Chemical Constituents of Phaius mishmensis

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Abstract: The partitioned n-hexane, CHCl₃, and EtOAc extracts from the crude MeOH extract of Phaius mishmensis showed considerable cytotoxicities against the human breast carcinoma (MCF-7), lung carcinoma (NCI-H460), and central nervous system carcinoma (SF-268) cell lines. Four new compounds, phaindole (1), (7′R,8′R)-phaithrene (2), methyl 3-hydroxy-4,5-dimethoxypropiophenone (3), and methyl hematinate (4), as well as 44 known compounds were isolated from the MeOH extract of Phaius mishmensis. The structures of the compounds were determined using spectroscopic methods.

Keywords: Phaius mishmensis; Orchidaceae; phaindole; (7′R,8′R)-phaithrene; methyl 3-hydroxy-4,5-dimethoxypropiophenone; methyl hematinate

1. Introduction

In our search for novel anticancer compounds from Taiwanese plants, the crude MeOH extract of Phaius mishmensis (Orchidaceae), a native orchid of Taiwan [1], showed considerable cytotoxicity against the human breast carcinoma (MCF-7), lung carcinoma (NCI-H460), and central nervous system carcinoma (SF-268) cell lines. Thus, the plant P. mishmensis was selected for purification based on the preliminary results. We isolated eight indoloquinazolinones, phaitanthrins A–E, methylisatoid, tryptanthrin, and candidine from the CHCl₃-soluble extract of the plant [2]. After extensive column and preparative thin-layer chromatographic separations, four new compounds, phaindole (1), (7′R,8′R)-phaithrene (2), 3-hydroxy-4,5-dimethoxypropiophenone (3), and methyl hematinate (4), as well as 44 known compounds were isolated from the MeOH extract of P. mishmensis. Herein we describe the isolation, substance elucidation, and cytotoxic properties of the isolated compounds.

2. Results and Discussion

2.1. Isolation of Chemical Constituents of P. mishmensis

Compound 1, isolated as a yellowish powder, was determined to have the molecular formula C₂₆H₂₂N₃O₆ as noted by the pseudo-molecular ion peak at m/z 472.1506 in high resolution electrospray ionization mass spectroscopy (HR-ESIMS). A broad infrared (IR) absorption at 3264 cm⁻¹ indicated the presence of a hydroxyl or amino functionality. Furthermore, four strong IR absorptions at 1702, 1697, 1659, and 1650 cm⁻¹ might indicate the presence of two carbonyl and two amodic functionalities. The ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectral data of compound 1 were shown in Table 1, and all chemical shifts (δ) were given in ppm. In the aromatic region of the ¹H- and COSY spectra, three sets of four mutually coupled proton signals at δ 7.34 ppm (t, J = 8.0 Hz, H-5), 7.40 ppm (t, J = 8.0 Hz, H-6), 7.52 ppm (d, J = 8.0 Hz, H-7), 8.14 ppm (d, J = 8.0 Hz, H-4), 7.22 ppm (t, J = 8.0 Hz, H-4′),
7.56 ppm (t, \( J = 8.0 \text{ Hz}, \text{H-5}' \)), 7.98 ppm (d, \( J = 8.0 \text{ Hz}, \text{H-3}' \)), 8.14 ppm (d, \( J = 8.0 \text{ Hz}, \text{H-6}' \)), 7.16 ppm (t, \( J = 8.0 \text{ Hz}, \text{H-4}' \)), 7.62 ppm (t, \( J = 8.0 \text{ Hz}, \text{H-5''} \)), 8.07 ppm (d, \( J = 8.0 \text{ Hz}, \text{H-3''} \)), and 8.86 ppm (d, \( J = 8.0 \text{ Hz}, \text{H-6''} \)) were observed. Combined with \(^{13}\)C, HMOC, and HMBC data, we determined that the first \( \alpha \)-disubstituted benzene was fused with a pyrrole ring to form an indole unit, as noted by the HMBC correlations of H-1 (\( \delta = 9.98 \text{ ppm, br s, NH} \)) with C-3 (\( \delta = 112.3 \text{ ppm} \)), C-7 (\( \delta = 112.5 \text{ ppm} \)), and C-9 (\( \delta = 125.7 \text{ ppm} \)), and H-4 with C-3, whereas the latter two signals belonged to two methyl 2-aminobenzoate moieties, as determined by the HMBC correlations of H-3' and 7'-OCH\(_3\) (\( \delta = 3.83 \text{ ppm} \)) with C-7' (\( \delta = 167.1 \text{ ppm} \)), H-3'' and 7''-OCH\(_3\) (\( \delta = 3.85 \text{ ppm} \)) with C-7'' (\( \delta = 168.4 \text{ ppm} \)), as well as 1''-NH (\( \delta = 12.55 \text{ ppm} \)) with C-6' (\( \delta = 124.8 \text{ ppm} \)) and 1'''-NH (\( \delta = 11.85 \text{ ppm} \)) with C-2' (\( \delta = 116.0 \text{ ppm} \)) and C-6'' (\( \delta = 121.6 \text{ ppm} \)). The remaining two carbonyl signals at \( \delta = 159.2 \text{ ppm} \) (C-10) and 164.7 ppm (C-11) were attributed to C-2 and C-3 of the indole ring, respectively, which formed an amide linkage with two methyl 2-aminobenzoate groups as determined by the HMBC correlations of 1''-NH with \( \delta = 159.2 \text{ ppm} \) (C-10) and 1'''-NH with \( \delta = 164.7 \text{ ppm} \) (C-11). The NOE correlations between H-1 and H-7, 1''-NH and H-6', 1'''-NH and H-4, and H-6'' revealed that the structure of 1 was 2,3-di(2-methoxycarbonylphenyl)carbamoylindole; this compound was named phaindole (Figure 1).

