Dearomatized Isoprenylated Acylphloroglucinol Derivatives with Potential Antitumor Activities from *Hypericum henryi*

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Abstract

A series of dearomatized isoprenylated acylphloroglucinol derivatives, hyperhenols A–E (1–5), as well as seven known analogues (6–12), were characterized from *Hypericum henryi*. Their structures were determined by combination of NMR, ECD spectroscopy, and X-ray diffraction analysis. Compounds 1 and 6–8 were tested to exhibit potential antitumor properties, of which 6 and 7 inhibited cell growth through inducing apoptosis and cell cycle arrest. In addition, these compounds could induce autophagy and PINK1/Parkin-mediated mitophagy in cancer cell lines, as well as suppress lung cancer A549 cells metastasis in vitro.

Keywords *Hypericum henryi* · Dearomatized isoprenylated acylphloroglucinols (DIAPs) · Apoptosis · Cell cycle arrest

1 Introduction

Natural phloroglucinol derivatives are widely distributed in Myrtaceae, Guttiferae, Euphorbiaceae, Aspidiaceae families as well as appeared in marine and microbial sources [1]. In which prenylated acylphloroglucinols are a special kind of hybrid natural products originated from a polyketide combined isoprenylation biosynthetic pathways, and were mainly reported from the plants of genus *Hypericum* and *Garcinia* in the family Guttiferae [2–4]. With their wide range of biological profiles and diverse molecular architectures exemplified by hyperforin [5], hypersubone B [6], hyperuralone A [7] and chinesins I/II [8], prenylated acylphloroglucinol derivatives have attracted great interest of chemists and pharmaceutists.

As a traditional folk medicine in China, *Hypericum henryi* has been used to treat hepatitis [9]. Previous investigations on this plant have reported structurally diverse polycyclic prenylated acylphloroglucinol derivatives (PPAPs) such as hyphenrones A–X [10–12]. As a part of systematic search on bioactive acylphloroglucinol derivatives, five new dearomatized isoprenylated acylphloroglucinols (DIAPs) derivatives, hyperhenols A–E (1–5) together with seven known analogues (6–12) were isolated from *H. henryi* (Fig. 1). In the bioactive study, compounds 1 and 6–8 were found to exhibit promising cytotoxic activities against three human cancer cell lines in vitro. And the further studies indicated...
of the 13C NMR spectroscopic data (Table 1) indicated the four methines, and ten quaternary carbons (three oxygen-27 carbon signals including seven methyls, six methylenes, prenyl and the methyl (δC 443.2803 [M − H] −, calcd. C27H39O5, 443.2803). The IR spectrum displayed bands Me-19 (δC 24.6, C-19) at C-5 was evidenced evidenced by HMBC correlations from Me-7 (δH 1.58) to C-8 (δC 151.0), C-5' (δC 47.1), and C-9' (δC 109.2); and from H2-9'' (δH 4.71, 4.67) to C-8' (δC 151.0), C-10' (δC 21.1), and C-5' (δC 47.1). The linkage of DIAP core and monoterpenoid moiety between C-1' and C-3 was evidenced by HMBC correlations from H-1' (δH 3.24) to

![Fig. 1 Structures of compounds 1-5](image)

compounds 6 and 7 could trigger autophagy, PINK1/Parkin-mediated mitophagy in cancer cell lines, and also suppress lung cancer A549 cells metastasis targeting Akt and coflin signaling pathways. In addition, 6 and 7 also displayed significant anti-proliferation activities by inducing apoptosis and cell cycle arrest. Herein, the isolation, structure elucidation, and bioactivities evaluation of these compounds were reported.

2 Results and Discussion

The MeOH extract was subjected to repeated column chromatography to yield five new DIAPs derivatives (1–5) together with seven known analogues hyphenone J (6) [13], hyphenone K (7) [13], hyphenone E (8) [12], hyphenone A (9) [12], hyphenone B (10) [12], hyphenone C (11) [12], and hyphenone D (12) [12].

