



**HAL**  
open science

## **A new phthalide derivative from *Peperomia nivalis***

Pedro Vásquez-Ocmín, Mohamed Haddad, Alice Gadea, Valérie Jullian, Denis Castillo, Lucie Paloque, Juan Pablo Cerapio, Geneviève Bourdy, Michel Sauvain

► **To cite this version:**

Pedro Vásquez-Ocmín, Mohamed Haddad, Alice Gadea, Valérie Jullian, Denis Castillo, et al.. A new phthalide derivative from *Peperomia nivalis*. *Natural Product Research*, 2017, 31 (2), pp.138-142. 10.1080/14786419.2016.1219857 . ird-01381569

**HAL Id: ird-01381569**

**<https://ird.hal.science/ird-01381569>**

Submitted on 17 Oct 2016

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## RESEARCH ARTICLE

## A new phthalide derivative from *Peperomia nivalis*

Pedro Vásquez-Ocmín<sup>a</sup>, Mohamed Haddad<sup>b</sup>, Alice Gadea<sup>a</sup>, Valérie Jullian<sup>b</sup>, Denis Castillo<sup>a</sup>, Lucie Paloque<sup>c,d</sup>, Juan Pablo Cerapio<sup>a</sup>, Geneviève Bourdy<sup>b</sup> and Michel Sauvain<sup>b</sup>

<sup>a</sup>Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru; <sup>b</sup>UMR 152 Pharma Dev, Université de Toulouse, IRD, UPS, France; <sup>c</sup>CNRS, LCC (Laboratoire de Chimie de Coordination) UPR8241, Toulouse, France; <sup>d</sup>Université de Toulouse, Toulouse, France

### ABSTRACT

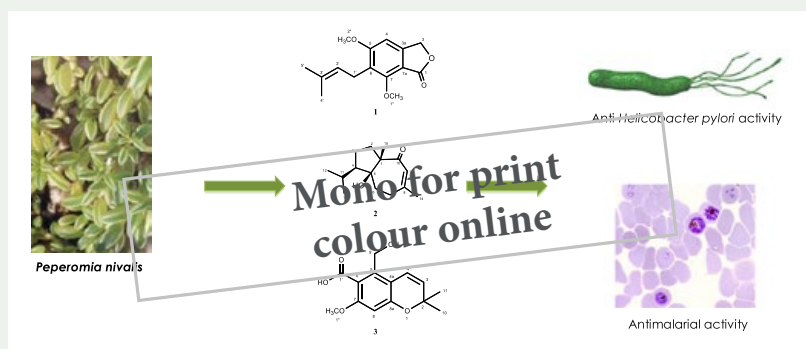
One new phthalide (**1**) was isolated from aerial parts of *Peperomia nivalis*, along with known compounds (**2** and **3**), reported in this species for the first time. The structure of the new compound was characterised on the basis of 1D (<sup>1</sup>H and <sup>13</sup>C NMR), 2D (COSY, HMQC, HMBC and NOESY) NMR and high-resolution mass spectral (HRMS) data. Compound **2** was isolated from a natural source for the first time but previously synthesised. Compounds **1–3** were evaluated for their anti-*Helicobacter pylori* and anti-*Plasmodium falciparum* activities. Compound **1** showed moderate activities against *H. pylori* (MIC 47.5 μM) and the F32-Tanzania strain of *P. falciparum* (IC<sub>50</sub> 8.5 μM). Compounds **2** and **3** exhibited weak anti-*H. pylori* activity (MIC 241.3 and 237.6 μM, respectively) and were inactive against *P. falciparum*.

### ARTICLE HISTORY

Received 22 January 2016  
Accepted 7 July 2016

### KEYWORDS

*Peperomia nivalis*; phthalide; secondary metabolites; NMR; *Helicobacter pylori*; *Plasmodium*



## 1. Introduction

*Peperomia*, a genus of the pepper family (Piperaceae), is one of the two large genera of the Piperaceae family, with more than 1500 recorded species of tropical and subtropical fleshy herbs, annuals as well as perennials. The *Peperomia* genus is the second most abundant source of bioactive compounds in the Piperaceae family (López et al. 2010). From this genus,

**CONTACT** Mohamed Haddad ✉ [mohamed.haddad@ird.fr](mailto:mohamed.haddad@ird.fr)

Supplemental data for this article can be accessed at <http://dx.doi.org/10.1080/14786419.2016.1219857>.