The optically active compound 2 ([\( \alpha \]D] \( -18.5^\circ \)) was obtained as a colorless powder and determined to have the molecular formula \( \text{C}_{27}\text{H}_{26}\text{O}_{10}\), owing to the pseudo-molecular ion peak at \( m/z \) 743.4883 in HR-FABMS. The IR spectrum showed a broad absorption at 3399 cm\(^{-1}\) corresponding to a hydroxyl group and a strong absorption at 1728 cm\(^{-1}\) corresponding to a carbonyl group. The \(^{1}\)H-NMR (300 MHz) and \(^{13}\)C-NMR (75 MHz) spectral data of compound 2 were shown in Table 1. In the aromatic region of the \(^{1}\)H-NMR and COSY spectra, three mutually coupled proton signals at \( \delta = 6.69 \text{ ppm} \) (d, \( J = 2.6 \text{ Hz}, \text{H-1} \)), 6.71 ppm (dd, \( J = 8.2, 2.6 \text{ Hz}, \text{H-3} \)), and 8.09 ppm (d, \( J = 8.2 \text{ Hz}, \text{H-4} \)) corresponding to a trisubstituted benzene ring and singlet proton signal at \( \delta = 6.53 \text{ ppm} \) (s, H-6) for a pentasubstituted benzene ring were observed. In the aliphatic region of the \(^{1}\)H-NMR spectrum, an ethylene proton signal at \( \delta = 2.66 \text{ ppm} \) (4H, m, H-9, and H-10) was observed. Using HMOC, HMBC, and NOESY, key long-range \(^{1}\)H-\(^{13}\)C correlations of H-1 with C-10 (\( \delta = 29.7 \text{ ppm} \)); H-4 with C-4b (\( \delta = 116.8 \text{ ppm} \)); H-9 with C-4b, C-8 (\( \delta = 113.9 \text{ ppm} \)) , C-10a (\( \delta = 139.3 \text{ ppm} \)); H-10 with C-1 (\( \delta = 114.1 \text{ ppm} \)) and C-8a (\( \delta = 136.8 \text{ ppm} \)), as well as a key NOE correlation between H-1 and H-10 revealed a 2,5,7,8-tetrasubstituted 9,10-dihydrophenanthrene nucleus. Three very down-field–shifted \(^{13}\)C- signals at C-2 (\( \delta = 153.4 \text{ ppm} \)), C-5 (\( \delta = 158.5 \text{ ppm} \)) , and C-7 (\( \delta = 159.0 \text{ ppm} \)) together with the NOE correlations between a hydroxyl group (\( \delta = 4.76 \text{ ppm} \)) and H-1, -3, a methoxyl group (\( \delta = 3.87 \text{ ppm} \)) and H-4, -6, revealed a hydroxyl (2-OH), a methoxyl (5-OCH\(_3\)), and oxygenated substituents (7-OR). The remaining three unresolved aromatic proton signals at \( \delta = 6.88 \text{ ppm} \) (3H, m, H-2', H-5', and H-6'), a hydroxyl signal at \( \delta = 5.63 \text{ ppm} \) (4'-OH), a methoxyl signal at \( \delta = 3.88 \text{ ppm} \) (3'-OCH\(_3\)), -CHCH- proton signals at \( \delta = 4.21 \text{ ppm} \) (d, \( J = 5.4 \text{ Hz, H-8} \)) and 5.93 ppm (d, \( J = 5.4 \text{ Hz, H-7} \)), together with a carbonyl signal at \( \delta = 172.4 \text{ ppm} \) (C-9'), revealed a 7''-oxigenated-8'-alkyl dihydroferulate moiety. The HMBC correlations of 4'-OH with C-3' (\( \delta = 146.7 \text{ ppm} \)), C-3' (\( \delta = 114.5 \text{ ppm} \)), 3'-OCH\(_3\) with C-3' (\( \delta = 146.7 \text{ ppm} \)); H-7' with C-2' (\( \delta = 108.0 \text{ ppm} \)), C-6' (\( \delta = 118.6 \text{ ppm} \)), and C-9'; H-8' with C-1' (\( \delta = 138.2 \text{ ppm} \)) and C-9 confirmed the presence of a ferulate moiety. A 22-long chain alcohol at \( \delta = 0.88 \text{ ppm} \) (3H, t, \( J = 6.8 \text{ Hz, H-22} \)), 1.25 ppm (3H, m, H-3'-H-21'), 1.62 ppm (2H, quintet, \( J = 6.9 \text{ Hz, H-2''} \)), and 4.15 ppm (2H, \( t, J = 6.9 \text{ Hz, H-1''} \)) formed docosyl ferulate, as noted by the HMBC correlation of H-1'' with C-9'. Finally, the HMBC correlation of H-7' with C-7, -8; H-8' with C-7; and the NOE correlation between H-8' and H-9 revealed that the ferulate was fused to the phenanthrene ring to form a furanophenanthrene. Based on a report by Juhasz [5], the absolute configuration was determined as follows: first, the smaller coupling constant 5.5 Hz between H-7' and H-8' suggested that the substituents on the dihydrofuran ring were in the \textit{trans} configuration. Second, a similar compound, (2S,3S)-methyl 2,3-dihydro-2-phenylbenzofuran-3-carboxylate (2a), with the S configuration at C-2 showed a negative Cotton effect at 280 nm by circular dichroism (CD) spectrometry. Compound 2 presented a positive Cotton effect at 280 nm by CD spectrometry, indicating the 7''R configuration. Consequently, the absolute configuration of (7''R,8'S'R) was deduced for 2 and the compound was named (7''R,8'S'R)-phaithrene.
Table 1. $^{13}$C- and $^1$H-NMR spectroscopic data for 1–4.