Hyphenol A (1) was isolated as yellow oil and assigned molecular formula of C27H40O5 with 8 degrees of unsaturation by HRESIMS (m/z 443.2803 [M − H]−, calcd. C27H39O5, 443.2803). The IR spectrum displayed bands for hydroxy (3417 cm⁻¹) and carbonyl groups (1636 cm⁻¹). The 13C NMR data along with DEPT experiments showed 27 carbon signals including seven methyls, six methylenes, four methines, and ten quaternary carbons (three oxygenated tertiary carbons and two carbonyls). Detailed analysis of the 13C NMR spectroscopic data (Table 1) indicated the presence of an isoprenyl (δC 40.3, C-14; 120.5, C-15; 135.3, C-16; 26.2, C-17; 18.2, C-18), a DIAPs core including an enolized 1,3-diketone group (δC 199.0, C-6; 107.4, C-1; 191.0, C-2), an enolic double bonds (δC 111.1, C-3; 179.4, C-4), a carbonyl (δC 207.9, C-7), and a quaternary carbon at δC 54.7 (C-5) [12, 13]. The location of the mentioned isoprenyl and the methyl (δC 24.6, C-19) at C-5 was evidenced by the HMBC correlations from H2-14 (δH 2.62, 2.56) and Me-19 (δH 1.32) to C-4 (δC 179.4), C-5 (δC 54.7) and C-6 (δC 199.0) (Fig. 2). Besides the aforementioned DIAP moiety, the remaining 14 carbon signals can be attributed to a sec-buty1 group (δC 43.4, C-8; δC 17.1, C-9; δC 27.7, C-10; δC 12.4, C-11) and another C10 unit.

Comparison of 1H and 13C NMR data indicated that the C10 unit of 1 shared a similar structure with the monoterpenoid moiety of callistilone B [14], which was confirmed by the 1H-1H COSY correlations of H-3'/H-4'/H-5'/H-6'/H-1', accompanied by HMBC correlations from Me-7 (δH 1.18) to C-1' (δC 42.7), C-2' (δC 73.3), and C-3' (δC 41.0); from Me-10' (δH 1.58) to C-8' (δC 151.0), C-5' (δC 47.1), and C-9' (δC 109.2); and from H2-9'' (δH 4.71, 4.67) to C-8' (δC 151.0), C-10' (δC 21.1), and C-5' (δC 47.1). The linkage of DIAP core and monoterpenoid moiety between C-1' and C-3 was evidenced by HMBC correlations from H-1' (δH 3.24) to

| No | 1a | 2b | 3a | 4b | 5b |
|----|----|----|----|----|----|
| 1  | 107.4 | 107.5 | 106.3 | 106.5 | 108.2 |
| 2  | 191.0 | 189.0 | 190.8 | 189.2 | 188.8 |
| 3  | 111.1 | 112.4 | 112.1 | 103.3 | 103.3 |
| 4  | 179.4 | 176.6 | 177.4 | 170.6 | 171.2 |
| 5  | 54.7 | 57.6 | 54.6 | 52.4 | 57.0 |
| 6  | 199.0 | 195.3 | 198.4 | 196.4 | 195.2 |
| 7  | 207.9 | 195.5 | 208.5 | 206.8 | 196.3 |
| 8  | 43.4 | 139.2 | 36.8 | 41.7 | 139.3 |
| 9  | 17.1 | 127.6 | 19.2 | 16.4 | 127.8 |
| 10 | 27.7 | 127.7 | 19.5 | 26.8 | 128.0 |
| 11 | 12.4 | 130.6 | 11.8 | 131.0 |
| 12 | 127.7 | | | | |
| 13 | 127.6 | | | | |
| 14 | 40.3 | 38.5 | 38.8 | 38.0 | 37.3 |
| 15 | 120.5 | 119.0 | 120.7 | 118.8 | 119.3 |
| 16 | 135.3 | 133.7 | 135.2 | 134.2 | 134.0 |
| 17 | 26.2 | 18.1 | 18.2 | 25.9 | 26.2 |
| 18 | 18.2 | 25.9 | 26.1 | 18.1 | 26.2 |
| 19 | 24.6 | 38.0 | 25.6 | 24.0 | 38.4 |
| 20 | 119.3 | | | | |
| 21 | 134.0 | | | | |
| 22 | 25.9 | | | | |
| 23 | 18.1 | | | | |
| 24 | 42.7 | 35.1 | 36.2 | 35.5 | 16.3 |
| 25 | 73.3 | 74.8 | 76.0 | 40.5 | 43.3 |
| 26 | 41.0 | 33.2 | 34.2 | 86.8 | 88.4 |
| 27 | 27.9 | 21.2 | 22.2 | 15.2 | 27.1 |
| 28 | 47.1 | 32.8 | 33.9 | 22.2 | 37.8 |
| 29 | 32.2 | 31.0 | 32.3 | 47.0 | 49.1 |
| 30 | 28.0 | 23.5 | 24.2 | 26.9 | 23.0 |
| 31 | 151.0 | 78.0 | 79.3 | 143.5 | 145.7 |
| 32 | 109.2 | 28.3 | 27.2 | 23.8 | 19.4 |
| 33 | 21.1 | 26.6 | 28.6 | 111.6 | 111.8 |

