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Structural elucidation, total synthesis, and cytotoxic activity of effphenol A†

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A highly substituted phenol derivative, effphenol A (1), was isolated from the deep-sea-derived fungus *Trichobotrys effuse* FS524. Its complete structural assignment was established through a combination of spectroscopic analysis together with single-crystal X-ray diffraction experiments and further unequivocally confirmed by a biomimetic total synthesis. Structurally, effphenol A possesses a poly-substituted 6-5/6/6 tetracyclic ring system, which represents the first case of such a skeleton found in nature. Furthermore, the cytotoxic activity of effphenol A (1) toward four human cancer cell lines was assayed.

Natural products have enjoyed tremendous growth in the repertoire of pharmaceutical and agrochemical industries and scientific investigations, and have been especially exemplified in acting as pharmacologically attractive lead compounds in biomedical research and clinical application for the assembly of novel therapeutic drugs.[1] Recent advances have demonstrated that natural products and their related derivatives could still behave as a powerful vehicle to fuel drug development, especially the natural products derived from microorganisms, plants, vertebrates, and invertebrates.[1,2] Among them, on account of complex genomes, marine fungi are more likely to produce natural molecules with rich varieties, novel structures, and remarkable activities,[3–6] thus providing more prodrug molecules and model structures for new drug development.[7–10]

As part of our continuing research program to discover structurally novel and biologically significant natural products from marine sources,[11–14] the organic extraction of *Trichobotrys effuse* were originally selected for chemical investigation due to its excellent anti-proliferative activity. Our previous chemical investigations on this marine fungus have led to the isolation of a number of phenol derivatives.[15] In the current study, a highly substituted phenol derivative, effphenol A (**1**), was characterized from the deep-sea-derived fungus FS524. Notably, effphenol A possessed an intriguing 6-5/6/6 tetracyclic ring system with a poly-substituted skeleton, which represents the first case of such a skeleton found in nature. Herein, we reported the isolation, full structural determination, biomimetic total synthesis, and biological activity of effphenol A.

Effphenol A was isolated as a dark red crystal, and its molecular formula was confirmed as $C_{26}H_{26}O_7$ on the basis of HRESIMS data (*m*/*z* 451.1739 $[M + H]^+$, calcd 451.1751), implying fourteen indices of hydrogen (IHDs). The ^{1}H NMR spectrum of effphenol A (Table 1) showed four aromatic proton signals at δ_H 7.50 (2H, d, J = 8.8 Hz, H-13, 17) and 6.76 (2H, d, J = 8.8 Hz, H-14, 16), ascribed to the presence of a *para*-substituted phenyl moiety. Moreover, two other aromatic protons appearing at δ_H 6.31 (1H, s, H-2) and 6.20 (1H, s, H-22), two methoxy protons at δ_H 3.56 (3H, s, H_3-7) and 3.53 (3H, s, H_3-26), and two methylene protons at δ_H 2.90 (2H, m) and 2.67 (2H, q, J = 7.3 Hz) assigned as H_2-8 and H_2-24, together with two methyl groups at δ_H 1.30 (3H, t, J = 7.5 Hz, H_3-9) and 1.11 (3H, t, J = 7.3 Hz, H_3-25) were also observed. The ^{13}C NMR data coupled with the HSQC spectrum resolved 25 carbon signals including thirteen quaternary carbons (eight oxygenated ones), six methines, two methylenes, and four methyls (two oxygenated ones). As twelve of the fourteen IHDs were accounted for by the three phenyl moieties, the remaining two IHDs indicated that effphenol A possessed an additional double bond with the formation of a fused ring system.

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Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **1** in CD_3COCD_3

	1			**1**	
No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
1		153.5, C	14	6.76 (d, 8.8)	116.0, CH
2	6.31 (s)	95.8, CH	15		157.9, C
3		153.5, C	16	6.76 (d, 8.8)	116.0, CH
4		106.7, C	17	7.50 (d, 8.8)	127.5, CH
5		154.9, C	18		102.2, C
6		114.3, C	19		155.3, C
7	3.56 (s)	56.0, CH_3	20		110.4, C
8	2.90 (q, 7.5)	17.3, CH_2	21		156.6, C
9	1.30 (t, 7.5)	14.6, CH_3	22	6.20 (s)	92.3, CH
10		—[a]	23		157.8, C
11		150.3, C	24	2.67 (q, 7.4)	17.2, CH_2
12		124.5, C	25	1.11 (t, 7.4)	14.6, CH_3
13	7.50 (d, 8.8)	127.5, CH	26	3.53 (s)	55.6, CH_3

[a] Not detected.

