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Flavonoids from *Perovskia atriplicifolia* and Their in Vitro Displacement of the Respective Radioligands for Human Opioid and Cannabinoid Receptors

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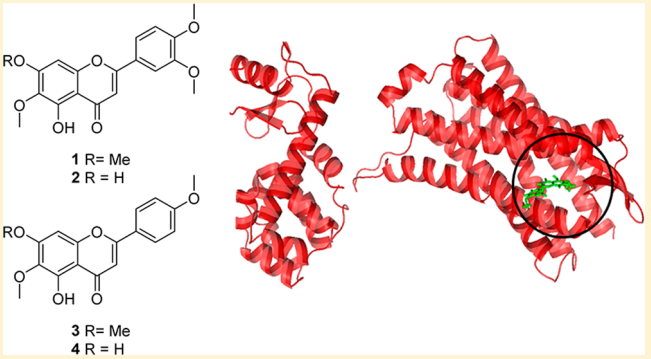
Supporting Information

ABSTRACT: Bioassay-guided fractionation of the leaves of *Perovskia atriplicifolia* (Russian sage) resulted in the isolation of four previously known flavonoid derivatives, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (**1**), 5,7-dihydroxy-6,3′,4′-trimethoxyflavone (**2**), 5-hydroxy-6,7,4′-trimethoxyflavone (**3**), and 5,7-dihydroxy-6,4′-dimethoxyflavone (**4**). Compounds **1**, **3**, and **4** showed displacement of the radioligand for the cloned human δ opioid receptor with K_i values ranging from 3.1 to 26.0 μM. In addition, the binding mode of the compounds in the active site of the δ opioid receptor was investigated through molecular modeling algorithms. This study may have implications in better understanding non-nitrogenous δ opioid receptor ligands.



Opioid and cannabinoid receptors are G-protein coupled receptors, which are a group of signaling receptors that are involved in the recognition of and transduction of messages across cell membranes.[1] Various subtypes of each receptor system have been recognized; the opioid receptor system mainly includes μ, κ, and δ receptors, while the cannabinoid receptor system includes CB_1 and CB_2 receptors. The opioid receptors are known to regulate various physiological functions including neurohormonal secretion of the adrenal and pituitary glands and also possess direct action on the adrenal glands. They play a major role in the central nervous system (CNS), as well as in cardiovascular, immune, reproductive, endocrine, and gastrointestinal systems.[2−4] Within the endocannabinoid systems, the CB_1 receptor is mainly expressed in the CNS, while CB_2 is primarily expressed in the peripheral nervous system, where it plays a crucial role in the stimulation of hematopoietic lineage growth.[2]

Numerous kinds of plants have been used for decades as folk medicines, food supplements, and food preservatives.[5] Many plants contain a wide variety of phenolic compounds, especially flavonoids, which have been reported to inhibit the propagation of free radical reactions and to protect the human body from certain diseases.[6]

Perovskia atriplicifolia Benth. (Lamiaceae), commonly known as Russian sage, is a silver-gray shrub reaching about 1.5 m high and comprising airy spires that bear small, edible lavender flowers. The plant is native to the rocky areas of Central Asia, including Pakistan, Afghanistan, Iran, and Tibet. It is mainly known for its ornamental and flavoring qualities. It is also smoked as a euphoriant and used as a decoction for chronic dysentery.[7] Recently, *P. atriplicifolia* has been explored as a possible remediative plant as a means to improve air and soil quality.[8]

In order to provide insight in the development of novel agents for managing neuropathic pain,[9] we used a radioligand binding receptor assay to identify natural products with selective affinity for specific opioid and cannabinoid receptors. An ethanolic extract of *P. atriplicifolia* leaves was found to have a percent displacement of a radioligand between 40% and 50% for the opioid receptors. Reported herein is a phytochemical study of the ethanolic extract of *P. atriplicifolia* leaves following a bioassay-guided fractionation strategy. Four previously known compounds, namely, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (**1**), 5,7-dihydroxy-6,3′,4′-trimethoxyflavone (**2**), 5-hydroxy-6,7,4′-trimethoxyflavone (**3**), and 5,7-dihydroxy-6,4′-dimethoxyflavone (**4**), were isolated, identified, and evaluated for their radioligand displacement affinity on opioid and cannabinoid

