

Total Synthesis and Biological Evaluation of the Marine Bromopyrrole Alkaloid Dispyrin: Elucidation of Discrete Molecular Targets with Therapeutic Potential

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The first total synthesis of dispyrin, a recently reported bromopyrrole alkaloid from *Agelas dispar* with an unprecedented bromopyrrole tyramine motif, was achieved in three steps on a gram scale (68.4% overall). No biological activity was reported for dispyrin, so we evaluated synthetic dispyrin against >200 discrete molecular targets in radioligand binding and functional assays. Unlike most marine natural products, dispyrin (**1**) possesses no antibacterial or anticancer activity, but was found to be a potent ligand and antagonist of several therapeutically relevant GPCRs, the α_{1D} and α_{2A} adrenergic receptors and the H2 and H3 histamine receptors.

Sponges of the genus *Agelas*, found throughout the world's tropical reefs, have provided a wealth of bromopyrrole carboxamide-containing alkaloids derived biosynthetically from oroidin. Examples include the tetracyclic alkaloid (–)-dibromophakelin and the tetrasubstituted cylobutane marine alkaloid (–)-sceptrin.¹ Recently, Crews and co-workers reported on the discovery of a new bromopyrrole alkaloid, dispyrin (**1**), from the Caribbean sponge *Agelas dispar* (Figure 1).² Unlike many laboratories that rely on biofractionation, triaging natural product extracts or sponge/soil samples for antibacterial or anticancer activities, the Crews effort was focused on discovering compounds with novel molecular architectures.^{2,3} Dispyrin (**1**) is unique in that it contains a novel bromopyrrole tyramine motif that has no precedent in marine natural products research. Moreover, unlike all bromopyrrole carboxamide alkaloids discovered from *Agelas* thus far, dispyrin is not biosynthetically derived from oroidin, but rather has an independent biosynthetic pathway.² Crews and co-workers did not ascribe any biological activity for dispyrin **1**; therefore, our laboratory initiated a program to synthesize dispyrin **1** and elucidate the molecular target(s) of this unique bromopyrrole carboxamide alkaloid.

As outlined in Scheme 1, our retrosynthetic analysis of dispyrin (**1**) envisaged an amide coupling between 4-bromo-2-carboxypyrrole (**2**) and 3-bromo-4-hydroxyphenethylamine (**3**), followed by an alkylation or Mitsunobu reaction with *N,N*-dimethyl-3-chloropropylamine or *N,N*-dimethyl-3-hydroxypropylamine (**4**), respectively. This expedited route should allow for the synthesis of gram quantities of dispyrin (**1**) to facilitate biological evaluation as well as an opportunity to readily prepare libraries of unnatural analogues.⁴

As shown in Scheme 2, our synthesis began with commercially available acid **2**, which was coupled to 3-bromo-4-methoxyphenethylamine (**5**) to provide **6** in 93% yield. Subsequent deprotection of the methyl ether with BBr_3 afforded **7** (92% yield). Multiple alkylation protocols were attempted, as well as Mitsunobu protocols, but all failed to deliver dispyrin in reasonable yields. Ultimately, phenol **7** was successfully alkylated with *N,N*-dimethyl-3-chloropropylamine (**4**), under a microwave-assisted protocol (160 °C, 20 min), to deliver dispyrin (**1**) in 80% isolated yield. Thus, the first total synthesis of dispyrin (**1**) was completed on a 1 g scale in three synthetic steps (overall yield of 68.4%).⁵ In the original disclosure by Crews et al., spectroscopic data were reported for what was depicted as the free base of dispyrin (**1**). The spectroscopic data (^1H , ^{13}C NMR and MS) we obtained for synthetic **1** were not in

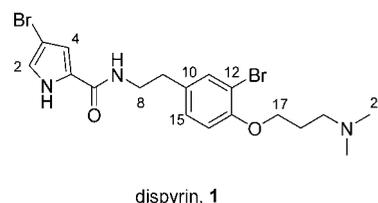
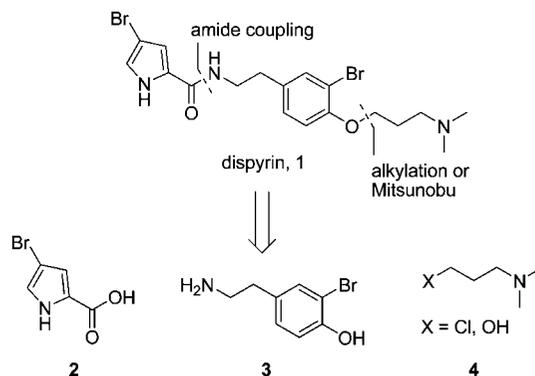


Figure 1. Structure of the dispyrin (**1**), a bromopyrrole alkaloid from *Agelas dispar*.

