JOURNAL OF NATURAL PRODUCTS

pubs.acs.org/jnp

Article

1-(2′-Bromobenzyl)-6,7-dihydroxy-*N*-methyl-tetrahydroisoquinoline and 1,2-Demethyl-nuciferine as Agonists in Human D_2 Dopamine Receptors

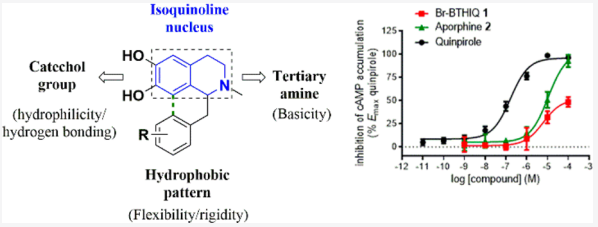
Andrea G. Silva, Laura Vila, Patrice Marques, Laura Moreno, Mabel Loza, María-Jesús Sanz, Diego Cortes,* Marián Castro,* and Nuria Cabedo*

Cite This: https://dx.doi.org/10.1021/acs.jnatprod.9b00921

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Certain D_2-like dopamine receptor (DR) agonists are useful therapeutically as antiparkinsonian drugs, whereas D_2-like DR antagonists or partial agonists are proven effective as antipsychotics. Two isoquinoline derivatives, 1-(2′-bromobenzyl)-6,7-dihydroxy-*N*-methyl-tetrahydroisoquinoline (Br-BTHIQ, **1**) and 1,2-demethyl-nuciferine (aporphine, **2**), were herein synthesized, and their dopaminergic affinity in cloned human D_2R, D_3R, and D_4R subtypes and their behavior as agonists/antagonists were evaluated. They showed affinity values (K_i) for hD_2, hD_3, and hD_4 DR within the nanomolar range. The trends in affinity were $hD_4R \gg hD_3R > hD_2R$ for Br-BTHIQ (**1**) and $hD_2R > hD_4R > hD_3R$ for 1,2-demethyl-nuciferine (**2**). The functional assays of cyclic adenosine monophosphate signaling at human D_2R showed a partial agonist effect for Br-BTHIQ (**1**) and full agonist behavior for aporphine (**2**), with half maximal effective concentration values of 2.95 and 10.2 μM, respectively. Therefore, both isoquinolines **1** and **2** have emerged as lead molecules for the synthesis of new therapeutic drugs that ultimately may be useful to prevent schizophrenia and Parkinson's disease, respectively.



Benzylisoquinoline alkaloids constitute a large structural group of secondary metabolites that are derived biosynthetically from tyrosine.[1] Condensation of dopamine and 4-hydroxyphenylacetaldehyde by norcoclaurine synthase produces (*S*)-norcoclaurine, which is considered the first 1-benzylisoquinoline alkaloid and the common precursor to isoquinoline derivatives, including 1-benzylisoquinolines and aporphines.[2] They are present mainly in Papaveraceae, Menispermaceae, Berberidaceae, and Ranunculaceae, among other plant families.[3] *Nelumbo nucifera* Gaertn., also called Asian lotus or sacred lotus, belongs to the family Nelumbonaceae. Its leaves and flower buds are an important source of aporphines (e.g., nuciferine and *N*-methylasimilobine) and 1-benzylisoquinoline alkaloids (e.g., armepavine and *N*-methylcoclaurine) (Figure 1).[3] These alkaloids have displayed a variety of biological activities, including antioxidant,[4] antimicrobial,[5] antiplatelet,[6] antidiabetic, antihyperlipidemic,[7] and antihypertensive[8] effects. Nuciferine has been described as producing an antipsychotic-like effect in rodents that is attributed, at least in part, to partial agonist activity on dopaminergic receptors (DRs).[9] In addition, the interaction with DRs of aporphines and 1-benzylisoquinolines of natural or synthetic origin has been extensively reported.[10] For instance, apomorphine (Figure 1), a semisynthetic derivative from morphine with dopaminergic agonist activity and selectivity for D_2-like DR, has some use in treating Parkinson's disease.[11]

Dopamine (3,4-dihydroxyphenethylamine) is one of the most important neurotransmitters involved in the control of essential functions, including locomotor activity, cognition, emotion, and memory. This catecholamine exerts its effects by acting on five subtypes of DRs, D_1−D_5, encoded in humans by genes DRD1−DRD5, respectively. DRs belong to the seven transmembrane G protein-coupled receptor (GPCR) families and are classified into two major classes, D_1-like DR (D_1 and D_5) and D_2-like DR (D_2, D_3, and D_4), based on the ability to regulate cyclic adenosine monophosphate (cAMP) production.[12] The D_2R subtype has two isoforms, D_{2S} (D_2 short) and D_{2L} (D_2 long), which differ in the presence of 29 additional amino acids in the third intracellular loop, whereas D_4R has many variants.[13,14] D_2-like DR subtypes share a high degree of sequence homology (D_2R shares 75 and 53% with D_3R and D_4R, respectively), with differences only in their tissue expression pattern, density, and physiological functions.[14] For this reason, despite the crystal structure elucidation of D_2R,[15] D_3R,[16] and D_4R[17] and the greatly improved design of ligands, it is difficult to obtain selective compounds for each receptor subtype. D_1-like DRs are coupled to G_s proteins, and they

Received: September 24, 2019

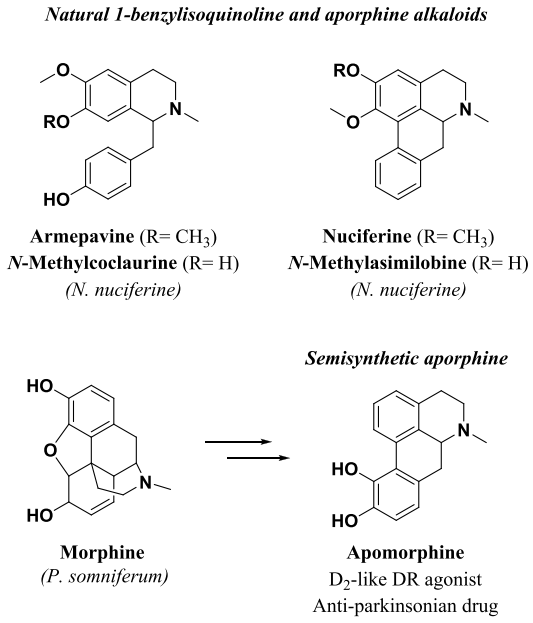


Figure 1. Natural and semisynthetic isoquinoline derivatives.