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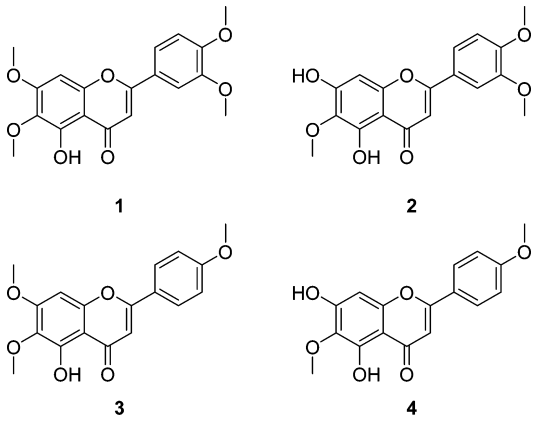
receptors. ^{1}H and ^{13}C NMR spectroscopic data (see Supporting Information) were compared with previously reported data for **1**–**4** and found to be consistent.[10−12] This study describes the first report of compounds **1**–**4** being isolated from a plant in this genus.



All pure compounds (**1**–**4**) were submitted for in vitro radioligand binding affinity assays using opioid receptors (subtypes μ, κ, and δ) and cannabinoid receptors (CB_1 and CB_2) following standard methods.[9] The results are shown in Table 1. At a concentration of 10 μM, compounds **1**, **3**, and **4** displaced the [^{3}H]-DPDPE radioligand by 86.0%, 31.6%, and 79.9%, respectively (Table 1). The K_i values (3.1 ± 0.3, 26.0 ± 7.0, and 8.4 ± 1.4 μM, for **1**, **3**, and **4**, respectively) are shown in Figure 1. In contrast, compound **2**, a closely related flavonoid, showed low radioligand binding displacement at 10 μM with an affinity of only 19.9% for the δ opioid receptor. In order to determine the functional efficacy of compounds **1**, **3**, and **4**, a modified GTPγ[^{35}S] functional assay was performed. At 300 μM, compounds **1**, **3**, and **4** reached 32%, 88%, and 48%, respectively, of maximal GTPγ[^{35}S] binding activity compared with the full agonist DPDPE. This suggest, therefore, that compounds **1**, **3**, and **4** are full or partial δ agonists (Figure S1, Supporting Information).

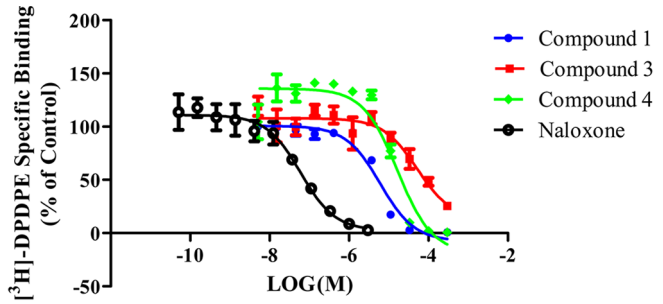
Table 1. Displacement Radioligand Assay of the Isolated Compounds (1−4) for Human Opioid Receptors (Subtypes δ, κ, and μ) and Cannabinoid Receptors (Subtypes CB_1 and CB_2)

	opioid receptors (%)			cannabinoid receptors (%)	
compound	δ	κ	μ	CB_1	CB_2
1	86.0	7.5	9.5	3.0	4.2
2	19.9	9.6	24.3	0.3	12.5
3	31.6	7.4		1.7	8.3
4	79.9		20.1	19.3	13.5
extract	42.8	37.6	52.2	12.2	26.8
naloxone[a]	106.4	101.6	97.0		
CP 55,940[b]				104.3	102.6

[a,b]Positive controls.