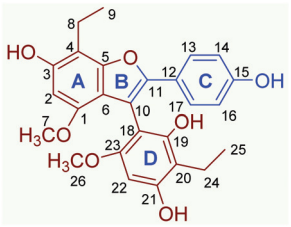


Fig. 1 Structure of effphenol A (**1**).

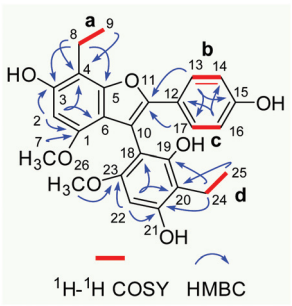


Fig. 2 ^{1}H–^{1}H COSY and key HMBC correlations of **1**.

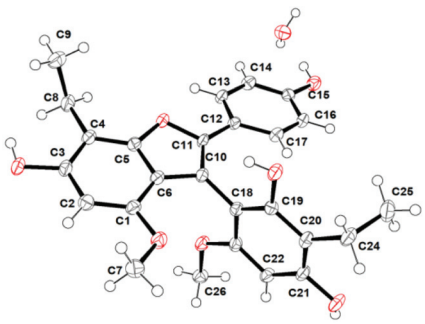


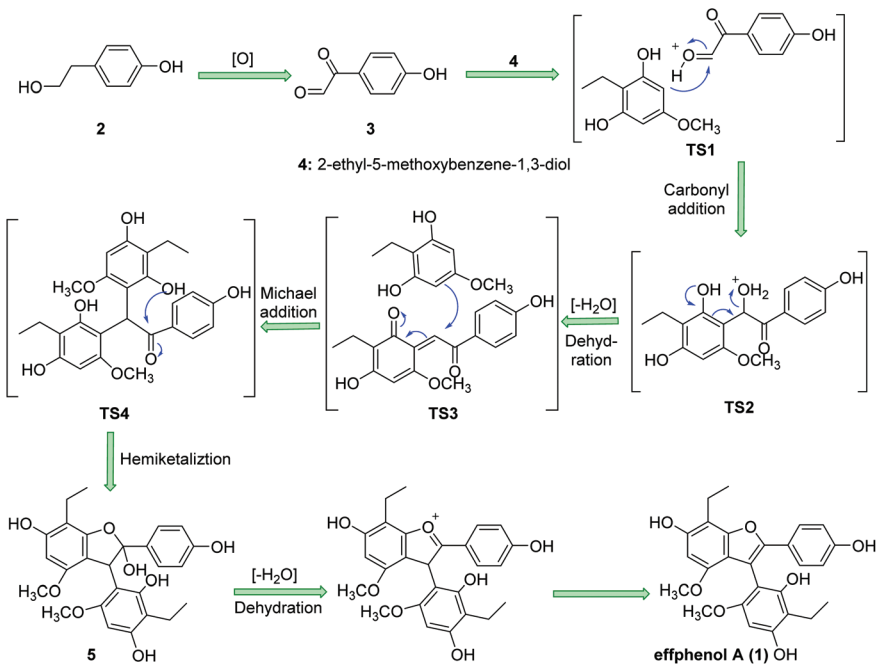
Fig. 3 X-ray crystallographic analysis of **1**.

Comprehensive interpretation of the 2D NMR spectra of **1** allowed the establishment of its planar structure. Readily, four spin systems **a** (H_2-8/H_3-9), **b** (H-13/H-14), **c** (H-16/H-17), and **d** (H_2-24/H_3-25) could be confirmed by the ^{1}H–^{1}H COSY correlations (Fig. 2). Moreover, the observed HMBC correlations from H-2 to C-1, C-3, C-4, and C-6; H_3-7 to C-1; H_2-8 to C-3, C-4, and C-5; and H_3-9 to C-4 and C-8 in conjunction with the additional spin system **a** suggested the presence of the pentasubstituted benzene ring A. Similarly, the pentasubstituted benzene ring D was also confirmed by the critical HMBC correlations from H-22 to C-18, C-20, C-21, and C-23; H_2-24 to C-19, C-20, and C-21; and H_3-25 to C-20 and C-24 along with the spin system **d**. Furthermore, the HMBC correlations from H-13 to C-11, C-14, C-15, and C-17, and H-14 to C-12, C-15, C-16, and C-17 along with the spin systems **b** and **c** logically confirmed the presence of a *para*-substituted phenyl moiety (ring C).