increase intracellular cAMP through adenylate cyclase (AC) activation, which results in the subsequent activation of protein kinase A (PKA). In contrast, D_2-like DRs are coupled to $G_{i/o}$ proteins, and they downregulate cAMP generation through AC inhibition, which leads to diminished PKA activity.[12,14] D_2-like DRs are implicated in numerous brain functions, including regulation of locomotor activity, cognition, and motivation.[13,14] This subfamily is clinically important as therapeutic targets for antiparkinsonian (agonists) and antipsychotic (antagonists or partial agonists) drugs.[13,14]

In the last 2 decades, our group has reported the synthesis and structure−activity relationship (SAR) studies of isoquinoline derivatives with affinity for D_1- and D_2-like DRs in rat striatal membranes.[18−25] These studies were supported by molecular modeling studies, which revealed the importance of the catechol group, secondary/tertiary amine, and hydrophobic substitution pattern in the isoquinoline nucleus to improve the affinity for each DR subfamily (Figure 2).[26−31] In previous studies into rat striatal membranes, it was determined that both 1-(2′-bromobenzyl)-6,7-dihydroxy-*N*-methyl-THIQ (Br-BTHIQ, **1**) and 1,2-demethyl-nuciferine (aporphine, **2**) showed high selectivity toward D_2-like DR.[25] In the present

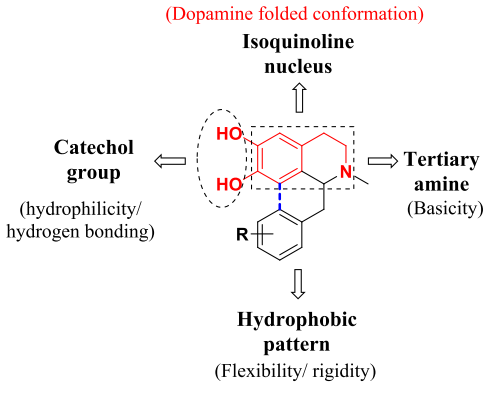


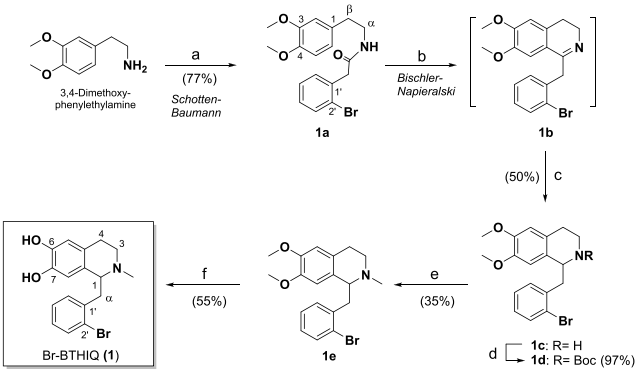
Figure 2. Structural features of D_2-like dopaminergic isoquinolines.

work, Br-BTHIQ (**1**) and aporphine (**2**) were synthesized to evaluate (i) their dopaminergic affinity for the cloned human D_2R, D_3R, and D_4R subtypes and (ii) their potential D_2-like DR agonist/antagonist behavior. These results should allow further lead compounds to be developed toward useful potential agents in neurological and/or neuropsychiatric disorders.

RESULTS AND DISCUSSION

Chemistry. The synthesis of 1-(2′-bromobenzyl)-6,7-dihydroxy-*N*-methyl-tetrahydroisoquinoline (**1**) and 1,2-demethyl-nuciferine (**2**) was carried out as previously reported.[25] The synthetic route is outlined in Schemes 1 and 2. Initially,

Scheme 1. Synthesis of 1-(2′-Bromobenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydro-isoquinoline (Br-BTHIQ, 1)[a]



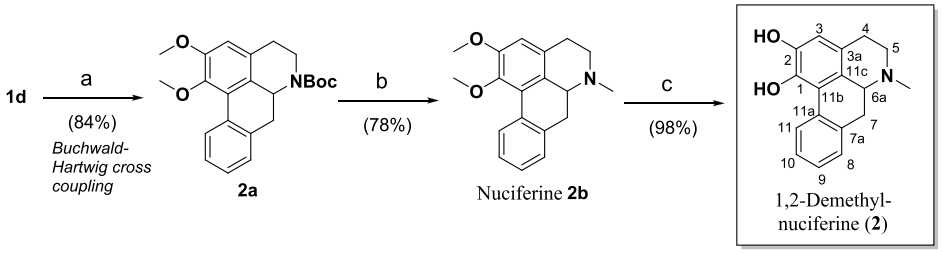
[a]Reaction conditions: (a) 2-$BrC_6H_4CH_2COCl$, CH_2Cl_2, 5% aqueous NaOH, room temperature, 16 h; (b) $POCl_3$, CH_3CN, N_2, reflux, 1 h; (c) $NaBH_4$, CH_3OH, N_2, room temperature, 2 h; (d) $[(CH_3)_3COCO]_2O$, CH_2Cl_2, room temperature, 2 h; (e) $LiAlH_4$, tetrahydrofuran (THF), reflux, 4 h; and (f) BBr_3, CH_2Cl_2, N_2, room temperature, 2 h.

the appropriate *β*-phenyl acetamide (**1a**) intermediate was prepared under Schotten−Baumann conditions. Next, Bischler−Napieralski cyclodehydration gave the isoquinoline nucleus bearing an imine function, which was reduced to give the corresponding brominated BTHIQ (**1c**). In a first approach, a reaction sequence of N-protection followed by carbamate reduction and subsequent O-demethylation gave brominated *N*-methyl-BTHIQ (**1**) (Scheme 1). In a second approach, the brominated *N*-Boc-BTHIQ (**1d**) intermediate was subjected to a Buchwald−Hartwig cross-coupling reaction toward carbon−carbon bond formation. Therefore, palladium-catalyzed direct arylation[32] with 2′-(diphenylphosphino)-*N*,*N*′-dimethyl-(1,1′-biphenyl)-2-amine (PhDave-Phos) and Pd-$(OAc)_2$ generated a good yield of *N*-Boc *nor*-nuciferine (**2a**). The carbamate-protecting group of compound **2a** was reduced to obtain nuciferine (**2b**), which was O-demethylated with boron tribromide to give 1,2-demethyl-nuciferine (**2**) (Scheme 2).

Affinity of Isoquinolines 1 and 2 for the Human D_2R, D_3R, and D_4R Subtypes. The *in vitro* binding affinities of the synthesized isoquinolines, 1-(2′-bromobenzyl)-6,7-dihydroxy-*N*-methyl-tetrahydroisoquinoline (Br-BTHIQ, **1**) and 1,2-demethyl-nuciferine (aporphine, **2**) were evaluated at the cloned human D_2R, D_3R, and D_4R subtypes. For this purpose, competition radioligand-binding assays were conducted on cell membranes stably expressing the three cloned human receptor

https://dx.doi.org/10.1021/acs.jnatprod.9b00921
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of 1,2-Demethyl-nuciferine (Aporphine, 2)[a]



[a]Reaction conditions: (a) PhDave-Phos, $Pd(OAc)_2$, K_2CO_3, DMA, 130 °C, 4 h; (b) $LiAlH_4$, THF, reflux, 4 h; and (c) BBr_3, CH_2Cl_2, N_2, room temperature, 2 h.

