.98, 4.02, 3.71, and 4.03 to C-4, C-5, C-3’, and C-6’, respectively. Significant NOESY correlations from 4-OCH$_3$ to H-3 ($\delta_H$ 7.17, s), 5-OCH$_3$ to H-6 ($\delta_H$ 7.56, s), 3’-OCH$_3$ to H-6’’ ($\delta_H$ 7.38, d, $J = 8.3$ Hz), and 6’-OCH$_3$ to H-5’ ($\delta_H$ 6.68, s), secured the positions of these methoxyl groups. Complete NMR analysis supported the full elucidation of the structure for compound 4 as shown in Figure 2.

Sanshamycin E (5) was isolated as a white powder. Its molecular formula was determined as C$_{19}$H$_{20}$O$_7$ (10 degrees of unsaturations) by HRESIMS (Figure S48) in combination with $^1$H and $^{13}$C NMR data. The $^1$H (Figure S41), $^{13}$C (Figure S42), DEPT135 (Figure S43), and HSQC (Figure S44) NMR spectra (Tables 1 and 2) showed resonances for 19 carbons, including 12 sp$^2$ carbons (five of which are protonated), one ester carbonyl carbon, one quaternary sp$^3$ carbon, two methoxyl carbons, one methylene sp$^2$ carbon, one methine sp$^3$ carbon, and one methyl carbon. Detailed analysis of the coupling constants of the protons in the $^1$H NMR spectrum and COSY correlations indicated the presence of a pentasubstituted and a 1,4-disubstituted benzene rings. Characteristic HMBC correlations (Figure S45) from H-2’’’/H-6’’ ($\delta_H$ 7.41, d, $J = 8.5$ Hz) to C-4’’ ($\delta_C$ 155.1) and C-4’ ($\delta_C$ 136.4) and from H-3’’’/H-5’’ ($\delta_H$ 6.90, d, $J = 8.5$ Hz) and H-5’ ($\delta_H$ 6.43, s) to C-1’’ ($\delta_C$ 130.5) secured the connectivity of C-1’’ to C-4’. HMBC correlations from the methoxyl proton signals at $\delta_H$
3.66 to C-3′ (δC 136.0), at δH 3.84 to C-6′ (δC 151.5), from H-5′ to C-3′ and C-6′, and NOESY correlations (Figure S47) from 6′-OCH3 to H-5′ and from 3′-OCH3 to H-2″/H-6″ confirmed the locations of the methoxyl groups at C-3′ and C-6′. So far, nine degrees of unsaturation have been assigned, while the last degree of unsaturation could be completed by the presence of a γ-butyrolactone moiety, which was deduced from the HMBC correlations from H-2 (δH 3.08, dd, J = 6.4, 4.5 Hz) to C-1 (δC 173.9) and C-4 (δC 118.3), H-3 (δH 3.96, dd, J = 6.4, 4.5 Hz) to C-1 and C-5 (δC 23.9), H-5 (δH 1.94, s) to C-3 (δC 45.7) and C-4, and 1H-1H COSY correlation (Figure S46) from H-2 to H-3. The high chemical shift value (δC 118.3) of C-4 is a characteristic of ketal carbon, which could suggest the connectivity of C-1 and C-4 through an ester bond. The γ-butyrolactone moiety was connected with the biphenyl moiety, which could be deduced from the HMBC correlations from H-2 to C-1′ (δC 114.6) and from H-3 to C-2′ (δC 149.9) and C-6′. We have tried to determine the absolute configurations of C-3 and C-4 by comparing the experimental and calculated ECD data, but unable to reach a conclusion. Finally, the structure for 5 was determined as shown in Figure 1. A plausible biosynthetic pathway for 5 was tentatively proposed in the Supplementary Materials (Figure S51).

The known compounds terphenyllin (6) [8], 3-hydroxyterphenyllin (7) [9], candidusin A (8) [10], 4,5-dimethoxycandidusin A (9) [11], and candidusin C (10) [12] were identified by comparison of their NMR data with those reported in the literature.