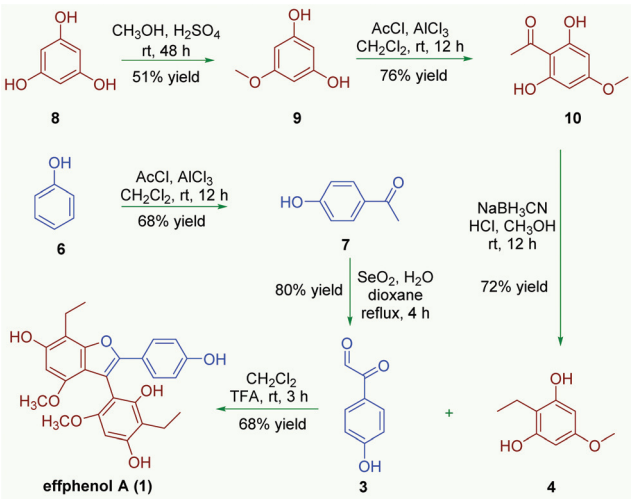
However, there were many quaternary carbons existing far away from the related conclusive protons, which resulted in the lack of necessary HMBC signals, thus bringing an unexpected challenge to distinguish the connection of ring A, ring C, and ring D. Fortunately, a single crystal of the natural product **1** suitable for X-ray diffraction analysis (Fig. 3) was obtained from a methanol/water mixture solvent system, which provided conclusive evidence for furan ring B and the linkage of C-10/C-18 and C-11/C-12. Thus, the structure of **1** with a natural 6/5-6-6 tetracyclic ring system was defined as shown in Fig. 1 and given the trivial name as effphenol A.

To the best of our knowledge, effphenol A (**1**) is the first example of a new class of benzofuran natural products with a poly-substituted skeleton. Regarding the fact that such a skeleton exactly like effphenol A has never been reported before, a plausible biogenetic pathway was proposed as shown in Scheme 1. Effphenol A might biogenetically originate from the bio-precursors 4-hydroxy-benzeneethanol and the phloroglucinol derivative **4**, which have also been discovered or disclosed as structurally constitutional units for many related natural products of *T. effuse* FS524 in our previous report.[15] In the proposed biosynthesis pathway, it mainly incorporated with a key spontaneous carbonyl addition/dehydration/Michael addition/hemiketalization cascade process[16] from **3** and **4** to furnish the rapid construction of the target scaffold for effphenol A through the transition states **TS1–TS4**, thus generating the pivotal intermediate **5**. Therefore, the substituted 2,3-dihydrobenzofuran **5** underwent dehydration to offer the targeted product effphenol A.

The novel structure and interesting biosynthetic pathway of effphenol A have fascinated us to explore its first biomimetic total synthesis. As shown in Scheme 2, aldehyde **3** could be directly derived through SeO_2-mediated oxidation[17] in 80% yield from 4′-hydroxyacetophenone **7**, which could be readily prepared from the commercially available phenol **6** through $AlCl_3$-mediated Friedel–Crafts acylation.[18] Meanwhile, the synthesis of phloroglucinol derivative **4** commenced with phloroglucin **8**, which could undergo methylation to afford phenol **9**



Scheme 1 Proposed biogenetic pathway of effphenol A.



Scheme 2 Total synthesis of effphenol A.

in a moderate yield. Further subjection of phenol **9** to Friedel–Crafts acylation followed by reduction using conditions modified from the literature[19] led to the desired phloroglucinol derivative **4** in about 55% overall yield for two steps. With the desired aldehyde **3** and phloroglucinol derivative **4** in hand, the sequential carbonyl addition/dehydration/Michael addition/hemiketalization tandem reaction was explored. Gratifyingly, the key reaction did proceed, successfully delivering the desired product **1** in 68% yield, which was then proved to be identical to effphenol A in all aspects.

Subsequently, cytotoxic evaluation against four human cancer cell lines SF-268 (human glioblastoma carcinoma), MCF-7 (breast cancer), HepG-2 (liver cancer), and A549 (lung cancer) was also tested with cisplatin as the positive control. Ultimately, it turned out that effphenol A (**1**) exhibited moderate activities against four human cancer cell lines with IC_{50} values ranging from 30.1 to 43.3 μM, while the values of cisplatin were 2.5–3.2 μM.