a Recorded in CD3OD
b Recorded in CDCl3
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C-2 (δC 191.0), C-3 (δC 111.1), and C-4 (δC 179.4) (Fig. 2). Finally, the sec-butyl group only can be attached at C-1.

The relative configuration of monoterpenoid moiety was determined by detailed interpretation of the ROESY spectrum. The NOE correlations of H-5′/H-1′ and H-1′/Me-7′ indicated that H-5′, H-1′ and Me-7′ were on the same side. However, due to the rotation of carbon–carbon single bond (C-3/C-1′) between DIAP core and monoterpenoid, determination of the configuration of C-5 is still challenging. For instance, an analogue of 1 (hyperhenone E, 8), has also been reported with the configuration of C-5 undefined [12]. In this study, hyperhenone E, as well as its crystals, was fortunately obtained, which unambiguously determined absolute configurations of 8 as 5S, 1′R, 2′R, 5′S (Fig. 3). Furthermore, the absolute configurations of C-5, C-1′, C-2′ and C-5′ in 1 were also determined to be the same with those of 8 via their well-matched ECD curves (Fig. 4).

Hyperhenol B (2) was obtained as yellow oil. A molecular formula of C33H42O5, was deduced by its 13C NMR and HRESIMS (m/z 519.3106 [M + H]+, calcd. C33H43O5 519.3105). The 1H and 13C NMR spectra of 2 and hyperhenone F are closely similar to each other [12]. Comparative analyses of their NMR data revealed that the isopropyl in hyperhenone F was replaced by a phenyl, which was supported by the HMBC correlations from H-9/H-13 (δH 7.43) to C-7 (δC 195.5) and C-8 (δC 139.2) in 2 (Fig. 2). Because of the rigidity of the bicyclic skeleton, cyclohexane moiety tended to form boat conformation. Hence, the ROESY correlations of H-1′/H-3′ (δH 1.96), H-4′/H-6′ (δH 2.10), and H-6′/H-1′ showed the same orientations of H-1′ and Me-7′ (Fig. 5).

Hyperhenol C (3) exhibited a molecular formula of C26H38O5, as assigned by HRESIMS (m/z 429.2653 [M − H]−, calcd. C26H37O5, 429.2646). The NMR spectra of 3 showed a close resemblance to those of hyperhenone F except that the signals for the isoprenyl at C-5 in
of H2O between hydroxyls of monoterpenoid and DIAPs degree of unsaturation [8], which could be derived from the loss C-2; its HRESIMS data (m/z 427.2855 [M + H]+, calcd. C27H39O4) showed molecular formulas (C27H38O4 for chinesin Ι; C27H38O4 for chinesin Ι) suggested that they shared closely similar plane 4 structures [8].