© 2016 Informa UK Limited, trading as Taylor & Francis Group

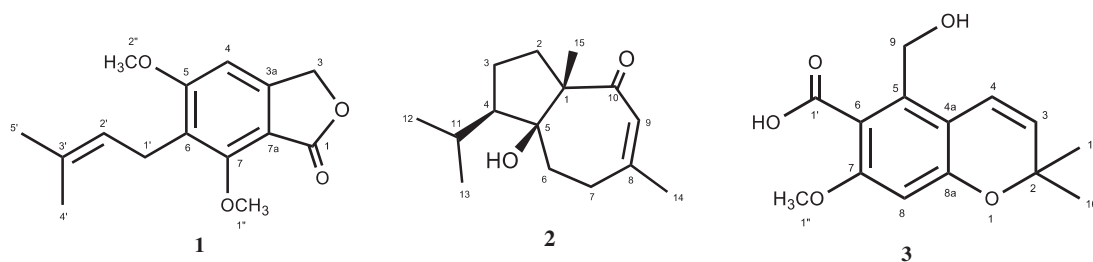
previous works have reported the isolation of bioactive compounds such as lignans, lactones and phenolic (Li et al. 2006; Mahiou et al. 1995). In Peru, numerous species of the *Peperomia* genus, such as *Peperomia galioides*, *P. congona* and *P. hartwegiana*, are used in traditional medicines to treat stomach problems (de la Cruz et al. 2014) or for their antibacterial activity (*P. galioides*) (Langfield et al. 2004; De Feo et al. 2008). However, some compounds such as prenylated phenols with antiparasitic activity were reported from *P. galioides* (Mahiou et al. 1995, 1996). In Cameroon, *P. vulcanica* and *P. fernandopoiana* are used as medicinal plants by traditional medicine practitioners to treat febrile illnesses (Ngemenya et al. 2015). Ngemenya et al. (2004) investigated *P. vulcanica* and observed moderate *in vitro* activity against *Plasmodium falciparum* (70% inhibition by 40 µg/mL crude extract).

Within the scope of our research line on isolation, structural characterisation and evaluation of the bioactivity of secondary metabolites from peruvian medicinal plants (Ruiz et al. 2011; Haddad et al. 2013; Cabanillas et al. 2015; Girardi et al. 2015), we investigated aerial parts of *Peperomia nivalis*. This plant, endemic to Peru, grows in rocky sunny places in high-altitude Andean areas and is used in traditional medicine to treat stomach ulcers, gastritis, internal pain and sensation of burning stomach (Pino et al. 2005). Phytochemical investigation led to the isolation of a new phthalide derivative along with two known compounds (2, 3). Compounds 1–3 were evaluated for their anti-*Helicobacter pylori* and anti-*P. falciparum* activities. To the best of our knowledge, this is the first phytochemical report on *P. nivalis*.

## 2. Results and discussion

Compound 1 (C<sub>15</sub>H<sub>18</sub>O<sub>4</sub>, HR-TOF-MS) was obtained as a white-coloured solid with molecular formula requiring seven degrees of unsaturation. It exhibited UV absorption bands at 248 and 301 nm, while the IR spectrum displayed characteristic absorption bands for a γ-lactone ring at 1760 cm<sup>-1</sup> and for alkene at 1619 and 1601 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum (Table S1) exhibited resonances for one aromatic (6.44, 1H, s) proton, two equivalent oxymethylene protons (δ<sub>H</sub> 5.12, 2H, s), one olefinic methine at δ<sub>H</sub> 5.09 (m), two methoxyl groups (δ<sub>H</sub> 4.00, 3H, s and 3.95, 3H, s), two equivalent allylic methylene protons at δ<sub>H</sub> 3.23 (d, *J* = 7.2 Hz) and two signals indicating terminal methyl at δ<sub>H</sub> 1.74 (3H, d, *J* = 0.9 Hz) and δ<sub>H</sub> 1.71 (3H, d, *J* = 1.4 Hz), respectively. The <sup>13</sup>C NMR spectrum (Table S1) showed one ester carbonyl (δ<sub>C</sub> 169.5), six resonances in the aromatic region (δ<sub>C</sub> 163.3, 158.4, 148.7, 115.6, 105.4 and 94.6), two olefinic (δ<sub>C</sub> 132.9 and 120.8), one methylene (δ<sub>C</sub> 68.0), two methoxy (δ<sub>C</sub> 56.1, 56.0), one allylic (δ<sub>C</sub> 24.4) and two methyl (δ<sub>C</sub> 25.7 and 17.7) carbons. Inspection on key HMBC correlations (Figure 1) as well as comparison with the literature data suggested the substitution of the aromatic cycle by two methoxyl groups and one alkenyl chain (Li et al., 2006). These data were confirmed by the HMBC correlations observed between H-1' (δ<sub>H</sub> 3.23, d) and C-6 (δ<sub>C</sub> 115.6) and C-5 (δ<sub>C</sub> 163.3) that established the connectivity of the alkenyl chain at C-6, in addition to those of H-1'' (δ<sub>H</sub> 4.00, 3H, s) and C-7 (δ<sub>C</sub> 158.4) and between H-2'' (δ<sub>H</sub> 3.95, 3H, s) and δ<sub>C</sub> 163.3 suggesting that the methoxyl groups are located at C-7 and C-5, respectively.

