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Synthesis, characterization, and biological assessment of the four stereoisomers of the H_3 receptor antagonist 5-fluoro-2-methyl-*N*-[2-methyl-4-(2-methyl[1,3′]bipyrrolidinyl-1′-yl)phenyl]benzamide

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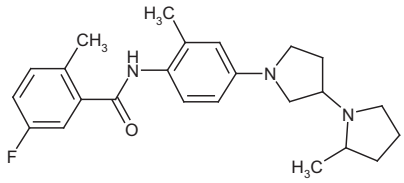
ABSTRACT

This Letter describes the asymmetric synthesis of the four stereoisomers (**8a**–**8d**) of a potent and highly selective histamine H_3 receptor (H_3R) antagonist, 5-fluoro-2-methyl-*N*-[2-methyl-4-(2-methyl[1,3′]bipyrrolidinyl-1′-yl) phenyl]benzamide (**1**). The physico-chemical properties, in vitro H_3R affinities and ADME of **8a**–**8d** were determined. Stereoisomer **8c** (2*S*,3′*S*) displayed superior in vitro H_3R affinity over other three stereoisomers and was selected for further profiling in in vivo PK and drug safety. Compound **8c** exhibited excellent PK properties with high exposure, desired brain to plasma ratio and reasonable brain half life. However, all stereoisomers showed similar unwanted hERG affinities.

In the past decade, histamine H_3 receptor (H_3R) has emerged as a promising therapeutic target for the treatment of obesity.[1–6] Obesity is a significant health problem worldwide. Global obesity rates have increased steadily in both developed and emerging countries over the past several decades with little sign of abating. Over 1.5 billion people[7] worldwide are overweight or obese and over 40 million children under the age of 5 are overweight. This condition is associated with a number of co-morbidities including cardiovascular diseases, type II diabetes mellitus, hypertension, cancers, and musculoskeletal disorders. Management strategies for weight reduction in obese individuals include changes in life style such as exercise and diet, behavioral therapy, and drug treatment, and in certain cases surgical intervention. Drug treatment offers a potential solution, at least as a possible adjunct to diet and exercise. In the past, several drugs were used as therapy of weight reduction including thyroid hormone, dinitrophenol, amphetamines and their analogs, for example fenfluramine. At present, orlistat[8] is the only FDA-approved medication for the longer term treatment (⩾24 weeks) of obesity. There exist unmet medical needs for safe and efficacious anti-obesity treatments.

Previous work[9,10] from our laboratories described a series of compounds, represented by 5-fluoro-2-methyl-*N*-[2-methyl-4-(2-methyl[1,3′]bipyrrolidinyl-1′-yl) phenyl]benzamide (**1**), (Fig. 1) which displayed oral activity in a mouse food intake inhibition model. The compound was potent in in vitro H_3R affinity and functional assays across species (human, rhesus monkey, and rat) and exhibited good in vitro metabolic stability and high permeability



1
rh-H_3R binding Ki = 1.2 nM
h-H_3R binding Ki = 8.6 nM
r-H_3R binding Ki = 16.5 nM
rh-H_3R GTPγS EC_{50} = 1.1 nM
solubility = 2.67 mg/mL
hERG IC_{50} = 0.48 μM

Figure 1. Structure of compound **1**.

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in Caco2, no inhibition of key cytochrome P450 isoenzymes (IC_{50} values >30 μM for CYP 1A2, 2C9, 2C19, 2D6, and 3A4), and high selectivity towards a panel of 78 GPCRs, ion channels, enzymes, and kinases, particularly human histamine H_1 receptor (h-H_1R), H_2 receptor (h-H_2R), and H_4 receptor (h-H_4R) (<50% inhibition @ 10 μM). However, compound **1** displayed unacceptably high affinity toward hERG channel (IC_{50} = 0.48 μM). Therefore, we needed to continue our optimization to identify a 'best in class' type of development candidate for treatment of obesity. Realizing that **1** is a mixture of four stereoisomers, before we embarked on the optimization, we decided to synthesize all four stereoisomers of **1** in order to determine if a specific stereoisomer retained high H_3R affinity while devoid of hERG channel inhibition. Herein, we describe the asymmetric synthesis, physico-chemical characterizations, and ADME properties, as well as biological activities of all four stereoisomers of **1**.