| Position | 1 in CDCl$_3$ (125 MHz/500 MHz) | 2 in CDCl$_3$ (75 MHz/300 MHz) | 3 in CDCl$_3$ (75 MHz/300 MHz) | 4 in CDCl$_3$ (75 MHz/300 MHz) |
|----------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|          | $\delta_C$ ppm | $\delta_H$ ppm (J in Hz) | $\delta_C$ ppm | $\delta_H$ ppm (J in Hz) | $\delta_C$ ppm | $\delta_H$ ppm (J in Hz) |
| 1        | 114.1 | 6.69 d (2.6) | 132.6 | 7.32 br s |
| 2        | 133.4 | 153.4 | 108.8 | 7.22 d (1.9) | 171.4 |
| 3        | 112.3 | 112.8 | 6.71 dd (8.2, 2.6) | 148.9 | 139.7 |
| 4        | 121.4 | 8.14 d (8.0) | 129.2 | 8.09 d (8.2) | 139.4 | 139.7 |
| 4a       | 125.8 | 8.14 d (8.0) | 129.2 | 8.09 d (8.2) | 139.4 | 139.7 |
| 4b       | 116.8 | 116.8 | 153.4 | 108.8 | 7.22 d (1.9) | 171.4 |
| 5        | 122.5 | 7.34 t (8.0) | 158.5 | 152.2 | 7.16 d (1.9) |
| 6        | 125.5 | 7.40 t (8.0) | 92.7 | 6.53 s | 103.7 | 7.16 d (1.9) |
| 7        | 112.5 | 7.52 d (8.0) | 148.9 | 139.7 | 199.9 |
| 8        | 134.4 | 113.9 | 31.6 | 2.94 q (7.2) |
| 8a       | 136.8 | 136.8 | 153.4 | 108.8 | 7.22 d (1.9) | 171.4 |
| 9        | 125.7 | 27.0 | 2.66 m | 8.4 | 1.21 t (7.2) |
| 10       | 139.2 | 29.7 | 2.66 m |
| 10a      | 139.3 | 139.3 | 153.4 | 108.8 | 7.22 d (1.9) | 171.4 |
| 11       | 164.7 | 164.7 | 153.4 | 108.8 | 7.22 d (1.9) | 171.4 |
| 1′       | 137.9 | 132.8 | 19.3 | 2.71 m |
| 2′       | 122.0 | 108.0 | 6.88 m | 31.7 | 2.61 m |
| 3′       | 130.9 | 7.98 d (8.0) | 146.7 | 172.5 |
| 4′       | 124.6 | 7.22 t (8.0) | 145.8 |
| 5′       | 133.0 | 7.56 t (8.0) | 114.5 | 6.88 m |
| 6′       | 124.8 | 8.14 d (8.0) | 118.6 | 6.88 m |
| 7′       | 167.1 | 87.4 | 5.93 d (5.4) |
| 8′       | 56.0 | 4.21 d (5.4) |
| 9′       | 172.4 |
| 1″       | 141.2 | 65.7 | 4.15 t (6.9) |
| 2″       | 116.0 | 28.6 | 1.62 quintet (6.9) |
| 3″       | 131.0 | 8.07 d (8.0) | 25.8 |
| 4″       | 123.1 | 7.16 t (8.0) |
| 5″       | 134.4 | 7.62 t (8.0) |
| 6″       | 121.6 | 8.86 d (8.0) | 29.5 | 1.25 m |
| 7″       | 168.4 |
| 8″–19″   | 31.9 | 22.7 |
| 20″      | 14.1 | 0.88 t (6.8) |
| 21″      | 22.7 |
| 22″      | 4.76 br s |
| 2-0H     | 5.87 br s |
| 3-0H     | 6.70 br s |
| 4-CH$_3$ | 8.7 | 2.00 s |
| 4-OCH$_3$ | 61.0 | 3.97 s |
| 5-OCH$_3$ | 55.6 | 3.87 s * | 56.0 | 3.91 s |
| 1′-NH     | 12.55 s |
| 3′-OCH$_3$ | 55.8 | 3.88 s * | 51.9 | 3.67 s |
| 4′-OCH$_3$ | 5.63 br s |
| 7′-OCH$_3$ | 52.2 | 3.83 s |
| 1″-NH     | 11.85 s |
| 7″-OCH$_3$ | 52.5 | 3.85 s |