These initial observations then led to an investigation on the interactions of flavonoids **1**–**4** with the human δ opioid receptor using molecular modeling algorithms. Compounds **1**–**4** were docked into the binding pocket of the known crystal structure complex of the mouse δ opioid. The complex contains the morphinan analogue naltrindole, which allowed better definition of the binding pocket.[13] Compounds **1** and **4** showed good docking scores (−8.6 and −8.4 kcal/mol, respectively) when compared to compounds **2** and **3** (−7.8 and −7.7 kcal/mol, respectively), which correlated with the experimental activities obtained (Table 1, Figure 1). Compound **4** (Figure 2) forms H-bonds with the key amino acids Tyr308, His278, and Tyr129 and shows interactions with the hydrophobic residues Met132, Val217, Phe218, Phe222, and Trp274. Similar interactions were observed in the case of compound **1**. One of the main questions was the apparent contradiction between the structural similarity of compounds **3** and **4** and their difference in percent displacement (Table 1). A minor structural modification of compound **4**, through conversion of the C-7 hydroxy group to a methoxy group, led to a decrease in the displacement potential. The same structural effects on activity are found in the case of compounds **1** and **2**. These observations can be explained, in part, by taking into consideration the thermodynamic properties of the active site in the apo-form (unbound protein). The active site shows two distinct regions of free energy, which affect the ligands' binding and hence distinguish between their biological activities. Figure 3 shows the negative free energy regions (blue), which support polar substituents and surround the polar hydroxy groups of compound **4**, as well as the hydrophobic regions of the compound that are well fitted inside the positive regions (red).

Based on the structural features of the flavones and the thermodynamic properties of the active site, a rationale can be provided for the activity of compound **1**. By adding methoxy groups at positions 7 and 3′, the binding mode of compound **1** would adjust itself to compensate for the hydrophobicity of the C-7 methoxy group. However, this will lead to the C-4′



	Compound 1	Compound 3	Compound 4	Naloxone
Ki ± SEM	3.1 ± 0.3 μM	> 26.0 ± 7.0 μM	8.4 ± 1.4 μM	0.030 ± 0.003 μM

Figure 1. Displacement of [^{3}H]-DPDPE binding by compounds **1**, **3**, and **4** on δ opioid receptors and K_i values.

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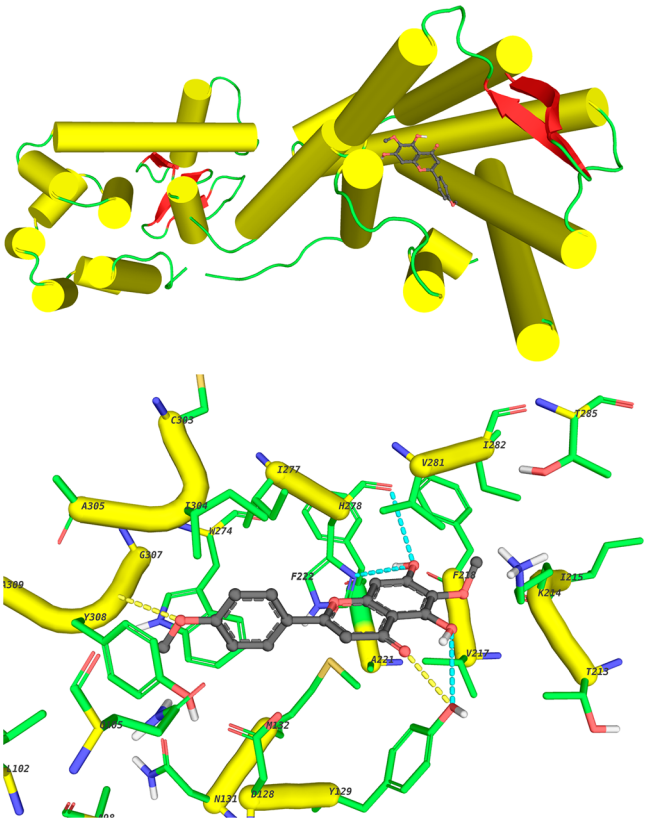

Figure 2. (Top) Protein complex: protein is shown as yellow cylindrical helices, red sheets, and green loops. The ligand is shown as ball and sticks. (Bottom) Interaction profile model of compound **4**: the amino acid residues in range of 5 Å of the ligand are shown as sticks, and hydrogen bonds are shown as dotted lines (cyan for hydrogen bond donor and yellow for hydrogen bond acceptor).

methoxy group being orientated in an unfavorable region. Furthermore, the C-3′ methoxy will fit very well in the positive free energy region, which in turn will compensate for the unfavorable positioning of the C-4′ methoxy. Compound **3** will have close behavior to compound **1** by adjusting the location of ring A in the active site, but on the other hand there is no compensatory effect like that found in compound **1**, which could explain its weak binding affinity to the active site. Compound **2** will follow the same pattern of compound **4**, but ring B will be in the unfavorable region, revealing this as a possible cause of the lower inhibitory potential of compound **2**.