Table 1. Affinity of Isoquinolines 1 and 2 in Human D_2R, D_3R, and D_4R

	hD_2R[a]			hD_3R[a]			hD_4R[a]		
compound	pK_i	K_i (nM)	percent inhibition at 10 μM (%)	pK_i	K_i (nM)	percent inhibition at 10 μM (%)	pK_i	K_i (nM)	percent inhibition at 10 μM (%)
Br-BTHIQ (1)	6.54 ± 0.08	286	95 ± 1	6.71 ± 0.09	197	93 ± 1	7.86 ± 0.05	13.8	96 ± 1
aporphine (2)	6.47 ± 0.03	340	91 ± 1	6.08 ± 0.03	838	93 ± 1	6.26 ± 0.06	551	73 ± 2
haloperidol	8.21 ± 0.04	6.22	103 ± 1	8.24 ± 0.15	5.16	101 ± 1	ND	ND	ND
clozapine	ND	ND	ND	ND	ND	ND	7.25 ± 0.04	56.9	96 ± 1

[a]Percent inhibition and affinity (pK_i and K_i) values were calculated by displacement of the specific binding of the radioligand [^{3}H]-spiperone by isoquinolines **1** and **2** on membranes from CHO-K1 cells stably expressing the cloned human D_2, D_3, and D_4 receptors. Haloperidol was used as a reference compound for the hD_2R and hD_3R assays, and clozapine was used as a reference compound for the hD_4R assay. Data are expressed as the mean ± standard error of the mean (SEM) (n = 2). ND = not determined.

subtypes.[33] The data obtained are depicted in Table 1. The competition curves of the isoquinolines for the specific binding of [^{3}H]-spiperone to human D_2R, D_3R, and D_4R are shown in Figure 3.

First, the displacement of the selective radioligand [^{3}H]-spiperone binding to hD_2R, hD_3R, or hD_4R by Br-BTHIQ (**1**) and aporphine (**2**) was evaluated at a single concentration of 10 μM. Haloperidol for D_2R and D_3R and clozapine for D_4R were used as reference compounds. The results showed that both compounds **1** and **2** displayed a high percentage of inhibition (>70%) of the specific radioligand binding (Table 1) at the three receptor subtypes. Next, the concentration response curves of the two isoquinoline compounds were constructed to determine their affinities (equilibrium dissociation constants, K_i) at the different receptors. Both Br-BTHIQ (**1**) and aporphine (**2**) were able to fully displace the specific radioligand binding at hD_2R, hD_3R, and hD_4R in a concentration-dependent manner (Figure 3). Br-BTHIQ (**1**), which contained a flexible appendage of the brominated benzyl moiety, exhibited a high binding affinity, with K_i values of 286, 197, and 13.8 nM for hD_2R, hD_3R, and hD_4R, respectively, and notable selectivity for hD_4 (Table 1). The semirigid 1,2-demethyl-nuciferine (aporphine, **2**) also displaced the selective radioligand from its specific binding sites in hD_2R, hD_3R, or hD_4R at nanomolar concentrations (K_i = 340, 838, and 551 nM, respectively), with a slight selectivity toward hD_2R (Table 1 and Figure 3).

Functional Activity of Isoquinolines 1 and 2 at D_2R. The behavior of Br-BTHIQ (**1**) and aporphine (**2**) as agonists or antagonists at human D_2R was evaluated using *in vitro* functional assays through cAMP signaling in Chinese hamster ovary (CHO)-K1 cells stably expressing the cloned human D_{2S} receptor. At the 10 μM concentration, Br-BTHIQ (**1**) and aporphine (**2**) inhibited forskolin-stimulated cAMP production to different extents (Table 2), as expected for D_2R agonists. In terms of their concentration–response curves, Br-BTHIQ (**1**) and aporphine (**2**) exhibited potency within the micromolar range (EC_{50} = 2.95 and 10.2 μM, respectively) and achieved an agonist efficacy at the highest assayed concentration (100 μM) of 48.4 and 92.7% of the maximal response of the D_2R full agonist quinpirole, respectively (Figure 4A). In view of these results and with the aim of confirming the partial agonist profile of Br-BTHIQ (**1**), the concentration–response curves of quinpirole (full agonist) and quinpirole in the presence of 10 μM Br-BTHIQ (**1**) were performed (Figure 4B). Br-BTHIQ (**1**) showed an agonistic effect at low quinpirole concentrations while an antagonistic effect at higher concentrations of the full agonist (100 nM quinpirole). Indeed, a shift of the quinpirole concentration–response curve to the right resulted in a ≈35-fold increase in the quinpirole EC_{50} values (Figure 4B). This response corresponds to that expected for a full agonist (quinpirole) in the presence of a partial agonist (1-Br-THIQ).

In summary, two isoquinoline derivatives, 1-(2′-bromobenzyl)-6,7-dihydroxy-tetrahydroisoquinoline (**1**) and 1,2-demethyl-nuciferine (**2**), were prepared within good yields. Both compounds **1** and **2** displayed affinity for the hD_2, hD_3, and hD_4 DR subtypes at nanomolar concentrations. The affinity order of Br-BTHIQ (**1**) for the human DR subtypes was $hD_4R \gg hD_3R > hD_2R$, whereas aporphine (**2**) showed a $hD_2R > hD_4R > hD_3R$ tendency. Br-BTHIQ (**1**) displayed a partial agonist effect at D_2R, whereas 1,2-demethyl-nuciferine (**2**) behaved as a full agonist. When these results are taken together, both isoquinolines **1** and **2** emerge as potential lead molecules for the synthesis of new useful therapeutic drugs for schizophrenia and Parkinson's disease, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Electron ionization mass spectrometry (EIMS) and high-resolution electron ionization mass spectrometry (HREIMS) were determined by a TripleTOF 5600 LC/