* Assignments may be interchangeable.
Compound 3 was isolated as yellowish needles, and was determined to have the molecular formula C_{11}H_{12}O_{4} by HR-EIMS at m/z 210.0894. A broad IR absorption at 3390 cm^{-1} and a strong IR absorption at 1680 cm^{-1} revealed the existence of hydroxyl and carbonyl groups, respectively. The $^1$H-NMR (300 MHz) and $^{13}$C-NMR (75 MHz) spectral data of compound 3 are shown in Table 1. The only two $^1$H-NMR signals at $\delta$ 7.16 ppm (d, $J = 1.9$ Hz, H-6) and 7.22 ppm (d, $J = 1.9$ Hz, H-2) indicated a 1,3,4,5-tetrasubstituted benzene nucleus. Three oxygenated substituents, namely a hydroxyl group at $\delta$ 5.87 ppm and two methoxyl groups at $\delta$ 3.91 ppm and 3.97 ppm, were observed. The fourth substituent was determined to be a propanoyl group due to an ethyl signal at $\delta$ 1.21 ppm (3H, t, H-9) and 2.94 ppm (2H, q, H-8) with a coupling constant of 7.2 Hz, which showed HMBC correlations with a carbonyl carbon at $\delta$ 199.8 ppm (C-7). The attachments were confirmed by the HMBC correlations between H-2, -6, and C-7, indicating that the propanoyl group was present on C-1; the HMBC correlations between the hydroxyl group at $\delta$ 5.87 ppm and H-2 indicated that the hydroxyl group was on C-3. Two methoxyl groups were present on C-4 and -5. Finally, the NOE correlation between a methoxyl group at $\delta$ 3.91 ppm (5-OCH$_3$) and H-6 and a methylene group at $\delta$ 2.94 ppm (H-8) and H-2, -6 confirmed that the structure of 3 was 3-hydroxy-4,5-dimethoxypropiophenone.