On the basis of the above results and by comparison of its NMR data with the literature values published for 5,7-dimethoxy-6-(3-methylbut-2-enyl)isobenzofuran-1(3H)-one (Li et al., 2006), and with other phthalides previously isolated from another genus (Li et al. 2012; Venditti et al. 2015), the structure of peperophthalide A was elucidated as 5,7-dimethoxy-6-(3-methylbut-2-enyl)-isobenzofuran-1(3H)-one (1).



**Figure 1.** Chemical structures of compounds 1–3.

15 Since *P. nivalis* is used in traditional medicine to treat stomach ulcers, gastritis, internal pain and sensation of burning stomach and since Marilone A, a phthalide derivative isolated from the sponge-derived fungus *Stachylidium* sp., and some chromenes were shown to have antiplasmodial activity against *Plasmodium berghei* (Oshimi et al. 2009; Almeida et al. 2011) and strong antiprotozoal activity (Harel et al. 2013), compounds 1–3 were evaluated for their  
20 anti-*H. pylori* and anti-*P. falciparum* activities.

Compound 1 was the most effective product against both biological models used in this work. It showed a moderate activity against *H. pylori* (MIC 47.5  $\mu$ M) and against the F32-Tanzania strain of *P. falciparum* (IC<sub>50</sub> 8.5  $\mu$ M), while compounds 2 and 3 exhibited weak anti-*H. pylori* activity (MIC 241.3 and 237.6  $\mu$ M, respectively) and were shown to be inactive against  
25 *P. falciparum*. According to the WHO guidelines, antiplasmodial activity was classified as weak at  $10 < IC_{50} < 100$   $\mu$ M. These results are in accordance with previous reports. On the one hand, previous screening programme designed to discover active compounds against *H. pylori* allowed the isolation of seven phthalide antibiotics with specific anti-*H. pylori* activity (Dekker et al. 1997; Radcliff et al. 2008). On the other hand, the antiplasmodial activity of peperophthalide 1 (IC<sub>50</sub> 8.5  $\mu$ M) was found to be close to the antiplasmodial activity of the phthalide derivative Marilone 1, isolated from the sponge-derived fungus *Stachylidium* sp.,  
30 with an IC<sub>50</sub> of 12.1  $\mu$ M against *P. berghei* (Almeida et al. 2011). Nevertheless, Sommart et al. (2012) isolated seven new phthalide derivatives, microsphaerophthalides A–G, which were shown to be inactive against *P. falciparum* (K1, multidrug-resistant strain). Furthermore, compound 3, a known chromene derivative, was shown to exhibit strong antifungal activity against *Cladosporium cladosporioides* (Malquichagua Salazar et al. 2005). In addition, some chromene derivatives, isolated from the genus *Peperomia* (Batista et al. 2011), and some chromenes containing structure, have shown to have a strong bacteriostatic activity against *Staphylococcus aureus* (Starks et al. 2014) and strong antiprotozoal activity (Harel et al. 2013).  
35 However, chromene 3 exhibited only a weak anti-*H. pylori* activity and no activity against *P. falciparum*. AQ11

### 5 3. Conclusion

Investigation of *P. nivalis* aerial part extract afforded the isolation of one new phthalide 1 derivative together with two known compounds. Compound 2 is reported here for the first time as a natural compound, and compound 3 has never been isolated before in *P. nivalis*. We reported for the first time the *in vitro* anti-*H. pylori* and anti-*P. falciparum* activity of *P. nivalis* derivatives. The moderate activity of compound 1 against *H. pylori* can be linked to the traditional use of the plant to treat stomach problem.

## Supplementary material

Experimental details relating to this paper are available online.

## Acknowledgement

15 The authors acknowledge the financial support of the LMI-LaVi (UPCH-IRD), for the development of this work, and the help of the Herbario at the Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (UNMSM) for plant determination.