Prisinzano[14] was one of the first to report the potential of flavonoids as a new scaffold for the development of novel opioid receptor ligands. Katavic et al. demonstrated that the biflavonoid amentoflavone and hyperoside have opioid κ antagonist activity. Another flavonoid, casticin, a taxonomical marker of the *Vitex* genus, showed selective agonist activation on δ opioid receptors.[15] Pharmacological studies in animal models demonstrated that the C-glycosylated flavone vitexin induced antinociceptive effects mediated by an opioid mechanism involving the μ, κ, and δ opioid receptors.[16] Recently, alpinetin activated δ opioid receptors to induce endogenous protection of myocardial cells via the PKC/ERK signaling pathway.[17]

EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were obtained on Bruker model AMX 500 and 400 NMR spectrometers with standard pulse sequences, operating at 500 and 400 MHz in ^{1}H and 125 and 100 MHz in ^{13}C, respectively. $CDCl_3$, DMSO-d_6, and CD_3OD were used as solvents, and TMS was used as internal standard. High-resolution mass spectra (HRMS) were recorded on a Micromass Q-Tof Micro mass spectrometer with a lock spray source. Column chromatography was carried out on silica gel (70−230 mesh, Merck) and Sephadex LH-20 (GE Healthcare,

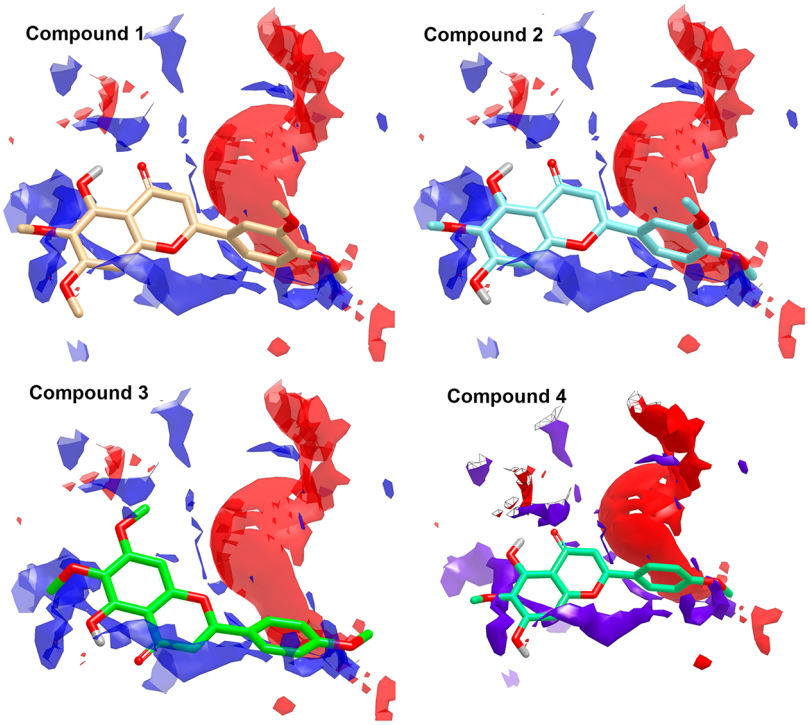

Figure 3. Molecular surface of the active site of δ opioid receptor pocket showing zones of negative free energy as blue surface and positive free energy as red, docked with the compounds **1**−**4**.

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Uppsala, Sweden). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F254). Preparative TLC was carried out on silica gel 60 PF254+366 plates (20 × 20 cm, 1 mm thick). All chemicals used were from Sigma-Aldrich (Poole, Dorset, U.K.) with the following exceptions. For the binding experiments, [^{3}H]CP 55,940 (174.8 Ci/mmol), [^{3}H]DAMGO (53.4 Ci/mmol), [^{3}H]U-69,593 (42.7 Ci/mmol), and [^{3}H]enkephalin (45 Ci/mmol) were obtained from PerkinElmer Life Sciences Inc. (Boston, MA, USA). CP 55,940, DAMGO, DPDPE, and naloxone hydrochloride were obtained from Tocris Bioscience (Ellisville, MO, USA).