Compound 4 was isolated as a white solid and was confirmed to have the molecular formula C$_9$H$_{12}$NO$_4$ by the HR-FABMS signal at m/z 198.0767. From the $^1$H NMR spectrum (Table 1), the structure of 4 was determined to possess a mutually coupled ethylene group at $\delta$ 2.61 ppm (H-2') and 2.71 ppm (H-1'), a methyl group at $\delta$ 2.00 ppm (4-CH$_3$), and a methoxyl group at $\delta$ 3.67 ppm (3'-OCH$_3$). The HMBC correlation of the 3'-OCH$_3$ with a carbonyl C-3' ($\delta$ 172.5 ppm); H-1', H-2', and 4-CH$_3$ with accidently coinciding olefinic carbons C-3 and C-4 ($\delta$ 139.7 ppm); as well as a NOE correlation between 4-CH$_3$ and H-1' indicated a cis CH$_3$C=CH$_2$CH$_2$COOCH$_3$ fragment. The remaining two carbonyl carbon signals at $\delta$ 171.4 ppm (C-2) and 171.5 ppm (C-5) and a broad NH proton signal at $\delta$ 7.52 ppm as well as the HMBC correlations between H-1' and C-2 and 4-CH$_3$ and C-5 formed an imido -O=C-NHC=O- fragment. By combining these two fragments, the structure of 3-(methoxycarbonyl)ethyl-4-methyl-2,5-pyrroledione was thus deduced as methyl hematinate (4). This compound has been obtained from the photooxygenation of biliverdin [4]. However, this is the first time it has been isolated naturally.
Other known compounds were also isolated from *P. mishmensis*, including 44 known compounds. The compounds were six indoles: isatin (5) [5], 3,3-dimethoxyisatin (6) [6], 2-methoxycarbonylindolinderivative (7) [7], indirubin (8) [8], cephalinone C (9) [9,10], 3-methoxycarbonylindole (10) [11]; 10 quinazolines: 1H,3H-quinazoline-2,4-dione (11) [12], 3-(2'-hydroxyphenyl)-3H-quinazolin-4-one (12) [13], tryptanthrin (13) [2], phaitanthrin-A (14) [2], phaitanthrin-B (15) [2], phaitanthrin-C (16) [2], phaitanthrin-D (17) [2], phaitanthrin-E (18) [2], methylisatoid (19) [2], candidine (20) [2]; one phenanthrene: cephalene A (21) [10]; one imide: 3-ethyl-4-methylpyrrole-2,5-dione (22) [14] (Figure 2).

![Figure 2. Structures of these known compounds 5–22.](image)

Moreover, 23 monocyclic aromatic hydrocarbons, 2-aminobenzonitrile (23) [15], 2-(aminocarbonyl)phenylcarbamate (24) [10], methyl anthranilate (25) [16], benzoic acid (26) [17], 4-hydroxybenzaldehyde (27) [18], methyl 4-hydroxybenzoate (28) [18], 4-hydroxyacetophenone (29) [18], vanillin (30) [10], vanillic acid (31) [10], methyl vanillate (32) [17], syringaldehyde (33) [10], 2-methyl-4-nitrophenol (34) [19], pisoninol L (35) [20], dihydrocinnamic acid (36) [21], p-dihydrocoumaric acid (37) [10], methyl p-dihydrocoumarate (38) [22], fucosid (39) [17], cinnamic acid (40) [18], ferulic acid (41) [10], methyl ferulate (42) [10], trans-p-coumaric acid (43) [23], methyl trans-p-coumarate (44) [18], and methyl cis-p-coumarate (45) [24], as well as 3-oxo-α-ionol (46) [25], dehydroamifolol (47) [26], and methyl hydrogen succinate (48) [27] were also isolated (Figure 3).
2-Aminobenzonitrile (23) 2-(aminocarbonyl)phenylcarbamate (24) Methyl anthranilate (25) Benzoic acid (26)