https://dx.doi.org/10.1021/acs.jnatprod.9b00921
J. Nat. Prod. XXXX, XXX, XXX−XXX

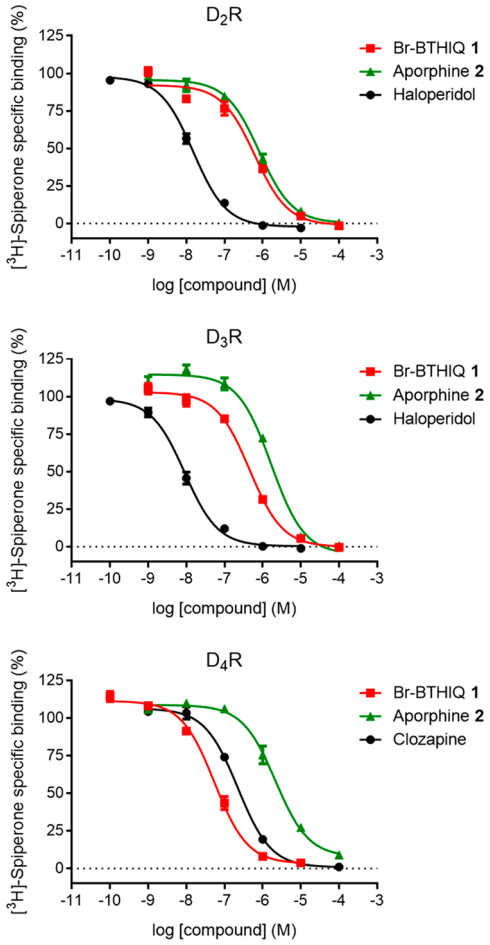


Figure 3. Radioligand displacement curves for isoquinolines **1** and **2** at human D_2R, D_3R, and D_4R subtypes. The graphs show the results (mean ± SEM) of $n = 2$.

Table 2. Evaluation of Efficacy and Potency of Isoquinolines 1 and 2 as Human D_2R Agonists in Functional Assays of cAMP Signaling in CHO-K1 Cells Stably Expressing the Receptors

	hD_2R[a]			
compound	E_{max} at 10 μM (%)	E_{max} at 100 μM (%)	pEC_{50}	EC_{50} (nM)
Br-BTHIQ (**1**)	31.9 ± 6.5	48.4 ± 5.3	5.53 ± 0.31	2951
aporphine (**2**)	50.7 ± 8.6	92.7 ± 6.5	4.99 ± 0.22	10233
quinpirole	98.8 ± 0.6	100	6.82 ± 0.12	150

[a]Efficacy of isoquinolines **1** and **2** at the indicated concentration was expressed as a percentage of the maximal quinpirole response (% E_{max}). Data are the mean ± SEM of $n = 3–4$ independent experiments performed in triplicate.

MS/MS system (AB SCIEX, Framingham, MA, U.S.A.) with the electron ionization (EI) method in the positive mode. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AC-300 or AC-500 spectrometer (Bruker Instruments, Darmstadt, Germany) using $CDCl_3$ (δ 7.26 for ^1H and δ 77.0 for ^{13}C) as the solvent. NMR assignments were performed using distortions enhancement by polarization transfer (DEPT), correlation spectroscopy with a 45° mixing pulse (COSY-45), heteronuclear single-quantum correlation (HSQC), and heteronuclear multiple-bond correlation (HMBC) experiments. All reactions were monitored by analytical thin-layer chromatography (TLC) with silica gel 60 F_{254} (Merck, Darmstadt, Germany). Residues were purified by silica gel

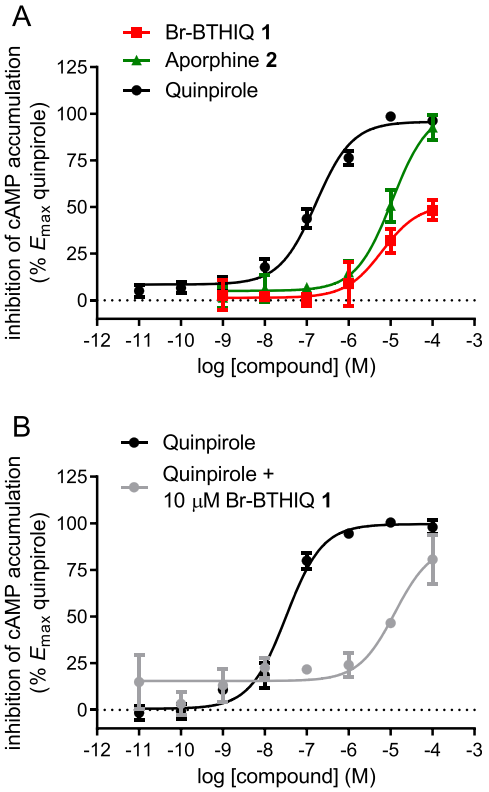


Figure 4. Functional assays of cAMP signaling at human D_2R. (A) Concentration–response (inhibition of forskolin-stimulated cAMP production) curves of isoquinolines **1** and **2** and the reference agonist quinpirole in CHO-K1 cells stably expressing the cloned human D_2 receptors. (B) Effect of Br-BTHIQ (**1**, 10 μM) on quinpirole concentration–response curves. The graphs show data (mean ± SEM) of $n = 3–4$ independent experiments performed in triplicate.

column chromatography using silica gel 60 from Merck (40–63 μm). Solvents were purchased from Scharlab SL (Barcelona, Spain) or Sigma-Aldrich (St. Louis, MO, U.S.A.) and used without further purification, unless otherwise noted. Dry and freshly distilled solvents were used in those reactions carried out under a nitrogen atmosphere.

Synthesis of Isoquinolines 1 and 2. The syntheses and spectroscopic characterization of Br-benzyltetrahydroisoquinoline (**1**) and 1,2-demethyl-nuciferine (**2**), including the intermediates **1a–1d**, **2a**, and **2b**, are described below.

*β-(3,4-Dimethoxyphenyl)ethyl-(2′-bromophenyl)acetamide (**1a**).* 2-Bromo-phenylacetyl chloride (1.2 mL, 8.07 mmol) was added dropwise to a solution of 3,4-dimethoxyphenethylamine (2 g, 11.04 mmol) in CH_2Cl_2 (15 mL) and 5% aqueous NaOH (2.5 mL) at 0 °C. The reaction was stirred at room temperature overnight and then extracted with CH_2Cl_2 (3 × 10 mL). The combination of the organic phases was washed with brine (2 × 10 mL) and H_2O (2 × 10 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified by silica gel column chromatography (hexane–EtOAc, 5:5), to afford 2.35 g of amide (**1a**) as a white powder (77%). ^1H NMR (500 MHz, $CDCl_3$): δ 7.48 (1H, d, $J = 7.8$ Hz, H-3′), 7.20 (2H, m, H-5′, H-6′), 7.05 (1H, m, H-4′), 6.66 (1H, d, $J = 8.1$ Hz, H-5), 6.56 (1H, d, $J = 1.9$ Hz, H-2), 6.52 (1H, dd, $J = 8.1$ and 1.9 Hz, H-6), 5.38 (1H, br s, CONH), 3.78 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.60 (2H, s, CH_2CO), 3.40 (2H, dd, $J = 12.8$ and 6.9 Hz, CH_2-α), 2.64 (2H, t, $J = 6.9$ Hz, CH_2-β). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.4 (CO), 149.0 (C-3), 147.6 (C-4), 134.8 (C-1′), 133.0 (CH-3′), 131.6 (CH-6′), 131.1 (C-1), 129.0 (CH-4′), 127.9 (CH-5′), 124.9 (C-2′), 120.5 (CH-6), 111.8 (CH-2), 111.3 (CH-5), 55.9 (OCH_3), 55.8 (OCH_3), 44.0 (CH_2CO), 40.7 (CH_2-α), 35.0 (CH_2-β). Electrospray mass spectrometry (ESMS): m/z 380 $[M + H]^+$.