2.2. In Vitro Evaluation of Type 4 Phosphodiesterase PDE4D Inhibitory Activity

p-Terphenyl compounds have been identified as phosphodiesterase (PDE) inhibitors. Terferol, derived from Streptomyces showdoensis SANK 65080, showed inhibitory activity towards cyclic adenosine 3′,5′-monophosphate phosphodiesterase (cAMP-PDE) and cyclic guanosine 3′,5′-monophosphate phosphodiesterase (cGMP-PDE) from various rat tissues [15]. Moreover, one metabolite with a similar structure to terferol from two different microbes was also found to possess inhibitory activities towards eukaryotic PDE11 and four PDE4s [14,16]. Given the close structural relationship of 1–10 with the known natural phosphodiesterase inhibitor terferol, the inhibitory activities of 1–6 and 9 were evaluated against the PDE4D, with rolipram as the positive control. As shown in Table 3, 3 was the most potent compound and displayed the best inhibition at 5 µM with inhibitory percentage of 49.4 %, while the other compounds showed weaker activities with inhibitory percentage of 4.8–23.2% (Table 3). Compound 3 was selected to test the concentration required for 50% inhibition of PDE4D (IC50), which gave an IC50 value of 5.543 ± 0.24 µM, while the positive drug rolipram exhibited an IC50 value of 0.588 ± 0.057 µM. Comparing with the other tested compounds, the two fused six-membered rings and the hydroxyl group at C-2′ in 3 may be responsible for the strong PDE4D inhibitory activity.

Table 3. Inhibitory activities of 1–6 and 9 towards PDE4D.

| Compounds | PDE4D Inhibitory Percentage (100%) |
|-----------|-----------------------------------|
| Rolipram  | 52.0                              |
| 1         | 4.8                               |
| 2         | 6.7                               |
| 3         | 49.4                              |
| 4         | 12.4                              |
| 5         | 5.1                               |
| 6         | 12.8                              |
| 9         | 23.2                              |

The test concentration of positive rolipram is 0.5 µM; The test concentrations of 1–6 and 9 are 5 µM.

3. Materials and Methods

3.1. General Experimental Procedures

One- and two-dimensional NMR spectra were measured on Bruker AVIII-500 NMR spectrometer (Bruker Corporation, Karlsruhe, Germany). The chemical shifts of 1H (500 MHz) and 13C (125 MHz) NMR data were given in δ (ppm) and referenced to the
solvent signal (CDCl$_3$, $\delta_H$ 7.26 and $\delta_C$ 77.16; acetone-$d_6$, $\delta_H$ 2.05 and $\delta_C$ 29.84). HRESIMS data were collected on an Agilent 6210 TOF LC-MS instrument (Agilent Technologies Inc., Palo Alto, CA, USA). Optical rotation value was recorded by a JASCO P-1020 digital polarimeter (JASCO, Tokyo, Japan). UV and IR data were measured on a UV-2550 spectrometer (Shimadzu, Kyoto, Japan) and Nicolet 380 Infrared Spectrometer (Thermo Electron Corporation, Madison, WI, USA), respectively. The ECD data were collected on JASCO J-715 spectropolarimeter (JASCO, Tokyo, Japan). The semi-preparative HPLC was conducted on a Waters 1525 HPLC equipped with a XBridge C18 column (5 µm, 250.0 mm × 10.0 mm; Waters Corporation, Milford, MA, USA). Thin-layer chromatography (TLC) was performed on pre-coated glass plates (silica gel GF$_{254}$, Qingdao Marine Chemical Inc., Qingdao, China). Column chromatography (CC) was performed on silica gel (45–75 µm; Qingdao Marine Chemical Inc., Qingdao, China), ODS (40–60 µm; Osaka Soda Co., Ltd., Hyogo, Japan) and Sephadex LH-20 (Cytiva, Upssala, Sweden).