## Disclosure statement

20 No potential conflict of interest was reported by the authors

## Funding

25 This work was supported by the LMI-LaVi (UPCH-IRD); Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (UNMSM).

## References

- 30
- 35 Almeida C, Kehraus S, Prudêncio M, König GM. 2011. Marilones A–C, phthalides from the sponge-derived fungus *Stachylidium* sp. *Beilstein J Org Chem*. 7:1636–1642. AQ12
- Batista JM Jr, Batista AN, Mota JS, Cass QB, Kato MJ, Bolzani VS, Freedman TB, López SN, Furlan M, Nafie LA. 2011. Structure elucidation and absolute stereochemistry of isomeric monoterpene chromane esters. *J Org Chem*. 76:2603–2612.
- 40 Benoit-Vical F, Lelièvre J, Berry A, Deymier C, Dechy-Cabaret O, Cazelles J, Loup C, Robert A, Magnaval J-F, Meunier B. 2007. Trioxaquinones are new antimalarial agents active on all erythrocytic forms including gametocytes. *Antimicrob Agents Chemother*. 51:1463–1472. AQ13
- Cabanillas B, Vásquez-Ocmín P, Zebiri I, Rengifo E, Sauvain M, Le HL, Vaisberg A, Voutquenne-Nazabadioko L, Haddad M. 2015. A new 5-alkylresorcinol glucoside derivative from *Cybianthus magnus*. *Nat Prod Res*. 16:1–6.
- 5 De Feo V, Juarez BA, Guerrero SJ, Senatore F, Formisano C. 2008. Antibacterial activity and composition of the essential oil of *Peperomia galioides* HBK (Piperaceae) from Perú. *Nat Prod Commun*. 3:1–5.
- Dekker KA, Inagaki T, Gootz TD, Kaneda K, Nomura E, Sakakibara T, Sakemi S, Sugie Y, Yamauchi Y, Yoshikawa N, Kojima N. 1997. CJ-12,954 and its congeners, new anti-*Helicobacter pylori* compounds produced by *Phanerochaete velutina*: fermentation, isolation, structural elucidation and biological activities. *J Antibiot*. 50:833–839.
- 10 **de la Cruz GM, Malpartida SB, Santiago HB, Jullian V, Bourdy G. 2014.** Hot and cold: medicinal plant uses in Quechua speaking communities in the high Andes (Callejón de Huaylas, Ancash, Perú). *J Ethnopharmacol*. 155:1093–1117.
- 15 Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob Agents Chemother*. 16:710–718. AQ14
- Ghisalberti EL. 1994. The daucane (carotane) class of sesquiterpenes. *Phytochemistry*. 37:597–623. AQ15
- Girardi C, Fabre N, Paloque L, Ramadani AP, Benoit-Vical F, González-Aspajo G, Haddad M, Rengifo E, Jullian V. 2015. Evaluation of antiplasmodial and antileishmanial activities of herbal medicine *Pseudelephantopus spiralis* (Less.) Cronquist and isolated hirsutinolide-type sesquiterpenoids. *J Ethnopharmacol*. 170:167–174.
- 20 Haddad M, Le Lamer AC, Vasquez-Ocmin PV, Vaisberg A, Sauvain M, Castillo D, Rojas R. 2013. Cytotoxic 13,28-epoxy-oleanane triterpene saponins from *Cybianthus magnus* (Mez) Pipoly. *Phytochem Lett*. 6:128–133.
- 25
- 30