https://dx.doi.org/10.1021/acs.jnatprod.9b00921
J. Nat. Prod. XXXX, XXX, XXX–XXX

*1-(2′-Bromobenzyl)-6,5-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1c**).* A solution of phenylacetamide (**1a**, 300 mg, 0.82 mmol) in dry CH_3CN (20 mL) was treated with $POCl_3$ (0.37 mL, 4.0 mmol) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was concentrated to dryness, redissolved in water, and made basic until pH ≈ 9. Then, the mixture was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic solution was dried over anhydrous Na_2SO_4 and evaporated to dryness to give imine (**1b**), which was used without further purification. The residue was dissolved in MeOH (25 mL) and treated with $NaBH_4$ (86 mg, 2.27 mmol) at room temperature. The reaction mixture was stirred for 2 h. Afterward, H_2O (5 mL) was added, and the organic solvent was removed under reduced pressure. The aqueous mixture was made basic, extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (toluene−EtOAc−MeOH−Et_3N, 6:3:1:0.1) to obtain 144 mg of THIQ (**1c**, 50%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52 (1H, d, J = 7.7 Hz, H-3′), 7.20 (2H, m, H-5′, H-6′), 7.05 (1H, m, H-4′), 6.65 (1H, s, H-8), 6.53 (1H, s, H-5), 4.20 (1H, dd, J = 9.8 and 3.9 Hz, H-1), 3.79 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.28 (1H, dd, J = 13.6 and 6.9 Hz, Ha-α), 3.20 (1H, m, Ha-3), 2.90 (2H, m, Hb-3, Hb-α), 2.68 (2H, t, J = 5.8 Hz, H-4). ^{13}C NMR (125 MHz, $CDCl_3$): δ 147.5 (C-6), 147.1 (C-7), 138.8 (C-1′), 133.0 (C-3′), 132.0 (CH-6′), 130.5 (C-8a), 128.2 (CH-4′), 127.4 (CH-5′), 127.1 (C-4a), 124.9 (C-2′), 111.7 (CH-5), 109.7 (CH-8), 55.9 (OCH_3), 55.8 (OCH_3), 54.9 (CH-1), 43.1 (CH_2-α), 39.5 (CH_2-3), 29.4 (CH_2-4). ESMS: m/z 362 $[M]^+$.

*1-(2′-Bromobenzyl)-N-tert-butyloxycarbonyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1d**).* A solution of di-*tert*-butyl dicarbonate (1.62 g, 7.42 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a solution of THIQ (**1c**, 2.23 g, 6.17 mmol) in CH_2Cl_2 (10 mL). After stirring at room temperature for 2 h, the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 97:3) to obtain 2.76 g of *N*-Boc-THIQ (**1d**, 97%) as a brown oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58 (1H, dd, J = 7.8 and 1.2 Hz, H-3′), 7.15 (3H, m, H-4′, H-5′, H-6′), 6.81 (1H, s, H-5), 6.62 (1H, s, H-8), 5.35 (1H, dd, J = 10.7 and 3.9 Hz, H-1), 4.37 (1H, m, Ha-3), 3.86 (6H, s, 2×OCH_3), 3.28 (2H, m, Hb-3, Ha-α), 3.03 (1H, m, Hb-α), 2.95 (1H, m, Ha-4), 2.67 (1H, m, Hb-4), 1.10 (9H, s, 3×CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.8 (CO), 148.2 (C-7), 147.8 (C-8), 138.5 (C-1′), 132.9 (CH-3′), 132.3 (CH-6′), 129.3 (C-8a), 128.6 (CH-4′), 127.9 (CH-5′), 126.8 (C-4a), 125.7 (C-2′), 110.1 (CH-8), 110.1 (CH-5), 79.8 (OC-*t*Bu), 56.3 (2×OCH_3), 54.1 (CH-1), 43.1 (CH_2-α), 36.5 (CH_2-3), 28.5 (CH_2-4), 27.82 (3×CH_3). ESMS: m/z 462 $[M]^+$.

*1-(2-Bromobenzyl)-N-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1e**).* A solution of *N*-Boc-THIQ (**1d**, 300 mg, 0.65 mmol) in dry THF (6 mL) was carefully treated with $LiAlH_4$ (98 mg, 2.58 mmol) at 0 °C. The mixture was heated to reflux overnight. Then, the reaction was cooled at 0 °C, quenched with water (1 mL) dropwise, followed by 1 M NaOH solution (1 mL), and then stirred at room temperature for 1 h. The suspension was diluted with EtOAc (10 mL) and filtered through Celite. The filtrate was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give 85 mg of *N*-methyl-Br-THIQ (**1e**, 35%) as a yellow oil, which was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54 (1H, dd, J = 7.9 and 1.3 Hz, H-3′), 7.16 (1H, m, H-5′), 7.14 (1H, m, H-4′), 7.06 (1H, m, H-6′), 6.58 (1H, s, H-8), 5.92 (1H, s, H-5), 3.85 (1H, m, H-1), 3.83 (3H, s, OCH_3), 3.50 (3H, s, OCH_3), 3.29 (2H, m, Ha-α, Ha-3), 2.92 (3H, m, Hb-α, Hb-3), 2.55 (2H, m, CH_2-4), 2.53 (3H, s, NCH_3). ESMS: m/z 377 $[M + H]^+$.