Plant Material. The leaves of *Perovskia atriplicifolia* were collected in the region of Quetta, Pakistan, in July 2012 and identified by Dr. Mudassir Asrar from the Medicinal Plant Garden at the University of Balochistan, Quetta, Pakistan. A voucher specimen (121/5, HBG/UOB) has been deposited in the Herbarium of the Botanical Garden, Quetta, Pakistan. A voucher specimen is also in the culture collection of the Department of BioMolecular Sciences, University of Mississippi (UM 072012).

Extraction and Isolation. The dried leaves were ground to a powder, yielding 370 g, which was extracted with ethanol after maceration for 3 days. Removal of the solvent afforded a viscous residue (40 g). The extract showed moderate radioligand displacement for opioid receptors (42.8% for δ and 52.5% for μ). The ethanolic extract (39.0 g) was fractionated on a flash silica gel column using a polarity gradient, resulting in five fractions: (100% hexanes, F-1; 100% dichloromethane, F-2; 100% ethyl acetate, F-3; 80% ethyl acetate in methanol, F-4; and 100% methanol, F-5). Fractions F-1 to F-5 were tested for their biological activity. F-3 showed 46.0% and 88.4% radioligand binding displacement for CB_2 and δ opioid receptors, respectively (Figure S2, Supporting Information). Accordingly, F-3 was further fractionated using a C_8 reversed-phase column. Six subfractions were obtained in order of inverse polarity with water to methanol [F-3A (100% water), F-3B (20% methanol in water), F-3C (40% methanol in water), F-3D (60% methanol in water), F-3E (80% methanol in water), F-3F (100% methanol)], and these subfractions were subjected to radioligand displacement bioassays. Subfractions F-3D and F-3E showed a high percentage of radioligand displacement for the δ opioid receptors with values of 62.8% and 78.5%, respectively. F-3D (60% methanol in water) was rechromatographed on a silica gel column eluted with hexanes to ethyl acetate in a step gradient to yield 15 subfractions, F-3D1 to F-3D15. From subfractions F-3D10 and F-3D12 a precipitate was obtained and crystallized separately to obtain flavones **1** (12 mg) and **2** (10 mg), respectively. Subfraction F-3E (80% methanol in water) was rechromatographed on a silica gel column eluted with hexanes to ethyl acetate in a step gradient to yield 13 subfractions, F-3E1 to F-3E13. A precipitate was obtained from subfractions F-3E5 to F-3E7. The solid showed two major spots using TLC plates (dichloromethane−ethyl acetate, 4:1); the subfractions were combined and subject to a column of Sephadex LH-20 eluted with dichloromethane−methanol (1:1) to furnish flavones **3** (5 mg) and **4** (3 mg).

Cell Culture and Membrane Preparation. HEK293 cells (ATCC) were stably transfected with plasmids containing cloned human cannabinoid receptor subtypes 1 and 2 (obtained from Origene, Rockville, MD, USA). These cells were maintained in a humidified incubator at 37 °C and 5% CO_2 in a Dulbecco's modified Eagle's medium (DMEM) nutrient mixture F-12 HAM supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 1000 IU/mL penicillin, and 1000 μg/mL of streptomycin, and 0.5 mg/mL G418 antibiotic solution. HEK293 cells stably transfected with opioid receptor subtypes μ, δ, and κ were used to perform the opioid receptor binding assays. These cells were maintained at 37 °C and 5% CO_2 in a DMEM nutrient mixture supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 1000 IU/mL penicillin, 1000 μg/mL of streptomycin, and either 0.5 mg/mL (κ) or 0.2 mg/mL (δ and μ) G418 antibiotic solution. Membranes for the radioligand binding assays were prepared by scraping the cells in cold Tris-HCl, pH 7.4, and then centrifuged at 5200*g* for 10 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in the same buffer, homogenized using a sonic dismembrator model 100 (Fisher Scientific, Pittsburgh, PA, USA) for 30 s, and then centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was saved, and the pellet underwent the suspension and sonication process two additional times under the same conditions. The supernatants were combined and centrifuged at 23300*g* for 40 min at 4 °C. The pellet was resuspended and aliquoted into 2 mL vials and stored at −80 °C. The total protein concentration was determined using a Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions.[18] The optimal membrane and radioligand (K_D) concentrations for each receptor batch were established through membrane evaluation and saturation binding experiments.