In conclusion, effphenol A (**1**), a new phenol derivative with a fascinating 6-5/6/6 tetracyclic ring system, was isolated from the deep-sea-derived fungus *T. effuse* FS524. Moreover, an efficient biomimetic total synthesis of **1** has been achieved, which confirmed the structure proposed by a combination of spectroscopic analysis, chemical techniques, and single-crystal X-ray diffraction experiments. Additionally, the cytotoxic activity of **1** toward four human cancer cell lines was assayed with moderate anti-proliferative activity. This research not only provided an efficient strategy and inspiration for the synthesis of similar phenol derivatives to enrich the structural library of these natural products but also shed light on a potential biosynthesis process for similar natural products.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 (*a*) G. M. Cragg, P. G. Grothaus and D. J. Newman, *Chem. Rev.*, 2009, **109**, 3012–3043; (*b*) D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2012, **75**, 311–335; (*c*) D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2020, **83**, 770–803; (*d*) D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2016, **79**, 629–661.

2 K. Zlotkowski, W. M. Hewitt, P. Yan, H. R. Bokesch, M. L. Peach, M. C. Nicklaus, B. R. O'Keefe, J. B. McMahon, K. R. Gustafson and J. S. Schneekloth Jr., *Org. Lett.*, 2017, **19**, 1726–1729.

3 G. M. Cragg and D. J. Newman, *Biochim. Biophys. Acta*, 2013, **1830**, 3670–3695.

4 (*a*) J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. G. Munro and M. R. Prinsep, *Nat. Prod. Rep.*, 2016, **33**, 382–431; (*b*) J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. G. Munro and M. R. Prinsep, *Nat. Prod. Rep.*, 2017, **34**, 235–294; (*c*) Y. Buijs, P. K. Bech, D. Vazquez-Albacete, M. Bentzon-Tilia, E. C. Sonnenschein, L. Gram and S. D. Zhang, *Nat. Prod. Rep.*, 2019, **36**, 1333–1350.

5 Y. H. Gui, W. H. Jiao, M. Zhou, Y. Zhang, D. Q. Zeng, H. R. Zhu, K. C. Liu, F. Sun, H. F. Chen and H. W. Lin, *Org. Lett.*, 2019, **21**, 767–770.

6 H. Wen, X. Yang, Q. Liu, S. Li, Q. Li, Y. Zang, C. Chen, J. Wang, H. Zhu and Y. Zhang, *J. Nat. Prod.*, 2020, **83**, 99–104.

7 C. Jiménez, *ACS Med. Chem. Lett.*, 2018, **9**, 959–961.

8 A. R. Carroll, B. R. Copp, R. A. Davis, R. A. Keyzers and M. R. Prinsep, *Nat. Prod. Rep.*, 2020, **37**, 175–223.

9 J. W. Blunt, A. R. Carroll, B. R. Copp, R. A. Davis, R. A. Keyzers and M. R. Prinsep, *Nat. Prod. Rep.*, 2018, **35**, 8–53.

10 W. H. Jiao, J. Li, M. M. Zhang, J. Cui, Y. H. Gui, Y. Zhang, J. Y. Li, K. C. Liu and H. W. Lin, *Org. Lett.*, 2019, **21**, 6190–6193.

11 S. Chen, Z. Liu, H. Tan, Y. Chen, S. Li, H. Li, S. Zhu, H. Liu and W. Zhang, *Org. Chem. Front.*, 2020, **7**, 557–562.

12 Z. Liu, Q. Wang, S. Li, H. Cui, Z. Sun, D. Chen, Y. Lu, H. Liu and W. Zhang, *J. Nat. Prod.*, 2019, **82**, 3440–3449.

13 S. Chen, Z. Liu, H. Tan, Y. Chen, S. Zhu, H. Liu and W. Zhang, *Org. Biomol. Chem.*, 2020, **18**, 642–645.

14 S. Chen, Z. Liu, Y. Chen, H. Tan, S. Zhu, H. Liu and W. Zhang, *Tetrahedron Lett.*, 2020, **61**, 151555.

15 S. Chen, Z. Liu, Y. Chen, H. Tan, S. Li, H. Liu, W. Zhang and S. Zhu, *Mar. Drugs*, 2020, **18**, 134.

16 G. X. He, J. M. Yuan, H. M. Zhu, K. Wei, L. Y. Wang, S. L. Kong, D. L. Mo, C. X. Pan and G. F. Su, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 1660–1664.

17 H. M. Kuo, Y. L. Chen, G. H. Lee and C. K. Lai, *Tetrahedron*, 2016, **72**, 6843–6853.

18 A. J. Grenning, J. H. Boyce and J. A. Porco, *J. Am. Chem. Soc.*, 2014, **136**, 11799–11804.

19 D. W. Chen, R. D. Chen, R. S. Wang, J. H. Li, K. B. Xie, C. C. Bian, L. L. Sun, X. L. Zhang, J. M. Liu, L. Yang, F. Ye, X. M. Yu and J. G. Dai, *Angew. Chem., Int. Ed.*, 2015, **54**, 12678–12682.