3.2. Fungal Material and Fermentation

The fungal strain ITBBc1 was isolated from a coral reef in the South China Sea in Sansha, Hainan, China. This fungal strain was identified as *Aspergillus* sp. by internal transcribed spacer (ITS) sequence (GenBank accession number OP614945). The voucher specimen of this strain was deposited in the Hainan Key Laboratory of Tropical Microbe Resources, Institute of Tropical Bioscience and Biotechnology, Haikou, P.R. China. The strain was cultivated on a PDA agar plate (consisting of potato extract 200 g/L, glucose 20 g/L, agar 15 g/L, chloramphenicol 0.1 g/L and 1 L sterilized deionized water) at 28 °C for 5 days. Then, the agar plugs with mycelia were added into 1 L Erlenmeyer flasks, each containing 200 mL ME liquid medium (consisting of malt extract 10.0 g/L, sucrose 10.0 g/L, peptone 1.0 g/L and 1 L sterilized deionized water), which was cultivated on a rotary shaker at 160 rpm/min at 28 °C. After 4 days of fermentation, 15 mL of the seed cultures were inoculated into the rice solid media (consisting of rice 30 g and seawater 45 mL) in 1L-Erlenmeyer flasks and fermented at 28 °C for 45 days under static conditions.

3.3. Extraction and Isolation

The whole fermentation materials of strain ITBBc1 were collected and extracted with ethyl acetate (EtOAc) at room temperature for four times to yield a crude extract (120.0 g). Then, the crude extract was fractionated by silica gel CC using gradient elution of petroleum ether/EtOAc mixtures (v/v, 100:1, 50:1, 25:1, 10:1, 5:1, 2:1, 1:1, 0:1) to give 8 fractions (Fr.A–Fr.H). The fraction Fr.E (petroleum ether/EtOAc, v/v, 5:1) was separated by ODS reversed-phase CC eluted with aqueous methanol (40% to 100%) to yield the Fr.E7 (85% aqueous methanol), which was further purified by Sephadex LH-20 (eluted with 100% methanol) and then silica gel CC [using gradient elution of petroleum ether/EtOAc mixtures (v/v, 53:1, 25:1, 10:2)] to give compounds 2 (6.6 mg), 3 (5.0 mg) and 4 (2.2 mg). The fraction Fr.F (petroleum ether/EtOAc, v/v, 2:1) was subjected to ODS reversed-phase CC eluted with aqueous methanol (45% to 100%) to produce nine subfractions Fr.F1–F9. The Fr.F1 (45% aqueous methanol) was further subjected to Sephadex LH-20 (eluted with 100% methanol) and then silica gel CC [using gradient elution of petroleum ether/EtOAc mixtures (v/v, 53:1, 25:1, 10:2)] to afford compound 5 (2.5 mg). The Fr.F3 (55% aqueous methanol) was purified by Sephadex LH-20 (eluted with 100% methanol) to give compound 1 (3.5 mg). The Fr.F5 (65% aqueous methanol) was subjected to Sephadex LH-20 (eluted with 100% methanol) and then purified by semi-preparative reverse-phase HPLC (eluted with 70% aqueous methanol; 3 mL/min, UV $\lambda_{max}$ 254 nm) to yield compound 10 (42.1 mg, $t_R$ 9.1 min). The Fr.F6 (75% aqueous methanol) was purified by Sephadex LH-20 (eluted with 100% methanol) to give compound 9 (0.9 mg). Compound 6 was recrystallized from the fraction Fr.G (petroleum ether/EtOAc, v/v, 1:1) and obtained as white crystals (90 mg). Then, the rest of the Fr.G was subjected to ODS reversed-phase CC eluted with aqueous methanol (40% to 100%) to yield seven subfractions Fr.G1–G7. The Fr.G3 (50% aqueous methanol) was separated by Sephadex LH-20 (eluted with 100% methanol) and then purified by
semi-preparative reverse-phase HPLC (3 mL/min, UV \( \lambda_{\text{max}} \) 254 nm) to give compounds 7 (59.1 mg, \( t_R \) 5.5 min; eluted with 60% aqueous methanol) and 8 (13.2 mg, \( t_R \) 8.5 min; eluted with 65% aqueous methanol).

Sanshamycin A (1): yellow powder; UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)): 304 (2.81), 332 (2.52), 342 (2.54) nm; IR(KBr) \( v_{\text{max}} \): 3415, 2918, 1652, 1403, 1118, 1075, 835, 759 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data, see Tables 1 and 2; HRESIMS \( m/z \) 389.1001 [M + Na]\(^+\) (calculated for C\(_{21}\)H\(_{18}\)NaO\(_6\), 389.0996).