4-Hydroxybenzaldehyde (27) Methyl 4-hydroxybenzoate (28) 4-Hydroxyacetophenone (29) Vanillin (30)

Vanillic acid (31) Methyl vanillate (32) Syringaldehyde (33) 2-Methyl-4-nitrophenol (34)

Pisoninol I (35) Dihydrocinnamic acid (36) p-Dihydrocoumaric acid (37) Methyl p-dihydrocoumarate (38)

Ficusol (39) Cinnamic acid (40) Ferulic acid (41) Methyl ferulate (42) trans-p-Coumaric acid (43)

Methyl trans-p-coumarate (44) Methyl cis-p-coumarate (45)

3-Oxo-α-ionol (46) Dehydrovamifoliol (47) Methyl hydrogen succinate (48)

Figure 3. Structures of monocyclic aromatic hydrocarbons 23–45 and compounds 46–48.
2.2. Cytotoxicity of Chemical Constituents of *P. mishmensis*

The partitioned *n*-hexane, CHCl₃, and EtOAc extracts from the crude MeOH extract of *P. mishmensis* showed considerable cytotoxicities against the MCF-7, NCI-H460, and SF-268 cell lines (Table 2). Unfortunately, most of the isolated compounds, except tryptanthrin (13) and phaitanthrin-A (14) [28], did not exhibit significant cytotoxicity against the tested cell lines. This result suggested that the practically insoluble tryptanthrin (13) could disperse in organic layers during extraction.

**Table 2.** Percentage inhibition of four partitioned extracts from the MeOH extract of *P. mishmensis* toward three cancer cell lines.

| Extracts | % Inhibition at 20 µg/mL |
|----------|--------------------------|
|          | MCF-7 ¹                  | NCI-H460 ² | SF-268 ³  |
| *n*-hexane | 3                     | 1          | 25        |
| CHCl₃     | 2                     | 1          | 4         |
| EtOAc     | 1                     | 1          | 1         |
| H₂O       | 134                   | 90         | 114       |

¹ MCF-7 = human breast tumor cell line. ² NCI-H460 = human lung tumor cell line. ³ SF-268 = human central nervous system tumor cell line.

3. Materials and Methods

3.1. General

Optical rotations were measured on a Jasco DIP-370 digital polarimeter (JASCO, Tokyo, Japan). CD spectra were determined on a Jasco J-715 spectropolarimeter (JASCO, Tokyo, Japan). UV spectra were recorded on an Agilent 8453 spectrophotometer (Agilent Technologies, m, CA, USA). The IR spectra measured using a Nicolet Magna FT-IR spectrophotometer (Nicolet Instrument, Inc., Madison, WI, USA). The ¹H-, ¹³C-, and 2D NMR spectra were recorded on Bruker Avance 300, AMX 400, and Avance-500 FT-NMR spectrometers (Bruker, Karlsruhe, Germany) at room temperature. All chemical shifts (δ) are given in ppm using tetramethylsilane as an internal standard. Mass spectra were obtained on a VG 70-250S spectrometer by a direct inlet system (Micromass Corp., Manchester, UK).

3.2. Plant Material

Whole plants of *P. mishmensis* were collected from Nanto Hsien, Taiwan, in October 2003. The collection was authenticated by Professor Chang-Sheng Kuoh, Department of Life Sciences, National Cheng Kung University, Tainan, Taiwan. A voucher specimen (No. PLW-0304) was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

3.3. Extraction and Isolation

The air-dried *P. mishmensis* plants (3.5 kg) were extracted with MeOH (7 × 8 L) under reflux. The combined extracts were concentrated under reduced pressure to give a dark brown syrup. The syrup was suspended in H₂O and then partitioned successively with *n*-hexane, CHCl₃, and EtOAc. These concentrated layers were stored in a refrigerator at −20 °C before they were purified.