Scheme 1. Retrosynthetic Analysis of Dispyrin (**1**)

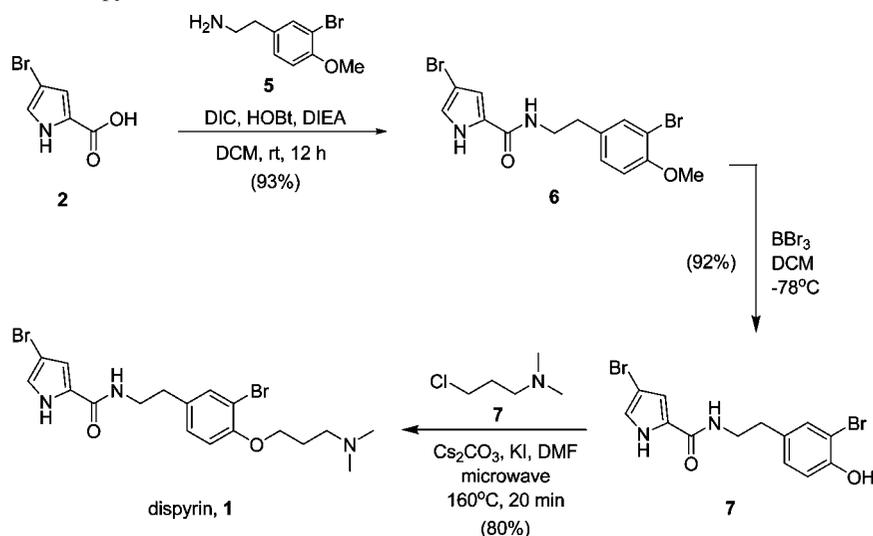
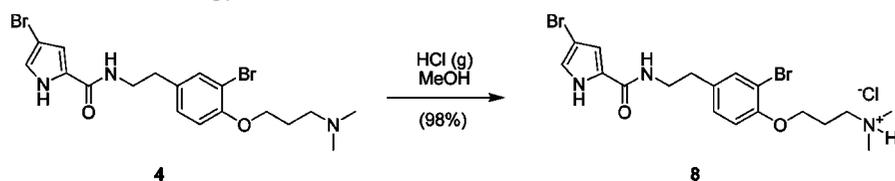


complete accordance with those reported for dispyrin (**1**) by Crews and co-workers.^{2,5}

We rationalized that the discrepancies observed could arise if the spectroscopic data reported for **1** were of the corresponding protonated salt form of the distal dimethylamino moiety. To evaluate this possibility, we synthesized the corresponding HCl salt of our synthetic dispyrin (**1**). As shown in Scheme 3, dispyrin (**1**) was dissolved in MeOH, and HCl gas was bubbled through the solution for 10 min. The reaction solution was concentrated and washed with dry Et_2O . The resulting white solid, the HCl salt of dispyrin (**8**), provided spectroscopic data (^1H , ^{13}C NMR and MS) in complete accordance with those reported for dispyrin (**1**) by Crews and co-workers; thus, the original report of natural dispyrin (**1**) characterized a protonated salt form, such as **8**, and not the free-base **1**.^{2,5}

With a large quantity of dispyrin (**1**) in hand, we embarked on the daunting task of identifying potential molecular target(s) for dispyrin (**1**). With thousands of discrete molecular targets known, it was a challenge to develop a plan to evaluate dispyrin (**1**) against all potential biological targets; moreover, unlike traditional natural products research, we did not want to focus

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Scheme 2. Total Synthesis of Dispyrin (**1**)**Scheme 3.** Synthesis of the HCl Salt of Dispyrin (**8**)

solely on antibacterial and anticancer activity. After careful consideration, we pursued multiple screening avenues. In the pharmaceutical industry, medicinal chemists evaluate late stage preclinical candidates against large panels of discrete molecular targets in an attempt to identify ancillary pharmacology and potential problems. In a paradigm change, we took advantage of this approach, but employed the power of these large panels of G protein-coupled receptors (GPCRs), ion channels, transporters, and kinases to potentially elucidate a molecular target(s) for dispyrin (**1**). Utilizing panels of radioligand binding assays from several companies, dispyrin (**1**) was evaluated against >200 discrete molecular targets over the course of two months. The MDS Pharma Services panel identified multiple activities for dispyrin (**1**).⁶ In the initial screen at a single 10 μM concentration, dispyrin (**1**), as we had anticipated based on its amphoteric properties, was found to provide modest inhibition (50–60% at 10 μM) of calcium (L-type) and potassium (hERG) ion channels, but none with significant activity after full concentration–response curves were obtained (no K_i 's or IC_{50} 's < 10 μM).^{5,6}