A comparison of the 1D NMR data of Fig. 5 ([12], which hyperhenone F was replaced by a methyl in 3) [12], which can be further confirmed by the HMBC correlations from Me-19 (δH 1.35) to C-5 (δC 103.3, C-3; δC 189.2; C-2; δC 170.6 C-4; δC 52.4 C-5; δC 196.4 C-6). A comparison of the 1D NMR data of 4 with those of chinesin Ι suggested that they shared closely similar plane structures [8]. The molecular formulas (C27H39O4 for chinesin Ι; C27H38O4 for 4) revealed that 4 possessed one more degree of unsaturation [8], which could be derived from the loss of H2O between hydroxyls of monoterpenoid and DIAPs core of chinesin Ι to afford 4. The ether linkage of C-4 and C-3′ was evidenced by indices of hydrogen deficiency, the downfield chemical shift of C-3′ (δC 86.8) and the ROESY correlation of Me-7/Me-17. The relative configurations of C-2′, C-3′, and C-6′ were elucidated by key ROESY correlations of Me-7′/H-2′, Me-7′/H-6′, and H-2′/H-6′. Unfortunately, the configuration of C-5 also could not be determined since the absence of sufficient evidence.

Hyperhenol E (5) was obtained as yellow oil, and its HRESIMS spectrum (m/z 501.3008 [M + H]+, calcd. C33H41O4 501.2999) showed a molecular formula of C33H40O4. The 1H NMR data of 5 (Table 2) exhibited a monosubstituted benzene (δH 7.30, 3H; 7.38, 2H), two isoprenyl (δH 4.75, t, J = 7.2 Hz; δH 4.81, t, J = 7.2 Hz). The NMR spectra of 5 showed a close resemblance to those of 4 except for the replacements of the sec-butyl group at C-7 and the methyl at C-5 in 4 by a phenyl and an isoprenyl in 5, respectively. This conclusion was verified via the 1H–1H COSY cross peak of H2-19/H-20 combined with the HMBC correlations of H2-19 (δH 7.2 Hz) and C-6 (δC 38.8). The similar NOE correlations of H-6′/H-3′ (δH 1.70), H-3′/H-4′ (δH 2.09), and H-4′/H-6′ (δH 1.61) in the ROESY spectrum, the obvious NOE correlation between Me-7′/H-10 (δH 4.02) and H-2′/H-10 (δH 1.61) showed that relative configurations were the same as those of 2 (Fig. 5). In the absence of sufficient evidence, configuration of C-5 could not be determined.

Hyperhenol D (4) was obtained as yellow oil. The molecular formula was established as C27H39O4 based on its HRESIMS data (m/z 427.2855 [M + H]+, calcd. C27H38O4 427.2843), implying 9 indices of hydrogen deficiency. The characteristic information for a DIAPs core was clearly observed in the 13C NMR spectra (δC 106.5, C-1; δC 189.2, C-2; δC 103.3, C-3; δC 170.6 C-4; δC 52.4 C-5; δC 196.4 C-6). A comparison of the 1D NMR data of 4 with those of chinesin Ι suggested that they shared closely similar plane structures [8]. The molecular formulas (C27H39O4 for chinesin Ι; C27H38O4 for 4) revealed that 4 possessed one more degree of unsaturation [8], which could be derived from the loss of H2O between hydroxyls of monoterpenoid and DIAPs core of chinesin Ι to afford 4. The ether linkage of C-4 and C-3′ was evidenced by indices of hydrogen deficiency, the downfield chemical shift of C-3′ (δC 86.8) and the ROESY correlation of Me-7/Me-17. The relative configurations of C-2′, C-3′, and C-6′ were elucidated by key ROESY correlations of Me-7′/H-2′, Me-7′/H-6′, and H-2′/H-6′. Unfortunately, the configuration of C-5 also could not be determined since the absence of sufficient evidence.