- Harel D, Schepmann D, Prinz H, Brun R, Schmidt J, Wünsch B. **2013**. Evaluation, and structure-activity relationships of novel chromene and chromane derivatives. *J Med Chem.* 56:7442–7448.
- Langfield RD, Scarano FJ, Heitzman ME, Kondo M, Hammond GB, Neto CC. **2004**. Use of a modified microplate bioassay method to investigate antibacterial activity in the Peruvian medicinal plant *Peperomia galioides*. *J Ethnopharmacol.* 94:279–281.
- 35 Li GH, Li L, Duan M, Zhang KQ. **2006**. The chemical constituents of the fungus *Stereum* sp. *Chem Biodivers.* 3:210–216. AQ16
- Li N, Wu J-L, Hasegawa T, Sakai J-U, Wang L-Y, Kakuta S, Furuya Y, Tomida A, Tsuruo T, Ando M. **2006**. Bioactive dibenzylbutyrolactone and dibenzylbutanediol lignans from *Peperomia duclouxii*. *J Nat Prod.* 69:234–239. AQ17
- 40 Li XN, Chen YY, Cheng DP, Tong SQ, Qu HP, Yan JZ. **2012**. Two phthalide dimers from the radix of *Angelica sinensis*. *Nat Prod Res.* 26:1782–1786.
- López SN, Lopes AA, Batista JJM, Flausino JO, Bolzani VDS, Kato MJ, Furlan M. **2010**. Geranylation of benzoic acid derivatives by enzymatic extracts from *Piper crassinervium* (Piperaceae). *Bioresour Technol.* 101:4251–4260.
- 45 López SN, Lopes AA, Batista JJM, Flausino JO, Bolzani VDS, Kato MJ, Furlan M. **2010**. Geranylation of benzoic acid derivatives by enzymatic extracts from *Piper crassinervium* (Piperaceae). *Bioresour Technol.* 101:4251–4260.
- Mahiou V, Roblot F, Hocquemiller R, Cave A, Barrios AA, Fournet A, Ducrot PH. **1995**. Peperogalin, a new prenylateddiphenol from *Peperomia galioides*. *J Nat Prod.* 58:324–328.
- 50 Mahiou V, Roblot F, Hocquemiller R, Cave A, De Arias AR, Inchausti A, Yaluff G, Fournet A. **1996**. New prenylated quinones from *Peperomia galioides*. *J Nat Prod.* 59:694–698.
- Malquichagua Salazar KJ, Delgado Paredes GE, Lluncor LR, Young MCM, Kato MJ. **2005**. Chromenes of polyketide origin from *Peperomia villipetiola*. *Phytochemistry.* 66:573–579.
- Ngemenya MN, Metuge HM, Mbah JA, Zofou D, Babiaka SB, Titanji VPK. **2015**. Isolation of natural product hits from *Peperomia* species with synergistic activity against Resistant *Plasmodium falciparum* strains. *Eur J Med Plants.* 5:77–87.
- Ngemenya MN, Tane P, Berzins K, Titanji VPK. **2004**. Antiplasmodial activity of some medicinal plants used in Cameroon: preliminary toxicity studies of highly active extracts; XIth Annual Conference of The Cameroon Bioscience Society 16–18 December, 2004.
- Oshimi S, Deguchi J, Hirasawa Y, Ekasari W, Widyawaruyanti A, Wahyuni TS, Zaini NC, Shiota O, Morita H. **2009**. Cassiarins C–E, antiplasmodial alkaloids from the flowers of *Cassia siamea*. *J Nat Prod.* 72:1899–1901.
- Pino G, Klopfenstein O, Cieza N. **2005**. Four new *Peperomia* from northern Peru. *Haseltonia.* 11:102–112.
- Radcliff FJ, Fraser JD, Wilson ZE, Heapy AM, Robinson JE, Bryant CJ, Flowers CL, Brimble MA. **2008**. Anti-*Helicobacter pylori* activity of derivatives of the phthalide-containing antibacterial agents spiroloxine methyl ether, CJ-12,954, CJ-13,013, CJ-13,102, CJ-13,104, CJ-13,108 and CJ-13,015. *Bioorg Med Chem.* 16:6179–6185.
- Ruiz C, Haddad M, Alban J, Bourdy G, Reategui R, Castillo D, Sauvain M, Deharo E, Estevez Y, Arevalo J, Rojas R. **2011**. Activity-guided isolation of antileishmanial compounds from *Piper hispidium*. *Phytochem Lett.* 4:363–366.
- Sommart U, Rukachaisirikul V, Tadpetch K, Sukpondma Y, Phongpaichit S, Hutadilok-Towatana N, Sakayaroj J. **2012**. Modiolin and phthalide derivatives from the endophytic fungus *Microsphaeropsis arundinis* PSU-G18. *Tetrahedron.* 68:10005–10010.
- Starks CM, Williams RB, Norman VL, Rice SM, O'Neil-Johnson M, Lawrence JA, Eldridge GR. **2014**. Antibacterial chromene and chromane stilbenoids from *Hymenocardia acida*. *Phytochemistry.* 98:216–222.
- Trager W, Jensen JB. **1976**. Human malaria parasites in continuous culture. *Science.* 193:673–675. AQ18
- Venditti A, Lattanzi C, Ornano L, Maggi F, Sanna C, Ballero M, Alvino A, Serafini M, Bianco A. **2015**. A new glucosidic phthalide from *Helichrysum microphyllum* subsp. *tyrrhenicum* from La Maddalena Island (Sardinia, Italy). *Nat Prod Res.* 30:789–795.
- Wiemer DF, Ales DC. **1981**. Lasidiol angelate: an ant repellent sesquiterpenoid from *Lasiantheae fruticosa*. *J Org Chem.* 46:5449–5450. AQ19