*1-(2-Bromobenzyl)-N-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**1**).* A solution of *N*-methyl-Br-THIQ (**1e**, 50 mg, 0.13 mmol) in dry CH_2Cl_2 (3 mL) was treated with BBr_3 (38 μL, 0.4 mmol) dropwise at −78 °C under a nitrogen atmosphere. Then, the reaction mixture was stirred to room temperature for 2 h. The reaction was cooled at −78 °C to be quenched by the addition of MeOH (0.5 mL) dropwise and then stirred at room temperature for 30 min. The solvent was removed under reduced pressure and purified by silica gel column chromatography (CH_2Cl_2−MeOH, 90:1) to obtain 25 mg of catechol-*N*-methyl-Br-THIQ (**1**, 55%) as a yellow oil. ^{1}H NMR (300 MHz, CD_3OD): δ 7.56 (1H, dd, J = 7.8 and 1.3 Hz, H-3′), 7.21 (1H, td, J = 7.5 and 1.3 Hz, H-5′), 7.12 (1H, td, J = 7.5 and 1.3 Hz, H-4′), 7.01 (1H, dd, J = 7.5 and 1.3 Hz, H-6′), 6.53 (1H, s, H-5), 5.86 (1H, s, H-8), 3.92 (1H, m, H-1), 3.31 (2H, m, Ha-α, Ha-3), 2.90 (3H, m, Hb-α, Hb-3), 2.64 (2H, m, CH_2-4), 2.51 (3H, s, NCH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 145.4 (C-6), 144.0 (C-7), 139.6 (C-1′), 133.8 (2C, CH-3′, CH-6′), 129.3 (CH-4′), 128.4 (CH-5′), 128.0 (C-2′), 125.9 (C-8a), 125.1 (C-4a), 116.1 (CH-5), 116.0 (CH-8), 63.4 (CH-1), 46.6 (CH_2-3), 42.3 (NCH_3), 41.1 (CH_2-α), 25.2 (CH_2-4). High-resolution electrospray ionization mass spectrometry (HRESIMS): m/z 348.0589 $[M + H]^+$ (calcd for $C_{17}H_{18}BrNO_2$, 348.0594).

*tert-Butyloxycarbonyl-nor-nuciferine (**2a**).* A mixture of *N*-Boc-THIQ (**1d**, 451 mg, 0.98 mmol), 2-diphenylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl (PhDave-Phos, 75 mg, 0.20 mmol), $Pd(OAc)_2$ (22 mg, 0.098 mmol), and K_2CO_3 (412 mg, 2.98 mmol) in *N*,*N*-dimethylacetamide (DMA, 5 mL) was heated at 130 °C overnight under a nitrogen atmosphere. Then, the reaction was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 99.5:0.5) to obtain 310 mg of *N*-Boc-*nor*-nuciferine (**2a**, 83%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.42 (1H, d, J = 7.8 Hz, H-11), 7.27 (3H, m, H-8, H-9, H-10), 6.67 (1H, s, H-3), 4.65 (1H, m, H-6a), 4.42 (1H, m, Ha-5), 3.90 (3H, s, OCH_3-2), 3.67 (3H, s, OCH_3-1), 2.75 (5H, m, CH_2-4, CH_2-7, Hb-5), 1.49 (9H, s, 3×CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.6 (CO), 151.9 (C-2), 145.5 (C-1), 137.0 (C-7a), 131.7 (C-11a), 129.8 (C-4a), 128.4 (CH-11), 128.0 (C-11b), 127.6 (CH-8), 127.5 (CH-10), 126.9 (CH-9), 126.5 (C-11c), 111.4 (CH-3), 79.8 (OC-*t*Bu), 59.9 (OCH_3), 55.8 (OCH_3), 51.5 (CH-6a), 38.4 (CH_2-5), 35.4 (CH_2-4), 30.4 (CH_2-7), 28.5 (3×CH_3). ESMS: m/z 381 $[M]^+$.

*Nuciferine (**2b**).*[34] A solution of *N*-Boc-nuciferine (**2a**, 200 mg, 0.52 mmol) in anhydrous THF (15 mL) was carefully treated with $LiAlH_4$ (102 mg, 2.69 mmol) under a nitrogen atmosphere at 0 °C. Afterward, the reaction mixture was heated at reflux for 4 h. The suspension was cooled at 0 °C, and water (1 mL) was added, followed by 1 M NaOH solution (1 mL). The suspension was stirred for additional 1 h at room temperature. The suspension was diluted with EtOAc (10 mL) and filtered through Celite. The filtrate was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 94:6) to afford 120 mg of nuciferine (**2b**) (78%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (1H, dd, J = 8.4 and 1.9 Hz, H-11), 7.25 (3H, m, H-8, H-9, H-10), 6.63 (1H, s, H-3), 3.89 (3H, s, OCH_3-2), 3.66 (3H, s, OCH_3-1), 3.10 (4H, m, H-6a, Ha-4, Ha-5, Ha-7), 2.67−2.53 (2H, m, Hb-7, Hb-4), 2.54 (3H, s, NCH_3), 2.47 (1H, m, Hb-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 151.9 (C-2), 145.1 (C-1), 136.3 (C-7a), 132.1 (C-11a), 128.5 (C-3a), 128.3 (CH-8), 127.8 (CH-9), 127.7 (C-11c), 127.3 (CH-10), 127.0 (CH-11), 126.8 (C-11b), 111.2 (CH-3), 62.3 (CH-6a), 60.2 (OCH_3), 55.9 (OCH_3), 53.3 (CH_2-5), 44.0 (NCH_3), 35.2 (CH_2-7), 29.3 (CH_2-4). ESMS: m/z 296 $[M + H]^+$.

*1,2-Demethyl-nuciferine (**2**).* Nuciferine (**2b**, 0.47 mmol, 130 mg) in dry CH_2Cl_2 (5 mL) was stirred at −78 °C. Then, 127 μL of BBr_3 (1.32 mmol) was added under a nitrogen atmosphere, and the resulting mixture was stirred for 2 h at room temperature. The reaction was cooled at −78 °C to be quenched by the addition of MeOH (0.5 mL) dropwise and then stirred at room temperature for 30 min. The solvent was evaporated to dryness and purified by silica gel column chromatography (CH_2Cl_2−MeOH, 85:15) to afford 115 mg of 1,2-demethyl-nuciferine (**2**, 98%) as a yellow oil. ^{1}H NMR (300 MHz, CD_3OD): δ 8.28 (1H, dd, J = 7.8 and 1.3 Hz, H-11), 7.13 (3H, m, H-8, H-9, H-10), 6.46 (1H, s, H-3), 3.04 (4H, m, H-6a, Ha-5, Ha-7, Ha-4), 2.53 (6H, m, Hb-5, Hb-7, Hb-4, NCH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 146.1 (C-1), 143.1 (C-2), 136.1 (C-11a), 134.0 (C-3a), 129.6 (CH-11), 128.7 (CH-8), 127.8 (CH-9), 127.7 (CH-10), 125.9, 123.8, 121.2 (3C, C-7a, C-11b, C-11c), 114.3 (CH-3),

https://dx.doi.org/10.1021/acs.jnatprod.9b00921
J. Nat. Prod. XXXX, XXX, XXX−XXX

64.0 (CH-6a), 54.5 (CH_2-5), 43.4 (NCH_3), 36.3 (CH_2-7), 28.6 (CH_2-4). HRESIMS: m/z 268.1325 $[M + H]^+$ (calcd for $C_{17}H_{17}NO_2$, 268.1332).