In the searching for their anticancer properties, compounds 1 and 6–8 were found to effectively inhibit cell growth in HeLa, A549, and MDAMB-231 cell lines (Table 3). Of which 6 and 7 could significant inhibit cancer cells growth with the IC50 up to 0.07 and 0.09 μM, respectively. Both the two compounds could also obviously increase mitochondrial fission and further activated the caspase-3, caspase-9, and increased PARP cleavage in HeLa cells (Fig. 6a, c). Treatment with 6 and 7 also increased the percentage of cells in G0/G1 phase and decreased in G2/M phase (Fig. 6b). Moreover, western blot results indicated that these two compounds efficiently suppressed the expression of cyclin D1 and Cdk 6 in HeLa cells, suggesting 6 and 7 induced cell cycle arrest. (Fig. 6c). Taken together, these

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**Fig. 4** Experimental ECD spectra of 1 and 8

**Fig. 5** Key ROESY correlations of 2, 4 and 5

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results demonstrated that these compounds inhibited cell growth through inducing apoptosis and cell cycle arrest.

Table 2 The $^1$H (600 MHz) NMR data of compounds 1–5 (δ in ppm and J in Hz)

| No | 1$^a$ | 2$^b$ | 3$^a$ | 4$^b$ | 5$^a$ |
|----|-------|-------|-------|-------|-------|
| 8  | 3.87, m | 3.98, sept (6.9) | 3.86, m |
| 9  | 1.09, d (6.6) | 1.11, d (6.9) | 1.10, d (6.9) | 7.30, overlap |
| 10 | 1.81, overlap | 1.10, d (6.9) | 1.68, overlap | 7.38, overlap |
| 11 | 0.89, m | 7.43, overlap | 7.38, overlap |
| 12 | 7.35, overlap | 7.38, overlap |
| 13 | 7.43, overlap | 7.30, overlap |
| 14 | 2.62, m | 2.61, overlap | 2.63, d (7.8) | 2.62, m | 2.57, m |
| 15 | 4.83, overlap | 4.88, overlap | 4.78, t (7.2) | 4.72, t (7.2) | 4.75, t (7.2) |
| 16 | 1.54, s | 1.58, s | 1.58, s | 1.54, s | 1.58, s |
| 17 | 1.58, s | 1.47 s | 1.51, s | 1.54, s | 1.55, s |
| 18 | 1.32, s | 2.61, overlap | 1.35, s | 1.28, s | 2.57, m |
| 19 | 4.88, overlap | 1.93, overlap | 1.92, dd (12.0, 6.5) |
| 20 | 1.37 s | 1.53, s |
| 21 | 2.61, overlap | 1.53, s |
| 1$'$ | 3.24, m | 3.35, m | 3.39, m | 1.81, m | 2.47, d (8.2) |
| 2$'$ | 1.96, m | 2.00, m | 1.92, dd (12.0, 6.5) |
| 3$'$ | 1.81, overlap | 1.68, overlap | 1.70, m |
| 4$'$ | 1.81, overlap | 1.68, overlap | 1.73, m | 1.68, overlap |
| 5$'$ | 2.09, m | 1.47, m | 1.61, overlap | 1.93, overlap | 2.03, ms |
| 6$'$ | 1.92, m | 2.10, m | 2.12, m | 2.79, m | 2.24, m |
| 7$'$ | 1.59, overlap | 1.59, overlap |
| 8$'$ | 1.18, s | 1.10, s | 1.45, s | 1.26, s |
| 9$'$ | 4.71, s | 1.37, s | 1.40, s | 1.76, s | 1.62, s |
| 10$'$ | 1.58, s | 1.30, s | 1.33, s | 4.93, s | 4.72, s |

$^a$Recorded in CD$_3$OD

$^b$Recorded in CDCl$_3$

Table 3 Cytotoxicity of the isolates on three cancer cell lines with IC$_{50}$ values (μM)

| Compound$^a$ | HeLa | A549 | MDA-MB-231 |
|--------------|------|------|-------------|
| 1            | 0.88 ± 0.042 | 3.53 ± 0.074 | 4.18 ± 0.43 |
| 6            | 0.07 ± 0.04 | 1.85 ± 0.18 | 1.37 ± 0.13 |
| 7            | 0.09 ± 0.099 | 3.10 ± 0.11 | 1.11 ± 0.06 |
| 8            | 0.89 ± 0.41 | 4.93 ± 1.08 | 22.16 ± 0.83 |
| Etoposide$^b$ | 9.46 ± 0.64 | 2.98 ± 1.08 | 31.31 ± 0.76 |