Importantly, the MDS panel identified four G protein-coupled receptors (adrenergic $\alpha 1\text{D}$, adrenergic $\alpha 2\text{A}$, H2, and H3 receptors) against which **1** showed promising therapeutic potential.^{7–10} Among the adrenergic family of GPCRs, the $\alpha 1\text{D}$ and $\alpha 2\text{A}$ subtypes are well-documented targets for hypertension, as they contribute to smooth muscle contraction and neural baroreflex control of blood pressure.^{11,12} A number of H2 receptor antagonists are on the market for the treatment of peptic ulcer disease, and the H3 receptor is a well-validated target for a number of CNS pathologies including depression, schizophrenia, ADHD, dementia, and sleep disorders.^{13,14} As shown in Table 1, dispyrin (**1**) displayed significant inhibition in a single-point screen at 10 μM against these four GPCRs, which justified obtaining full dose–response curves. Dispyrin (**1**) showed mid to high nanomolar binding and inhibition of both the adrenergic $\alpha 1\text{D}$ receptor ($K_i = 275$ nM, $\text{IC}_{50} = 560$ nM) and the $\alpha 2\text{A}$ receptor ($K_i = 69$ nM, $\text{IC}_{50} = 185$ nM), while affording low micromolar binding and inhibition of both the H2 receptor ($K_i = 1.02$ μM , $\text{IC}_{50} = 1.25$ μM) and the H3 receptor ($K_i = 1.04$ μM , $\text{IC}_{50} = 2.35$ μM).^{5,6} Thus, dispyrin (**1**) represents a new chemotype and a potential novel lead compound for these therapeutically important

Table 1. Biological Evaluation of Dispyrin (**1**)

target	% inhibition (10 μM) ^a	K_i (μM) ^a	IC_{50} (μM) ^a
Adren $\alpha 1\text{D}$	91	0.275	0.560
Adren $\alpha 2\text{A}$	97	0.069	0.185
H2	91	1.02	1.25
H3	91	1.04	2.35

^a All determinations are the average of at least three independent experiments.^{7–10}

molecular targets, and a rare example of a marine natural product as a ligand for such GPCRs.

In order to evaluate functional activity, dispyrin (**1**) was also placed on the screening deck of the Vanderbilt Screening Center for GPCRs, Ion Channels and Transporters, a member of the Molecular Library Screening Center Network, which employs cell-based functional assays.¹⁵ Thus far, **1** has been evaluated against nine targets in agonist, antagonist, and potentiator mode; however, dispyrin (**1**) has yet to be identified as a hit, but it will remain on the screening deck and will be evaluated in ~ 20 assays/year.

In summary, we have completed the first total synthesis of dispyrin (**1**) on a 1 g scale and demonstrated that the spectroscopic data reported for the natural product **1** were that of a protonated salt form, such as **8**, and not the free base (as reported), and that dispyrin is a potent ligand for therapeutically important GPCRs (the adrenergic $\alpha 1\text{D}$, adrenergic $\alpha 2\text{A}$, H2, and H3 receptors). Although the role of natural products discovery efforts within the pharmaceutical industry is being significantly reduced, despite overwhelming success, the biological activity of dispyrin argues further that natural products are viable leads for therapeutically relevant targets.

Experimental Section

General Experimental Procedures. All NMR spectra were recorded on a 400 MHz Bruker AMX NMR spectrometer. ¹H chemical shifts are reported in δ values in ppm downfield from TMS as the internal standard in DMSO-*d*₆. ¹³C chemical shifts are reported as δ values in ppm with the DMSO-*d*₆ carbon peak set to 39.5 ppm. UV absorption was recorded on a DU 800 spectrophotometer and reported in nm. IR spectra were recorded on KBr plates using a Thermo IR 100

spectrometer and reported in cm^{-1} . Low-resolution mass spectra were obtained on an Agilent 1200 LCMS with electrospray ionization. High-resolution mass spectra were recorded on a Waters QToF-API-US plus Acquity system with electrospray ionization. Analytical thin-layer chromatography was performed on 250 μM silica gel 60 F₂₅₄ plates. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. Analytical HPLC was performed on an Agilent 1200 analytical LCMS with UV detection at 214 and 254 nm along with ELSD detection. All reactions were carried out under an argon atmosphere employing standard chemical techniques. Solvents for extraction, washing, and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. at the highest commercial quality and were used without purification. Microwave-assisted reactions were conducted using a Biotage Initiator-60. All yields refer to analytically pure and fully characterized materials (¹H NMR, ¹³C NMR, analytical LCMS, and HRMS).