The concentrated *n*-hexane layer (81 g) was fractionated on a silica gel column by eluting with a gradient of *n*-hexane and Me₂CO (9:1, v/v to 100% Me₂CO) to obtain eight fractions. Fractions 1–5 were included fatty acids, chlorophylls, sitosterol, and stigmasterol. Fraction 6 was chromatographed on a silica gel column with *n*-hexane–EtOAc (4:1, v/v) to obtain 3-methoxycarbonylindole (10) (3.9 mg), phaindole (1) (3.4 mg), tryptanthrin (13) (total 350 mg), cephalthrene A (21) (2.4 mg), (⁷R,⁸R)-phaithrene (2) (18.1 mg), 3-hydroxy-4,5-dimethoxypropiophenone (3) (4.8 mg), and methyl ferulate (42) (6.8 mg).
The concentrated CHCl₃ layer (15 g) was chromatographed on a silica gel column by eluting with a gradient of CHCl₃ and MeOH (20:1, v/v to 100% MeOH) to yield seven fractions. After repeated chromatography on silica gel followed by preparative TLC, fraction 2 gave 2-methoxy carbonylindolin-3-one (7) (3.0 mg), tryptanthrin (13) (total 350 mg), phaitanthrin-A (14) (30.0 mg), candidine (20) (9.8 mg), 2-aminobenzonitrile (23) (2.9 mg), methyl anthranilate (25) (3.1 mg), and methyl vanillate (32) (1.2 mg). Fraction 3 yielded isatin (5) (2.7 mg), 3,3-dimethoxyisatin (6) (1.5 mg), tryptanthrin (13) (total 350 mg), methylisatoid (19) (1.8 mg), phaitanthrin-D (17) (11.8 mg), phaitanthrin-E (18) (2.0 mg), 3-ethyl-4-methylpyrrole-2,5-dione (22) (1.0 mg), methyl hematinate (4) (8.6 mg), 4-hydroxybenzaldehyde (27) (11.1 mg), methyl 4-hydroxybenzoate (28) (7.8 mg), 4-hydroxyacetophenone (29) (4.2 mg), vanillin (30) (31.3 mg), syringaldehyde (33) (12.2 mg), 2-methyl-4-nitrophenol (35) (0.5 mg), and methyl p-dihydrocoumarate (38) (54.4 mg) by eluting with n-hexane and Me₂CO (4:1, v/v to 100% Me₂CO). Fraction 4 was chromatographed on a silica gel column by eluting with a gradient of CHCl₃ and MeOH (50:1 to 20:1, v/v) to yield 3-(2′-hydroxyphenyl)-3H-quinazolin-4-one (12) (57.0 mg), tryptanthrin (13) (total 350 mg), phaitanthrin-C (16) (3.1 mg), 2-(aminocarbonyl)phenylcarbamate (24) (285.3 mg), benzoic acid (26) (1.3 mg), pisonol (35) (5.2 mg), dihydrocinamic acid (36) (6.1 mg), fiscol (39) (9.0 mg), cinnamic acid (40) (1.2 mg), 3-oxo-α-ionol (46) (2.4 mg), and dehydrovamifoliol (47) (2.5 mg). Fraction 5 was chromatographed on a silica gel column by eluting with a gradient of CHCl₃ and MeOH (50:1 to 4:1, v/v) to obtain indirubin (8) (36.8 mg), cephalinone C (9) (19.8 mg), tryptanthrin (13) (total 350 mg), phaitanthrin-B (15) (3.6 mg), methyl trans-p-coumarate (44) (0.7 mg), and methyl cis-p-coumarate (45) (0.5 mg).

The concentrated EtOAc layer (17 g) was chromatographed on a silica gel column by eluting with a gradient of CHCl₃ and MeOH (10:1, v/v) and 2% H₂O to yield six fractions. Fraction 2 was chromatographed on a silica gel column by eluting with a gradient of CHCl₃ and MeOH (50:1, v/v) to give tryptanthrin (13) (total 350 mg). Fraction 2 was separated on a silica gel column by eluting with a gradient of CHCl₃ and MeOH (50:1, v/v) to obtain tryptanthrin (13) (total 350 mg), vanillic acid (31) (55.3 mg), and methyl hydrogen succinate (48) (261.1 mg). Fraction 3 was chromatographed on a silica gel column by eluting with a gradient of CHCl₃ and MeOH (50:1, v/v) to yield p-dihydrocoumaric acid (37) (8.4 mg), trans-p-coumaric acid (43) (1.6 mg), and ferulic acid (41) (0.7 mg). In addition, a solid (345.5 mg) insoluble in CHCl₃ and MeOH was identified as 1H,3H-quinazoline-2,4-dione (11).