Sanshamycin B (2): yellowish powder; UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) : 258 (2.60), 298 (2.81) nm; IR(KBr) \( v_{\text{max}} \): 3404, 2931, 1599, 1465, 1389, 1191, 1079, 1025, 616, 547 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data, see Tables 1 and 2; HRESIMS \( m/z \) 427.1928 [M + Na]\(^+\) (calculated for C\(_{26}\)H\(_{20}\)NaO\(_4\), 427.1880).

Sanshamycin C (3): yellow powder; UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) : 273 (2.68), 302 (2.83) nm; IR(KBr) \( v_{\text{max}} \): 3416, 2934, 2434, 1629, 1468, 1404, 1103, 1072, 795 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data, see Tables 1 and 2; HRESIMS \( m/z \) 389.1752 [M + H]\(^+\) (calculated for C\(_{25}\)H\(_{25}\)O\(_4\), 389.1747).

Sanshamycin D (4): white powder; UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) : 258 (2.64), 310 (2.92), 337 (2.96) nm; IR(KBr) \( v_{\text{max}} \): 3411, 2919, 1617, 1480, 1384, 1129, 1023, 617 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data, see Tables 1 and 2; HRESIMS \( m/z \) 447.1801 [M + H]\(^+\) (calculated for C\(_{27}\)H\(_{27}\)O\(_5\), 447.1802).

Sanshamycin E (5): white powder; \([\alpha]_{D}^{25}\) 68 (c 0.10, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) : 231 (2.50), 266 (2.67) nm; IR(KBr) \( v_{\text{max}} \): 3430, 2922, 1628, 1382, 1097, 638, 534 cm\(^{-1}\); ECD (MeOH) \( \lambda_{\text{max}} \) (\( \Delta \varepsilon \)) : 190 (−19.29), 199 (−21.27), 208 (−17.37), 234 (−0.33) nm; \(^1\)H and \(^{13}\)C NMR data, see Tables 1 and 2; HRESIMS \( m/z \) 383.1080 [M + Na]\(^+\) (calculated for C\(_{19}\)H\(_{20}\)NaO\(_7\), 383.1101).

3.4. Type 4 Phosphodiesterase PDE4D Inhibitory Screening Assay

The type 4 phosphodiesterase PDE4D inhibitor assays were performed as described previously [30–32]. The inhibition of PDE4D by compounds 1–6 and 9 were assayed by a PerkinElmer 2910 (PerkinElmer, Inc., Waltham, MA, USA) liquid scintillation counter. Rolipram was used as a positive drug. Three independent experiments were conducted for the measurement of the inhibitory effect of compound 3 against PDE4D. The experimental data were analyzed via GraphPad Prism 5.1 (GraphPad Software, San Diego, CA, USA), and the IC\(_{50}\) values were calculated by nonlinear regression.

4. Conclusions

In conclusion, five new \( p \)-terphenyl derivatives, sanshamycins A–E (1–5), and five known analogues (6–10) were isolated and characterized from the coral-associated fungus Aspergillus sp. ITBBc1. The structures of the unreported compounds (1–5) were elucidated by interpretation of their 1D and 2D NMR and HRESIMS data whiles the structures of the previously reported compounds (6–10) were identified by comparison of their NMR data with those reported in literature. Sanshamycin A (1) represents the first example of \( p \)-terphenyls with an aldehyde substitution on the benzene ring. Sanshamycins B–D (2–4) feature varying methoxyl and isopentenyl substitutions, while sanshamycin E (5) features a five-membered lactone linked to a biphenyl. The inhibitory effects of 1–6 and 9 on PDE4D were assayed in vitro and 3 exhibited potent PDE4D inhibitory activity with an IC\(_{50}\) value of 5.543 \( \mu \)M. The current study revealed a new natural type 4 phosphodiesterase PDE4 inhibitor from the marine-derived fungus, Aspergillus sp. strain ITBBc1, could serve as a new structural motif for the future PDE4 inhibitor design. In general, the results of this study expand the knowledge of the chemical and biological diversity of the family of \( p \)-terphenyl natural products, and may provide a promising lead structure for the future development of PDE4 inhibitors. The inhibitory activity against other PDE members and the PDE4-inhibitory mechanism of sanshamycin C (3) deserves further investigation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/md20110679/s1, Figures S1–S51: 1D, 2D NMR, MS, UV, and IR spectra of compounds 1–5.
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