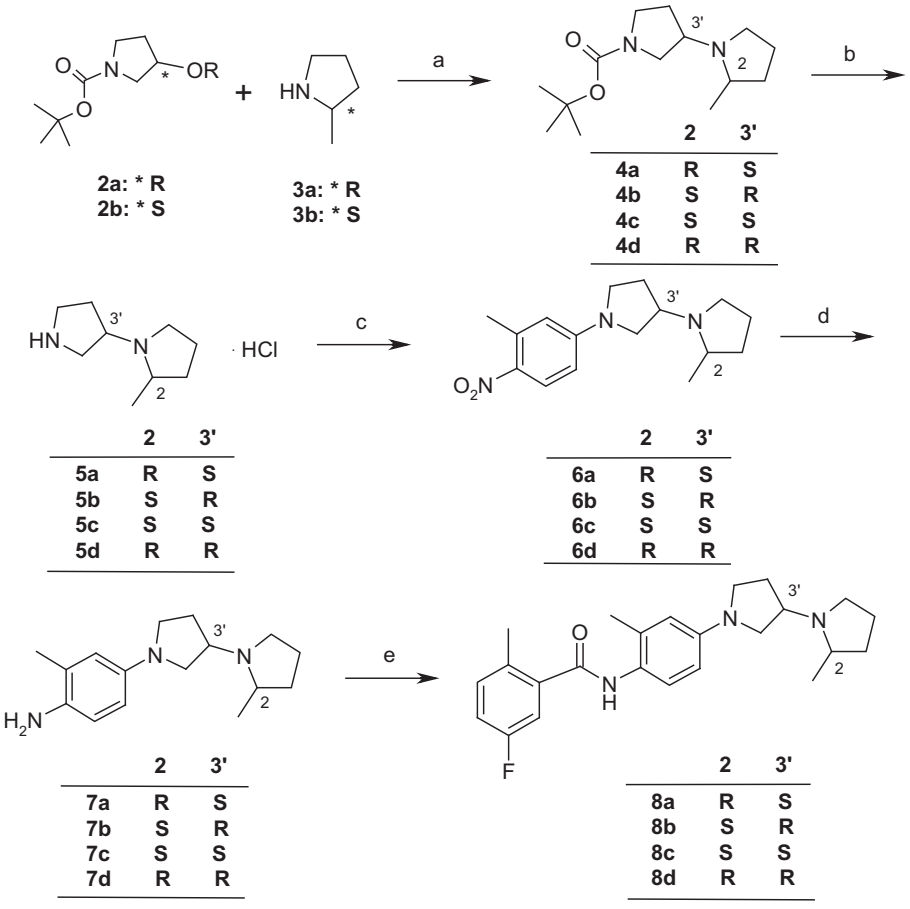
The syntheses of the stereoisomers of **1** were carried out as outlined in Scheme 1. The commercially available (*R*)- or (*S*)-3-BOC-pyrrolidin-3-ol (**2a**) or (**2b**) (R = H) were transformed into corresponding tosylate **2a** or **2b** (R = Ts), respectively. These intermediates (**2a** and **2b**, R = Ts) were condensed with commercially available (*R*)- or (*S*)-2-methyl-pyrrolidines, **3a** or **3b**, respectively, to obtain the four optically pure isomers **4a**–**4d** in good yield (64–68%). These four intermediates were individually treated with HCl in dioxane to release the free amines **5a**–**5d**, which were condensed with 5-fluoro-2-nitrotoluene to yield the four optically pure intermediates **6a**–**6d**. The optical purities of these four compounds were assessed at this stage using chiral HPLC equipped with UV detector. The results (Table 1) showed that the optical purities were >99% for all four stereoisomers **6a**–**6d**.

Table 1
Chiral HPLC analyses of intermediates **6a**–**6d**[a]

Stereoisomer	t_R (min)	Optical purities (%)
6a	10.92	>99
6b	11.93	>99
6c	8.16	>99
6d	8.95	>99

[a] Chiral HPLC conditions: instrument used was Mettler-Toledo AutoChem Inc. Berger SFC Analytix–Minigram; column: Chiralpak AS, 10 μM, 250 × 4.6 mm; eluent: 90% CO_2/10% iso-propanol with 0.5% isopropylamine; outlet pressure: 150 bars; flow rate: 5.0 mL/min.; detection: UV 224 nm; injection volume: 20 μL; concentration: ~1.0 mg/mL.

The nitro group of intermediates **6a**–**6d** was hydrogenated to obtain the anilines **7a**–**7d** which were coupled with 2-methyl-5-fluorobenzoic acid or acid chloride under standard coupling conditions to obtain the desired final stereoisomers **8a**–**8d**. The structures of these stereoisomers were characterized spectroscopically.[11] Their physico-chemical properties were also determined (Table 2). The specific rotations, within experimental error, were in good alignment as two enantiomeric pairs (**8a** vs **8b** and **8c** vs **8d**). Crystals of all four stereoisomers were obtained by recrystallization from DCM and methyl *t*-butyl ether. The physical states were characterized as crystlline material for **8a**–**8d** by polarized light microscopy and X-ray powder diffraction (XRPD). The DSC thermogram of the compounds exhibited one endothermic peak with onset



Scheme 1. Syntheses of the stereoisomers of **1**. Reagents and conditions: (a) (1) optically pure (*R*) or (*S*)- 3-Boc-pyrrolidin-3-ol, TsCl, TEA, DMAP, DCM, 75%; (2) optically pure (*R*)- or (*S*)-2-methyl-pyrrolidine, K_2CO_3, CH_3CN, 75 °C, 20 h, 64–68%; (b) 4 M HCl/dioxane, 2 equiv, 100%; (b) 5-fluoro-2-nitrotoluene, K_2CO_3, DMSO, 85 °C, overnight; (d) H_2, 10% Pd/C, MeOH, rt, 4 h, 85%; (e) 2-methyl-5-fluoro-benzoic acid, EDC·HCl, HOBt, *N*-methyl morpholine, DCM, DMF, 0 °C to rt, 82%.