**Phaindole (1):** yellowish powder, UV (CHCl₃) λ_max (log ε) 254 (3.89), 320 (3.90), 412 (2.68) nm; IR (KBr) ν_max 3264, 1702, 1697, 1659, 1650, 1605 cm⁻¹; EIMS m/z (rel. int.) 471 (2, [M⁺]), 86 (100); HR-ESIMS m/z 472.1506 [M + H]⁺ (calcd for C₂₆H₂₂N₃O₆, 472.1506).

(7R,8R)-Phaithrene (2): colorless amorphous powders; [α]D = -18.5° (c 0.08, CHCl₃); UV (CHCl₃) λ_max (log ε) 240 (3.94), 282 (4.01) nm; IR (KBr) ν_max 3399, 1728, 1609 cm⁻¹; FABMS m/z (rel. int.) 743 (25, [M + H⁺]), 389 (100); HR-FABMS m/z 743.4883 [M + H]⁺ (calcd for C₂₇H₁₇O₇, 743.4886).

3-Hydroxy-4,5-dimethoxypropionophenone (3): colorless needles, UV (CHCl₃) λ_max (log ε) 238 (3.47), 274 (3.82) nm; IR (KBr) ν_max 3390, 1680 cm⁻¹; EIMS m/z (rel. int.) 210 (49, [M⁺]), 181 (100); HR-EIMS m/z 210.0894 [M⁺]⁺ (calcd for C₁₃H₁₂O₄, 210.0892).

**Methyl hematinate (4):** white powder; UV (MeOH) λ_max (log ε) 230 (3.80), 270 (3.06) nm; IR (film) ν_max 3295, 2955, 1776, 1731, 1714 cm⁻¹; FABMS m/z (rel. int.) 198 (18 [M + H]⁺, 22), 149 (100); HR-FABMS m/z 198.0767 [M + H]⁺ (calcd for C₉H₁₂NO₄, 198.0766).

### 3.4. Cytotoxicity Assay

The cytotoxicity assay was carried out according to a procedure described previously [29]. Carcinoma cells MCF-7 and SF-268 were maintained in DMEM (Dulbecco’s Modified Eagle Medium, Fisher Scientific, HyClone, Logan, UT, USA) and NCI-H460 were maintained in RPMI (Roswell Park Memorial Institute, MP Biomedicals, Inc., Solon, OH, USA) medium supplemented with 10% fetal...
bovine serum (Biological Industries Inc., Cromwell, CT, USA). Firstly, the MCF-7, NCI-H460, and SF-268 cells were plated at a density of $5 \times 10^3$ cells per well in 96-well plates overnight and then treated with different concentrations of the isolated compounds. After 48 h, MTS cell proliferation assay kit (Promega, Madison, WI, USA) was added to each well; then, the experiment was performed as the manufacturer recommended (Promega). The absorbance was measured at 490 nm on a MQX200R microplate reader (BioTek, Winooski, VT, USA).

4. Conclusions

Four new compounds, phaindole (1), ($7^R,8^R$)-phaithrene (2), methyl 3-hydroxy-4,5-dimethoxypropophenone (3), and methyl hematinate (4), and 44 known compounds were isolated from $P. mishmensis$. Most of the isolated compounds, except tryptanthrin (13) and phaitanthrin-A (14), did not exhibit any significant cytotoxicity against the MCF-7, NCI-H460, and SF-268 cell lines. Phaitanthrin-A (14), an aldol adduct of tryptanthrin with acetone, exhibited better solubility than compound 13 in commonly used solvents (e.g., chloroform, ethyl acetate, and methanol). Derivatives using tryptanthrin as a template are being produced in our laboratories to obtain tryptanthrin derivatives with better solubilities and stronger tumor-selective toxicities.

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Sample Availability: Samples of the compounds 1–17, 19–21, 23–34, 36–45, 47, and 48 are available from the authors.

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