4-Bromo-N-(3-bromo-4-methoxyphenethyl)-1H-pyrrole-2-carboxamide (6). To a stirred solution of acid **1** (1.00 g, 5.3 mmol), *N*-hydroxybenzotriazole (HOBt) (1.50 g, 11.0 mmol), and amine **5** (1.21 g, 5.3 mmol) in 9:1 $\text{CH}_2\text{Cl}_2/\text{DIEA}$ at 25 °C was added diisopropylcarbodiimide (DIC) (1.33 g, 10.6 mmol), and the mixture was stirred overnight. After quenching with 250 mL of H_2O , the reaction was added to a 500 mL separatory funnel and washed with 3×200 mL of CH_2Cl_2 . The organic layers were combined and washed with 500 mL of saturated aqueous brine solution. The organic layer was dried over MgSO_4 and concentrated *in vacuo* to yield the crude coupled product. The crude material was then subjected to flash chromatography (EtOAc/hexanes, 1:1) to give pure **6** as a white solid (1.98 g, 4.9 mmol, 93% yield): UV (MeOH) λ_{max} (log ϵ) 288 (2.06) nm; IR (KBr) ν_{max} 3224, 2941, 1631, 1565, 1521, 1495, 1455, 1429, 1383, 1345, 1325, 1276, 1255, 1130, 1055, 1019, 921, 823, 759, 744, 600 cm^{-1} ; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.13 (t, *J* = 5.6 Hz, 1H), 7.43 (d, *J* = 2.0 Hz, 1H), 7.17 (dd, *J* = 1.6, 8.4 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.96 (m, 1H), 6.82 (s, 1H), 3.80 (s, 3H), 3.40 (q, *J* = 6.8 Hz, 2H), 2.74 (t, *J* = 7.2 Hz, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 159.5, 153.7, 133.3, 132.9, 129.1, 126.9, 121.1, 112.5, 111.3, 110.4, 94.9, 56.1, 40.1, 33.8; HRMS (Q-TOF) *m/z* 400.9517 (calc for $\text{C}_{14}\text{H}_{14}\text{Br}_2\text{N}_2\text{O}_2$, 400.9500).

4-Bromo-N-(3-bromo-4-hydroxyphenethyl)-1H-pyrrole-2-carboxamide (7). To a stirred solution of coupled material **6** (1.00 g, 2.5 mmol) in anhydrous CH_2Cl_2 under argon at -78 °C was added BBR_3 (10 mL, 10 mmol, 1.0 M solution in CH_2Cl_2) over 20 min. The solution was stirred at -78 °C for 30 min and then allowed to warm to 25 °C for 1.5 h. The reaction was slowly quenched with saturated aqueous NaHCO_3 until slightly basic by pH paper. This solution was added to a 1 L separatory funnel containing 500 mL of H_2O and extracted with 3×300 mL of CH_2Cl_2 . The combined organic layers were washed with 500 mL of saturated aqueous brine solution. The organic layer was dried over MgSO_4 and concentrated *in vacuo* to yield the deprotected product **7** (0.89 g, 2.3 mmol, 92% yield). This material was used without further purification. UV (MeOH) λ_{max} (log ϵ) 288 (1.57) nm; IR (KBr) ν_{max} 3409, 1608, 1564, 1508, 1425, 1384, 1327, 921, 600 cm^{-1} ; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.98 (br s, 1H) 8.11 (t, *J* = 5.2 Hz, 1H), 7.33 (d, *J* = 1.6 Hz, 1H), 7.02 (dd, *J* = 2.0, 8.4 Hz, 1H), 6.95 (m, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.80 (d, *J* = 2.0 Hz, 1H), 3.37 (m, 2H), 2.68 (t, *J* = 6.8 Hz, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 159.5, 152.3, 132.7, 131.6, 128.9, 126.9, 121.0, 116.2, 111.3, 109.0, 94.9, 40.2, 33.9; HRMS (Q-TOF) *m/z* 386.9359 (calc for $\text{C}_{13}\text{H}_{12}\text{Br}_2\text{N}_2\text{O}_2$, 386.9344).