$^a$Other selected ones not listed in the table were inactive (IC$_{50}$ > 40 μM) for cell lines

$^b$Etoposide was used as positive controls

Autophagy is widely implicated in human diseases, offering a potential target for drug discovery [15]. Then, the effects of 6 and 7 on autophagy were assessed. GFP-LC3 puncta were significantly increased upon these compounds treatment (Fig. 7a). Western blot analysis showed that 6 and 7 inhibited autophagy, as assessed by the increased expression of LC3 II and P62 (Fig. 7b). Similar to CCCP (mitophagy inducer) treatment, 6 and 7 also increased the YFP-Parkin puncta formation (Fig. 7c). These data suggested that the compounds could induce PINK1/Parkin-mediated mitophagy. In addition, the antimetastasis effects
of these compounds were also studied. As shown in Fig. 8, wound healing and migration assay suggested 6 and 7 could efficiently suppress cell metastasis consistent with sorafenib (SFB) treated, which also decreased the expression of vimentin, p-AKT and cofilin (Fig. 8). Together, these results indicated that these isolates could suppress lung cancer A549 cells metastasis in vitro and may affect tumor metastasis targeted by Akt and cofilin signaling pathways.

In summary, five new and seven known DIAPs derivatives were isolated from H. henryi. Structurally, these compounds were characterized by a dearomatized isoprenylated acylphloroglucinol core combined a functionalized cyclohexane or cyclopentane skeleton. It is worthy to note...
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that several isolates exhibited significant cytotoxic activities in vitro. In addition, they also possess inducing autophagy, mitophagy, and anti-metastasis activities, which provided sufficient information on the potential application of these compounds on future drug development. Therefore, the finding of these DIAPs derivatives with potential antitumor properties may provide a new clue for the discovery of anti-tumor lead compounds, which should attract great interest from pharmacological and total synthetic communities.

3 Experimental

3.1 General Experimental Procedures

Optical rotations were measured on a Jasco P-1020 polarimeter. UV spectra were detected on a Shmadzu UV-2401PC spectrometer. IR spectra were determined on a Bruker FT-IR Tensor-27 infrared spectrophotometer with KBr disks. All 1D and 2D NMR spectra were recorded on Bruker DRX-600 spectrometers using TMS as an internal standard. Unless
otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESIMS and HRESIMS analysis were carried out on Waters Xevo TQS and Aglient G6230 TOF mass spectrometers, respectively. Semi-preparative HPLC was performed on an Aglient 1100 HPLC with a ZORBAX SB-C18 (9.4×250 mm) column and a Waters 2695 HPLC with a CHIRALCEL OJ-RH column [4.6×150 mm cellulose tris-(4-methylbenzoate) coated on 5 μM silica-gel]. Silica gel (100–200, 200–300 mesh, Qingdao Marine Chemical Co., Ltd., People’s Republic of China), and MCI gel (75–150 μM, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography. Fractions were monitored by TLC (GF 254, Qingdao Marine Chemical Co., Ltd.), and spots were visualized by heating silica gel plates sprayed with 10% H2SO4 in EtOH.

Fig. 8  

a Wound healing assay. A549 cells were treated with compounds 6 and 7 and positive control Sorafenib (20 μM) and monitored with an inverted microscope. b Cell migration was measured by transwell assays. The summary data for transwell migration assay was presented as the means ± S.D. Scale bars: 10 μM. c A549 cells were treated with compounds 4–7 for 24 h and then analyzed by western blot.
3.2 Plant Material

The plants of *Hypericum henryi* were collected in Dongchuan prefecture (Yunnan Province, People’s Republic of China) in September 2018. The plant was identified by ZHANG Yong-Zeng. A voucher specimen (No. 2018H01) was deposited in Kunming Institute of Botany.