Cell Cultures. CHO-K1 cells stably expressing human $D_{2S}R$ were generated in-house as described.[33] In brief, the CHO-K1 parental cell line (Leibniz Institute DSMZ−German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) was stably transfected with the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, U.S.A.) containing the cDNA of human $D_{2S}R$ using the calcium phosphate method and selected in medium containing 400 μg/mL Geneticin (G-418). Cells were maintained in Dulbecco's modified Eagle's medium−GlutaMAX-I (Gibco, Thermo Fisher Scientific, Madrid, Spain) supplemented with 10% (v/v) fetal bovine serum (Sigma-Aldrich, Madrid, Spain), 100 units/mL penicillin/0.1 mg/mL streptomycin (Sigma-Aldrich, Madrid, Spain), 2 mM L-glutamine (Sigma-Aldrich, Madrid, Spain), and 500 μg/mL Geneticin G418 (Gibco, Thermo Fisher Scientific, Madrid, Spain).

Radioligand-Binding Assays. Competition radioligand-binding assays for isoquinolines **1** and **2** were conducted in membranes from CHO-K1 cells stably expressing the cloned human $D_{2S}R$ (isoform D_2 short) or the cloned human D_3R or D_4R (isoform $D_{4.2}$) (PerkinElmer, Waltham, MA, U.S.A.), following protocols described previously.[33] In brief, [^{3}H]-spiperone was employed as a radioligand, whereas non-specific binding was assessed in the presence of 10 μM sulpiride (D_2R), 1 μM haloperidol (D_3R), or 25 μM haloperidol (D_4R). Isoquinolines **1** and **2** were evaluated in displacement curves at six different concentrations ranging from 1 nM to 100 μM or from 0.1 nM to 10 μM. Haloperidol (from 0.1 nM to 10 μM) or clozapine (from 1 nM to 100 μM) were included in the assays as reference competitor ligands for both hD_2R and hD_3R binding (haloperidol) or hD_4R binding (clozapine). The affinities of isoquinolines expressed as an equilibrium dissociation constant (K_i) were calculated using Prism 6 software (GraphPad, San Diego, CA, U.S.A.), by fitting the data from competition binding curves to a single binding site competition model using the equations $\log EC_{50} = \log(10^{\log K_i(1 + HotNM/HotKdNM)})$ and Y = bottom + (top − bottom)/$(1 + 10^{X \log EC_{50}})$, where Y is binding, HotNM is the concentration of radioligand in the assay, HotKdNM is the equilibrium dissociation constant (K_d) of the radioligand as determined in saturation binding experiments, and X is the log molar concentration of the unlabeled compound.

cAMP Functional Assay at D_2R. The behavior of isoquinolines **1** and **2** as agonists or antagonists of D_2R receptors was evaluated in *in vitro* assays of cAMP signaling in the CHO-K1 cell line stably expressing the cloned human D_{2S} receptor employed in radioligand-binding assays. Cellular cAMP levels were quantified using the homogeneous time-resolved fluorescence (HTRF)-based cAMP kit cAMP-Gs Dynamic HTRF Kit (Cisbio, Bioassays, Codolet, France) following the protocol of the manufacturer. The possible agonist effect of the compounds was evaluated by their ability to inhibit forskolin-stimulated cAMP production either at a single concentration (10 μM) and/or in concentration (from 1 nM to 100 μM)−response curves. Cells seeded in 384-well plates in stimulation buffer containing 500 μM 3-isobutyl-1-methylxanthine (IBMX) were incubated with the test compounds for 10 min at 37 °C. Then, forskolin (10 μM) was added, and the incubation was continued for 5 min. After this time, intracellular cAMP levels were quantified. Quinpirole (from 10 pM to 100 μM) was included as a control agonist in these assays. When the effects of Br-BTHIQ (**1**, 10 μM) on quinpirole concentration−response curves were investigated, compound **1** and quinpirole were added simultaneously to the cells and the assay proceeded as described above. For assessment of a possible antagonist effect, the test compound was added to the cells 5 min prior to the addition of the reference agonist quinpirole (100 nM) and assays were subsequently carried out as described above. In all cases, basal cAMP levels were determined in control wells in the absence of compound, quinpirole, and forskolin.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b00921.

^{1}H and ^{13}C NMR spectra of synthesized compounds (**1a−1d**, **1**, **2a**, **2b**, and **2**) (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Diego Cortes − *University of Valencia, Valencia, Spain*; Phone: 34-96-3544975; Email: dcortes@uv.es

Marián Castro − *Universidad de Santiago de Compostela, Santiago de Compostela, Spain*; Phone: 34-881-815458; Email: marian.castro@usc.es

Nuria Cabedo − *University Clinic Hospital of Valencia, Valencia, Spain, University of Valencia, Valencia, Spain*; orcid.org/0000-0001-6729-8057; Phone: 34-96-3544975; Email: ncabedo@uv.es

Other Authors

Andrea G. Silva − *Universidad de Santiago de Compostela, Santiago de Compostela, Spain*

Laura Vila − *University Clinic Hospital of Valencia, Valencia, Spain*

Patrice Marques − *University Clinic Hospital of Valencia, Valencia, Spain, University of Valencia, Valencia, Spain*

Laura Moreno − *University of Valencia, Valencia, Spain*

Mabel Loza − *Universidad de Santiago de Compostela, Santiago de Compostela, Spain*

María-Jesús Sanz − *University Clinic Hospital of Valencia, Valencia, Spain, University of Valencia, Valencia, Spain*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.9b00921

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Grants SAF2014-57138-C2-1-R, SAF2014-57845-R, SAF2017-89714-R, CP15/00150, and PI18/01450 from the Spanish Ministry of Economy and Competiveness, the Carlos III Health Institute, and the European Regional Development Fund. Nuria Cabedo was funded by the Miguel Servet Program, Carlos III Institute of Health, co-funded by the European Fund for Regional Development Fund and the European Social Fund. Patrice Marques was funded by a pre-doctoral grant from the Spanish Ministry of Economy and Competitiveness (FPI). Andrea G. Silva was funded by a pre-doctoral grant from Xunta de Galicia (Spain).

■ REFERENCES

(1) Hagel, J. M.; Facchini, P. J. *Plant Cell Physiol.* **2013**, *54*, 647−672.

(2) Stadler, R.; Kutchan, T. M.; Zenk, M. H. *Phytochemistry* **1989**, *28*, 1083−1086.

(3) Menendez-Perdomo, I. M.; Facchini, P. J. *Molecules* **2018**, *23*, 2899.

(4) Cho, E. J.; Yokozawa, T.; Rhyu, D. Y.; Kim, S. C.; Shibahara, N.; Park, J. C. *Phytomedicine* **2003**, *10*, 544−551.