Radioligand Displacement for Cannabinoid Receptor Subtypes. Compounds evaluated in this assay were run in competition binding with both cannabinoid receptor subtypes, CB_1 and CB_2.[19] Cannabinoid receptor binding screening was performed under the following conditions: 10 μM of each compound from independent triplicate dilutions was incubated with 1.6975 nM (CB_1) or 1.959 nM (CB_2). [^{3}H]-CP 55,940, a potent cannabinoid agonist with affinity for both receptor subtypes, and 5 μg of CB_1 or 1 μg of CB_2 membrane were incubated for 90 min at 37 °C with gentle agitation in a 96-well plate in a 0.2 mL final volume of 50 mM Tris-HCl, 20 mM EDTA, 154 mM NaCl, and 0.2% radioimmunoassay grade BSA, pH 7.4. The reaction was terminated via rapid vacuum filtration through a UniFilter 96 GF/C filter (PerkinElmer Life Sciences Inc., Boston, MA, USA), presoaked with 0.3% polyethylenimine, followed by 10 washes with 50 mM Tris-HCl, pH 7.4, buffer containing 0.2% BSA. Filters were dried, 25 μL of MicroScint20 was added, and the plates were read using a TopCount NXT microplate scintillation counter (PerkinElmer Life Sciences Inc., Boston, MA, USA). Total binding was defined as binding in the presence of vehicle (1.0% DMSO). Nonspecific binding was the binding observed in the presence of 10.0 μM CP-55,940. Specific binding was defined as the difference between total and nonspecific binding. Percent displacement was calculated using the following formula:

$$100 - (\text{binding of compound} - \text{nonspecific binding}) \times 100/\text{specific binding}$$

Radioligand Displacement for Opioid Receptor Subtypes. All compounds evaluated in the assay were run in competition binding assays against the opioid receptor subtypes (δ, κ, μ). Opioid binding assays were performed under the following conditions: independent triplicate dilutions of 10 μM compound were incubated with 0.85 nM [^{3}H]-DAMGO (μ), 0.91 nM [phenyl-3,4-^{3}H]-U-69,593 (κ), or 0.99 nM [^{3}H]-DPDPE (δ) for 60 min in a 96-well plate in a 0.2 mL final volume of 50 mM Tris-HCl, pH 7.4, with 15 μg (κ) or 20 μg (μ and δ) of membrane. The reaction was terminated via rapid vacuum filtration through a UniFilter 96 GF/B filter presoaked with 0.3% BSA, followed by 10 washes with 4 °C 50 mM Tris-HCl, pH 7.4. Filters were dried, 50 μL of MicroScint20 was added, and the plates were read using a TopCount NXT microplate scintillation counter. Total binding, specific binding, and percent displacement were calculated using the following formula:

$$100 - (\text{binding of compound} - \text{nonspecific binding}) \times 100/\text{specific binding}$$

Nonspecific binding was the binding observed in the presence of 10 μM DAMGO (μ), U-69,593 (κ), or DPDPE (δ).

The competitive binding assay was performed by testing 12 triplicate 3-fold serial dilutions of 300 μM compound and 3 μM control (naloxone hydrochloride) with 15 μg of δ membrane and 1.87 nM [^{3}H]-DPDPE. The K_i values were calculated by a nonlinear curve fit model using GraphPad Prism 5.0 software (GraphPad, La Jolla, CA, USA).

Molecular Modeling and Docking Study. The protein structural file (PDB ID: 4EJ4) was downloaded from the protein databank (http://www.rcsb.org). The protein was subjected to an extensive structural preparation step to correct missing atoms, bond orders, and tautomeric and rotameric states. The receptor grid was