3.3 Extraction and Isolation

The sample (20.0 kg) was extracted with MeOH at room temperature and filtered, and the solvent was evaporated in vacuo. The crude extract was subjected to silica gel chromatography eluted with CHCl₃ to afford a fraction (3.2 kg). The fraction was deposited in Kunming Institute of Botany.

3.3.1 Hyperhenol (1)

Yellow oil; [α] + 250.8 (c 0.35, MeOH); UV (MeOH) λ_max (log ε) 202 (4.14), 225 (4.10), 346 (4.02) nm; IR (KBr) ν_max 3417, 2968, 2932, 1636, 1520, 1462, 1379 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 519 [M + H]^⁺; HRESIMS m/z [M + H]^⁺; 519.3105 (calcd for C₃₃H₄₀O₅, 519.3105).

3.3.2 Hyperhenol (2)

Yellow oil; [α] + 53.8 (c 0.24, MeOH); UV (MeOH) λ_max (log ε) 203 (4.40), 227 (4.20), 343 (3.90) nm; IR (KBr) ν_max 3427, 2969, 2927, 1505, 1448, 1257, 1202 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 519 [M + H]^⁺; HRESIMS m/z [M + H]^⁺; 519.3106 (calcd for C₃₃H₄₀O₅, 519.3105).

3.3.3 Hyperhenol (3)

Yellow oil; [α] + 199.0 (c 0.31, MeOH); UV (MeOH) λ_max (log ε) 202 (4.08), 228 (3.98), 239 (3.97), 281 (3.83), 326 (3.91) nm; IR (KBr) ν_max 3431, 2970, 2932, 2878, 1651, 1522, 1470, 1437 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 429 [M − H]^−; HRESIMS m/z 429.2653 [M − H]^−; (calcd for C₂₉H₃₇O₅, 429.2646).

3.3.4 Hyperhenol (4)

Yellow oil; [α] + 5.3 (c 0.26, MeOH); UV (MeOH) λ_max (log ε) 320 (2.14), 275 (2.30), 241 (2.42), 197 (2.50), 310 (2.17), 269 (2.30), 215 (2.34) nm; IR (KBr) ν_max 3422, 2970, 2935, 2876, 1657, 1618, 1462, 1379 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 427 [M + H]^⁺; HRESIMS m/z 427.2855 [M + H]^⁺ (calcd for C₂₇H₃₈O₄, 426.2843).

3.3.5 Hyperhenol (5)

Yellow oil; [α] − 45.0 (c 0.12, MeOH); UV (MeOH) λ_max (log ε) 354(2.40), 287(2.17), 231(2.53), 197(2.94), 300(2.15), 276(2.16), 228(2.53), 193(2.81) nm; IR (KBr) ν_max 3429, 2967, 2926, 2854, 1727, 1659, 1622, 1587, 1512, 1448 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 501 [M + H]^⁺; HRESIMS m/z 501.3008 [M + H]^⁺ (calcd for C₂₇H₃₈O₄, 501.2999).

3.3.6 X-ray Crystallographic Analysis of Hyperhenone E (8)

C₂₇H₃₉O₅, M = 430.56, a = 22.6496(4) Å, b = 9.4550(2) Å, c = 23.8898(4) Å, α = 90°, β = 94.6410(10)°, γ = 90°, V = 5099.27(16) Å³, T = 100(2) K, space group P21, Z = 8, μ (CuKa) = 0.609 mm⁻¹, 56,500 reflections measured, 17,671 independent reflections (R_int = 0.0269). The final R₁ values were 0.0363 (I > 2σ(I)). The final wR² values were 0.0766 (I > 2σ(I)). The final R₁ values were 0.0366 (all data). The final wR² values were 0.0971 (all data). The goodness of fit on F² was 1.056. Flack parameter = 0.022. Crystallographic data for the structure of 8 have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CDCC 1,941,889).