4-Bromo-N-(3-bromo-4-(3-(dimethylamino)propoxy)phenethyl)-1H-pyrrole-2-carboxamide, Dispyrin (1). In a 20 mL microwave vial containing **4** (1.0 g, 2.6 mmol), amine **7** (0.49 g, 3.1 mmol), KI (1.29 g, 7.8 mmol), and Cs_2CO_3 (2.54 g, 7.8 mmol) was added anhydrous DMF (15 mL). This was heated under microwave conditions at 160 °C for 20 min. The reaction was filtered, concentrated *in vacuo*, and purified via reversed-phase HPLC to obtain pure dispyrin as the TFA salt. This material was dissolved in a minimal amount of MeOH and added to a 60 mL SCX solid-phase extraction column, which was washed with 2 column volumes of MeOH. The material was removed from the column by eluting with 2 column volumes of 2 M NH_3 in MeOH. This was again concentrated *in vacuo* to obtain pure dispyrin (**1**) as the free base (0.98 g, 2.1 mmol, 80% yield): UV (MeOH) λ_{max} (log ϵ) 293 (2.40) nm; IR (KBr) ν_{max} 3223, 2948, 2864, 2825, 2780, 2360, 2340, 1629, 1565, 1523, 1495, 1467, 1386, 1325, 1279, 1253, 1054, 668 cm^{-1} ; ¹H NMR (400 MHz, MeOH-*d*₄) δ 7.42 (d, *J* = 2.0

Hz, 1H), 7.14 (dd, *J* = 2.0, 8.4 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 1.2 Hz, 1H), 6.70 (d, *J* = 1.6 Hz, 1H), 4.05 (t, *J* = 6.0 Hz, 2H), 3.47 (t, *J* = 7.6 Hz, 2H), 2.78 (t, *J* = 7.2 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 2.30 (s, 6H), 1.99 (m, 2H); ¹³C NMR (100 MHz, MeOH-*d*₄) δ 162.5, 155.2, 134.5, 130.1, 127.6, 122.7, 114.6, 113.1, 112.9, 97.4, 68.2, 57.4, 49.0, 45.4, 42.0, 35.6, 28.0; HRMS (Q-TOF) *m/z* 472.0243 (calc for $\text{C}_{18}\text{H}_{23}\text{Br}_2\text{N}_3\text{O}_2$, 472.0235).

4-Bromo-N-(3-bromo-4-(3-(dimethylamino)propoxy)phenethyl)-1H-pyrrole-2-carboxamide Hydrochloride, Dispyrin-HCl (8). Dispyrin (**1**) (400 mg, 845 mmol) was dissolved in MeOH (20 mL). HCl gas was bubbled through the solution for 10 min. The solvent was removed *in situ* and washed with anhydrous ether (3×20 mL) to afford Dispyrin-HCl (**8**) as a white solid (420 mg, 98%): UV (MeOH) λ_{max} (log ϵ) 287 (1.48) nm; IR (KBr) ν_{max} 3232, 2955, 2360, 2341, 1676, 1629, 1566, 1496, 1466, 1253, 1202, 1132, 1055, 799, 721 cm^{-1} ; ¹H NMR (400 MHz, MeOH-*d*₄) δ 7.45 (d, *J* = 2.0 Hz, 1H), 7.17 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 1.6 Hz, 1H), 6.72 (d, *J* = 1.6 Hz, 1H), 4.15 (t, *J* = 5.6 Hz, 2H), 3.48 (t, *J* = 7.6 Hz, 2H), 3.39 (t, *J* = 7.6 Hz, 2H), 2.96 (s, 6H), 2.80 (t, *J* = 7.2 Hz, 2H), 2.25 (m, 2H); ¹³C NMR (100 MHz, MeOH-*d*₄) δ 162.6, 154.7, 135.3, 134.6, 130.4, 127.6, 122.8, 114.8, 113.3, 112.9, 97.5, 67.5, 57.2, 43.8, 42.0, 35.6, 25.7; HRMS (Q-TOF) *m/z* 472.0235 (calc for $\text{C}_{18}\text{H}_{24}\text{Br}_2\text{N}_3\text{O}_2$, 472.0234).