(5) Agnihotri, V. K.; ElSohly, H. N.; Khan, S. I.; Jacob, M. R.; Joshi, V. C.; Smillie, T.; Khan, I. A.; Walker, L. A. *Phytochem. Lett.* **2008**, *1*, 89−93.
(6) Chen, K.-S.; Ko, F.-N.; Teng, C.-M.; Wu, Y.-C. *J. Nat. Prod.* **1996**, *59*, 531−534.
(7) Zhang, C.; Deng, J.; Liu, D.; Tuo, X.; Xiao, L.; Lai, B.; Yao, Q.; Liu, J.; Yang, H.; Wang, N. *Br. J. Pharmacol.* **2018**, *175*, 4218−4228.
(8) Morales, M.; Bustamante, S.; Brito, G.; Paz, D.; Cassels, B. K. *Phytother. Res.* **1998**, *12*, 103−109.
(9) Farrell, M. S.; McCorvy, J. D.; Huang, X.-P.; Urban, D. J.; White, K. L.; Giguere, P. M.; Doak, A. K.; Bernstein, A. I.; Stout, K. A.; Park, S. M.; Rodriguiz, R. M.; Gray, B. W.; Hyatt, W. S.; Norwood, A. P.; Webster, K. A.; Gannon, B. M.; Miller, G. W.; Porter, J. H.; Shoichet, B. K.; Fantegrossi, W. E.; Wetsel, W. C.; Roth, B. L. *PLoS One* **2016**, *11*, No. e0150602.
(10) Cabedo, N.; Berenguer, I.; Figadère, B.; Cortes, D. *Curr. Med. Chem.* **2009**, *16*, 2441−2467.
(11) Millan, M. J.; Maiofiss, L.; Cussac, D.; Audinot, V.; Boutin, J. A.; Newman-Tancredi, A. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 791−804.
(12) Kebabian, J. W. *Life Sci.* **1978**, *23*, 479−483.
(13) Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. *Physiol. Rev.* **1998**, *78*, 189−225.
(14) Beaulieu, J. M.; Espinoza, S.; Gainetdinov, R. R. *Br. J. Pharmacol.* **2015**, *172*, 1−23.
(15) Wang, S.; Che, T.; Levit, A.; Shoichet, B. K.; Wacker, D.; Roth, B. L. *Nature* **2018**, *555*, 269−273.
(16) Chien, E. Y. T.; Liu, W.; Zhao, Q.; Katritch, V.; Won Han, G.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. *Science* **2010**, *330*, 1091−1095.
(17) Wang, S.; Wacker, D.; Levit, A.; Che, T.; Betz, R. M.; McCorvy, J. D.; Venkatakrishnan, A. J.; Huang, X.-P.; Dror, R. O.; Shoichet, B. K.; Roth, B. L. *Science* **2017**, *358*, 381−386.
(18) Cabedo, N.; Protais, P.; Cassels, B. K.; Cortes, D. *J. Nat. Prod.* **1998**, *61*, 709−712.
(19) Andreu, I.; Cortes, D.; Protais, P.; Cassels, B. K.; Chagraoui, A.; Cabedo, N. *Bioorg. Med. Chem.* **2000**, *8*, 889−895.
(20) Cabedo, N.; Andreu, I.; Ramírez de Arellano, M. C.; Chagraoui, A.; Serrano, A.; Bermejo, A.; Protais, P.; Cortes, D. *J. Med. Chem.* **2001**, *44*, 1794−1801.
(21) Andreu, I.; Cabedo, N.; Torres, G.; Chagraoui, A.; Carmen Ramírez de Arellano, M.; Gil, S.; Bermejo, A.; Valpuesta, M.; Protais, P.; Cortes, D. *Tetrahedron* **2002**, *58*, 10173−10179.
(22) El Aouad, N.; Berenguer, I.; Romero, V.; Marín, P.; Serrano, A.; Andujar, S.; Suvire, F.; Bermejo, A.; Ivorra, M. D.; Enriz, R. D.; Cabedo, N.; Cortes, D. *Eur. J. Med. Chem.* **2009**, *44*, 4616−4621.
(23) Berenguer, I.; Aouad, N. E.; Andujar, S.; Romero, V.; Suvire, F.; Freret, T.; Bermejo, A.; Ivorra, M. D.; Enriz, R. D.; Boulouard, M.; Cabedo, N.; Cortes, D. *Bioorg. Med. Chem.* **2009**, *17*, 4968−4980.
(24) Párraga, J.; Cabedo, N.; Andujar, S.; Piqueras, L.; Moreno, L.; Galán, A.; Angelina, E.; Enriz, R. D.; Ivorra, M. D.; Sanz, M. J.; Cortes, D. *Eur. J. Med. Chem.* **2013**, *68*, 150−166.
(25) Moreno, L.; Cabedo, N.; Ivorra, M. D.; Sanz, M.-J.; Castel, A. L.; Álvarez, M. C.; Cortes, D. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4824−4827.
(26) Suvire, F. D.; Cabedo, N.; Chagraoui, A.; Zamora, M. A.; Cortes, D.; Enriz, R. D. *J. Mol. Struct.: THEOCHEM* **2003**, *666*−*667*, 455−467.
(27) Andujar, S. A.; Tosso, R. D.; Suvire, F. D.; Angelina, E.; Peruchena, N.; Cabedo, N.; Cortes, D.; Enriz, R. D. *J. Chem. Inf. Model.* **2012**, *52*, 99−112.
(28) Andujar, S.; Suvire, F.; Berenguer, I.; Cabedo, N.; Marín, P.; Moreno, L.; Ivorra, M. D.; Cortes, D.; Enriz, R. D. *J. Mol. Model.* **2012**, *18*, 419−431.
(29) Browman, K. E.; Curzon, P.; Pan, J. B.; Molesky, A. L.; Komater, V. A.; Decker, M. W.; Brioni, J. D.; Moreland, R. B.; Fox, G. B. *Pharmacol., Biochem. Behav.* **2005**, *82*, 148−55.
(30) Newman-Tancredi, A.; Heusler, P.; Martel, J.-C.; Ormière, A.-M.; Leduc, N.; Cussac, D. *Int. J. Neuropsychopharmacol.* **2008**, *11*, 293−307.
(31) Gerlach, M.; Double, K.; Arzberger, T.; Leblhuber, F.; Tatschner, T.; Riederer, P. *J. Neural. Transm.* **2003**, *110*, 1119−1127.
(32) Lafrance, M.; Blaquiere, N.; Fagnou, K. *Eur. J. Org. Chem.* **2007**, *2007*, 811−825.
(33) Selent, J.; Marti-Solano, M.; Rodriguez, J.; Atanes, P.; Brea, J.; Castro, M.; Sanz, F.; Loza, M. I.; Pastor, M. *Eur. J. Med. Chem.* **2014**, *77*, 91−95.
(34) Guinaudeau, H.; Leboeuf, M.; Cave, A. *Lloydia* **1975**, *38*, 275−338.

https://dx.doi.org/10.1021/acs.jnatprod.9b00921
J. Nat. Prod. XXXX, XXX, XXX−XXX