3.3.7 Cell Culture

HeLa cells, GFP-LC3 HeLa cells, YFP-Parkin HeLa cells and A549 cells were maintained in DMEM (Gibco,
3.3.7.1 MTT Assay and Determination of IC₅₀
The cells were seeded in a 96-well tissue culture plate at a predetermined density in 100 μL of complete medium, attached overnight, and then treated with a series of concentrations of compound for 72 h. At the end of the incubation period, 10 μL MTT solution was added into each well of a 96-well plate for 4 h at 37°C. After the medium was removed, 100 μL DMSO was added to dissolve the purple crystals. After shaking for 5 min, the optical densities at 490 nm were measured using a Microplate Reader.

3.3.7.2 MitoTracker Red Staining
HeLa cells were seeded on coverslips and treated with compounds 6 and 7 for 48 h. We then removed the media from the dish and added staining solution containing MitoTracker red (100 nM) incubation 30 min at 37 °C. The cells were fixed with 4% PFA in PBS for 15 min and observed using a fluorescence microscope.

3.3.7.3 Flow Cytometry Analysis
HeLa cells were treated with various concentrations of 7 and 8 for 48 h. Subsequently, the cells were harvested, washed with PBS and fixed with 70% alcohol at 4 °C overnight. Then cells were washed with PBS and stained with 20 μg/mL PI/RNase staining buffer for 30 min and analyzed using a fluorescence microscope (Becton Dickinson, USA).

3.3.8 Immunofluorescence Microscopy
The GFP-LC3 or YFP-Parkin HeLa cells were treated with compounds for the indicated time point, and then the cells were fixed with 4% PFA in PBS for 15 min at room temperature. The cells were observed under a fluorescence microscope (Olympus, IX83).

3.3.9 Wound Healing Assay
Wound healing was used to evaluate cell motility as our previous study [16]. Briefly, A549 cells were seeded into a 24-well culture plate. When the cells grew to 90% confluence, then a scratch was gently created through the cell monolayer by sterile 10 μL pipette tips and loose cells were washed away. The cell migration was observed and imaged under an IX83 microscope for each condition and timepoint (0, 48 h). (Olympus, Tokyo, Japan).

3.3.10 Cell Migration Assay
Cell migration assay were performed as described previously [17]. In brief, cell migration was estimated using transwell chambers (Millicell, Germany) with a pore size of 8 μM. For the migration assay, 4.5×10⁴ A549 cells resuspended in 100 μL serum-free medium were seeded in the upper chamber with serum-containing medium in the lower chamber of 24-well transwell plates (BD Biosciences, San Jose, CA). After 24 h, the experiment was terminated by wiping the cells from the wells with a cotton swab and fixed and stained with 0.05% crystal violet for 20 min, scored under a light microscope in five random fields.

3.3.11 Western Blotting Analysis
Cells were harvested and lysed in a lysis buffer (62.5 mM Tris at pH 6.8, 20% glycerol, 2% SDS, phosphatase inhibitor), proteins were separated on SDS polyacrylamide gels and transferred to PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% nonfat milk, and immunoblotted with primary antibodies at 4 °C overnight. After washed three times with TBST, membranes were incubated for 1 h with appropriate secondary antibodies at room temperature. The follow antibodies were used in our experiments: Caspase-3 (CST, 9662), Cleaved-caspase-3 (CST, 9661), Caspase-9 (CST, 9502), PARP (CST, 9542), LC3 (Sigma, L7543), P62 (BML, PM045), PINK1 (CST, 6946), Tim23 (BD, 611222), Tom20 (sc-17764), E-cadherin (CST, 3195), Vimentin (CST, 5741), pAKT (Ser473, CST, 9171), AKT (CST, 9272), Cofilin (CST, 5175) and GAPDH (CST, 5174). GAPDH was used as the loading control. Membranes were visualized with Image Quant LAS 4000 (General Electric Company).

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Compliance with Ethical Standards
Conflict of interest All authors declare no conflict of interest.

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