Biological Assays. All biological assays were conducted at MDS Pharma according to published protocols.^{7–10}

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Supporting Information Available: General experimental procedures, preparation of compounds **1**, **6**, **7**, and **8**, along with ¹H NMR, ¹³C NMR, and high-resolution mass spectral data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) (a) Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc., Chem. Commun.* **1971**, 1129–1130. (b) Garcia, E. E.; Benjamin, R. I.; Fryer, R. I. *J. Chem. Soc., Chem. Commun.* **1973**, 78–79. (c) Keiffer, P. A.; Schwartz, R. E.; Koker, M. E.; Hughes, R. G.; Rittschof, D.; Reinhart, K. L. *J. Org. Chem.* **1977**, *42*, 2965–2875. (d) Gautschi, J. T.; Whitman, S.; Holman, T. R.; Crews, P. *J. Nat. Prod.* **2004**, *67*, 1256–1261.
- (2) Pina, I. C.; White, K. N.; Cabrera, G.; Rivero, E.; Crews, P. *J. Nat. Prod.* **2007**, *70*, 613–617.
- (3) Miller, C. A.; Kauffman, P. R.; Jensen, Fenical, W. *J. Org. Chem.* **2007**, *72*, 323–330.
- (4) (a) Tan, D. S.; Foley, M.; Shair, M. D.; Shreiber, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 422–423. (b) Pelish, H. E.; Westwood, N.; Feng, Y.; Kirchhausen, T.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 6740–6741. (c) Lindsley, C. W.; Bogusky, M. J.; Leister, W. H.; McClain, R. T.; Robinson, R. R.; Barnett, S. F.; Defeo-Jones, D.; Ross, C. W.; Hartman, G. D. *Tetrahedron. Lett.* **2005**, *46*, 2279–2782.
- (5) See Supporting Information for full experimental details.
- (6) For information on MDS Pharma Services, assay details, and available molecular targets see: www.mdsp.com.
- (7) Kenny, B. A.; Chalmers, D. H.; Philpott, P. C.; Naylor, A. M. *Br. J. Pharmacol.* **1995**, *115*, 981–986.
- (8) Uhlen, S.; Porter, A. C.; Neubig, R. R. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1558–1565.
- (9) Ruat, M.; Traiffort, E.; Bouthenet, M. L.; Schwartz, J. C.; Hirschfeld, J.; Buschauer, A.; Schunack, W. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1658–1662.
- (10) (a) Yanai, K.; Ryu, J. H.; Sakai, N.; Takahashi, T.; Iwata, R.; Ido, T.; Murakami, K.; Wantanbe, T. *Jpn. J. Pharmacol.* **1994**, *65*, 107–112. (b) Zhu, Y.; Machalovich, D.; Wu, H.-L.; Tan, K. B.; Dytko, G. M.; Mannan, I. J.; Boyce, R. J.; Alston, J.; Tierney, L. A.; Li, X.; Herrity, N. C.; Vawter, L.; Sarau, H. M.; Ames, R. S.; Davenport, C. M.; Hieble, J. P.; Wilson, S.; Bergsma, D. J.; Fitzgerald, L. R. *Mol. Pharmacol.* **2001**, *59*, 434–441.
- (11) (a) Piascik, M. T.; Guarino, R. D.; Smith, M. S.; Soltis, E. E.; Saussy, D. L.; Perez, D. M. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 1583–1588. (b) Chu, C.-P.; Kunitake, T.; Kato, K.; Watanabe, S.; Qui, D.-L.; Tanoue, A.; Kannan, H. *Neurosci. Lett.* **2004**, *356*, 33–36.
- (12) Makartus, K.; Johns, C.; Gavras, I.; Altman, J. D.; Handy, D. E.; Bresnahan, M. R.; Gavras, H. *Hypertension* **1999**, *34* (3), 403–407.
- (13) Ganellin, C. R. *Analogous-Based Drug Discovery* **2006**, 71–80.

- (14) Berlin, M.; Boyce, C. W. *Exp. Opin. Ther. Patent* **2007**, *17*, 675–687.
- (15) (a) For detailed information on the NIH Roadmap and the MLSCN see: <http://mli.nih.gov/mlscn/index.php/RAJIV15>. (b) For details on the Vanderbilt Screening Center for GPCRs Ion Channels and

Transporters see: www.vanderbilt.edu/MLSCN. (c) Lindsley, C. W.; Weaver, D.; Jones, C.; Marnett, L.; Conn, P. J. *ACS Chem. Biol.* **2007**, *2*, 17–20.

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