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prepared using the coordinates of the bound ligand. The Fred[20] module of the OpenEye software (www.eyesopen.com) was used for the docking step. All docking poses were then subjected to thermodynamic calculations using SZMAP.[21] The hydrogen atoms were explicitly added to the protein complexes, and then the partial charges were assigned. OH, S, and Met-CH_3 were allowed to rotate during the course of calculation. By using explicit water molecules as probes, SZMAP calculates the free energy of the active site surface.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Comparative functional experiment (Figure S1), bioassay-guided fractionation process (Figure S2), as well as ^1H and ^{13}C NMR and HRESIMS spectra of compounds **1**–**4** (Figures S3–S14). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00218.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Bockaert, J.; Pin, J. P. *EMBO J.* **1999**, *18*, 1723–1729.
(2) Reisine, T.; Brownstein, M. J. *Curr. Opin. Neurobiol.* **1994**, *4*, 406–412.
(3) Felder, C. C.; Joyce, K. E.; Briley, E. M.; Mansouri, J.; Mackie, K.; Lai, Y.; Ma, A. L.; Mitchell, R. L. *Mol. Pharmacol.* **1995**, *48*, 443–450.
(4) MacDonald, J. *Br. J. Pharmacol.* **2006**, *148*, 385–386.
(5) Bagetta, G.; Cosentino, M.; Corasaniti, M. T.; Sakurada, S. *Herbal Medicines. Development and Validation of Plant-Derived Medicines for Human Health*; CRC Press, Taylor & Francis Group: Boca Raton, FL, 2012; p 478.
(6) Terao, J.; Piskula, M. K. In *Flavonoids in Health and Disease*; Rice-Evans, C. A.; Packer, L., Eds.; Marcel Dekker: New York, 1997; pp 277–295.
(7) Tareen, R. B.; Bibi, T.; Khan, M. A.; Ahmad, M.; Zafar, M. *Pak. J. Bot.* **2010**, *42*, 1465–1485.
(8) Zamfirache, M. M.; Burzo, I.; Gostin, I.; Stefan, M.; Padurariu, C.; Olteanu, Z.; Badea, M. L.; Lamban, C.; Truta, E.; Ivanescu, L.; Cojocaru, D. *Carpath. J. Earth Environ.* **2011**, *6*, 261–268.
(9) León, F.; Gao, J.; Dale, O. R.; Wu, Y.; Habib, E.; Husni, A. S.; Hill, R. A.; Cutler, S. J. *Planta Med.* **2013**, *79*, 1756–1761.
(10) Horie, T.; Ohtsuru, Y.; Kenichi, S.; Yamashita, K.; Tsukayama, M.; Kawamura, Y. *Phytochemistry* **1998**, *47*, 865–874.
(11) Suleimenow, E. M.; Smagulova, F. M.; Morozova, O. V.; Raldugin, V. A.; Bagryanskaya, Y.; Gatilov, Y. V.; Yamovoi, V. I.; Adekenov, S. M. *Chem. Nat. Compd.* **2005**, *41*, 689–691.
(12) Zhao, H. Y.; Yang, L.; Wei, J.; Huang, M.; Jiang, J. G. *Food Chem.* **2012**, *135*, 2175–2181.
(13) Granier, S.; Manglik, A.; Kruse, A. C.; Kobilka, T. S.; Thian, F. S.; Weis, W. I.; Kobilka, B. K. *Nature* **2012**, *485*, 400–404.
(14) Katavic, P. L.; Lamb, K.; Navarro, H.; Prisinzano, T. E. *J. Nat. Prod.* **2007**, *70*, 1278–1282.
(15) Webster, D. E.; He, Y.; Chen, S.-N.; Pauli, G. P.; Farnsworth, N. R.; Wang, Z. J. *Biochem. Pharmacol.* **2011**, *81*, 170–177.
(16) Özkay, Ü. D.; Can, Ö. D. *Pharmacol., Biochem. Behav.* **2013**, *109*, 23–30.
(17) Suo, C.; Sun, L.; Yang, S. *Exp. Ther. Med.* **2014**, *7*, 109–116.
(18) Atta-ur-Rahman; Choudhary, M. I.; Thomsen, W. J. *Bioassay Techniques for Drug Development*; Harwood Academic Publisher: Amsterdam, The Netherlands, 2005; pp 167–188.
(19) Ross, R. A.; Gibson, T. M.; Stevenson, L. A.; Saha, B.; Crocker, P.; Razdan, R. K.; Pertwee, R. G. *Br. J. Pharmacol.* **1999**, *128*, 735–743.
(20) *OEDOCKING*, version 3.0.1; OpenEye Scientific Software, Inc.: Santa Fe, NM, http://www.eyesopen.com.
(21) *SZMAP*, version 1.2.0.7; OpenEye Scientific Software, Inc.: Santa Fe, NM, http://www.eyesopen.com.

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