Table 2
Physico-chemical characterization of the stereoisomers **8a**–**8d**

	8a	8b	8c	8d
Stereochemistry	2*R*,3′*S*	2*S*,3′*R*	2*S*,3′*S*	2*R*,3′*R*
Specific rotation[a]	−28.49°	+27.14°	+22.70°	−24.41°
Melting point[b] (°C)	149.0	149.4	123.0	123.5
Solubility in PBS (pH 7.4) (μg/mL)	ND	88.5	293.4	ND
pKa_1[c]	6.3	6.3	6.3	6.3
pKa_2[c]	8.9	8.9	8.9	8.9
log*P*[c]	ND	3.55	3.55	ND
log*D*[c]	ND	2.09	2.09	ND

[a] Solvent: MeOH; concentration (w/v): compound **8a**: 0.516%; compound **8b**: 0.560%; compound **8c**: 0.540%; compound **8d**, 0.504%.
[b] Melting points were determined by DSC using instrument of 2910 M DSC V4.4E, scanning range of −10 to 300 °C.
[c] Determined experimentally. ND = not determined.

Table 3
H3R binding affinity, functional antagonism activity, and ADMET characteristics

Assays	Species	Compounds			
		8a	8b	8c	8d
H3R binding Ki[a] (nM)	Rhesus	121.0	7.3	2.5	147.0
	Human	819.7	18.0	3.4	300.0
	Rat	93.0	11.0	3.0	73.7
H3R FLIPR IC50[b] (nM)	Human	0.66	0.0092	0.0060	0.71
Microsomal lability[c] (%)	Human	7.0	5.0	3.0	8.0
	Mouse	29.0	20.0	19.0	17.0
	Rat	60.0	36.0	20.0	27.0

[a] Binding assay was performed as in Ref. 10; K_i data for all the species were presented as a range of multiple experiments ($n \geqslant 3$). ND = not determined.
[b] Human H_3R FLIPR data is reported as an averge of $n = 3$.
[c] Microsomal lability was performed using human, mouse, and rat liver microsomes preparation (in house) incubated at 37 °C for 20 min with 5 μM concentration of the substrate. The data was expressed as the percentage of the parent compounds metabolized.

temperature which was confirmed as a melting event by microscopic hotstage. The TGA thermogram did not show a significant weight loss for all the stereoisomers **8a**–**8d**.[12]

The affinity of **8a**–**8d** for the H_3R was evaluated in binding assays by displacement of [^{3}H]*N*-α-methylhistamine in membranes isolated from a CHO cell line stably transfected with the recombinant human H_3 receptor (h-H_3R), the rhesus monkey H_3 receptor (rh-H_3R) or the rat H_3 receptor (r-H_3R) (Table 3). These stereoisomers were found to be very different in H_3R affinity. The affinity of **8c** toward rh-H_3R is 59-fold higher than **8d**, 48-fold higher than **8a**, and 3-fold higher than **8b**. The stereoisomer **8c** also displayed higher affinity toward h-H_3R and r-H_3R than the rest of the stereoisomers. The same trend was also observed in functional behavior (FLPR data, Table 3). These results indicated that 2*S* stereochemistry was preferred for activity (compare **8c** vs **8a**).[13] In contrast, there was little to no preference in activity for either *R* or *S* substituents at 3'-position. All four stereoisomers showed acceptable in vitro metabolic stabilities. However, the differences were appreciable. For example, in rat liver microsome preparations, **8c** was 3-fold more stable than **8a**.

Due to the superiority of **8c** in affinity and in vitro PK parameters, this stereoisomer was selected for further profiling. The compound showed P450 enzyme inhibition IC_{50} value >30 μM for all the isozymes tested (CYP 1A2, 2B6, 2C9, 2C19, 2D6, and 3A4). In a mouse PK experiment, **8c** showed high exposure in plasma ($AUC_{0-\infty}$ of 4000 ng h/mL) and in brain ($AUC_{0-\infty}$ = 18,000 ng h/mL) when dosed orally at 10 mg/kg. The compound showed short on-board time (p.o., t_{max} of 0.17 h to plasma, t_{max} of 1.0 h to brain), good $t_{1/2}$ (p.o., plasma $t_{1/2}$ = 3.6 h, brain $t_{1/2}$ = 3.5 h) for potential use in CNS indications (Table 4). The brain to plasma ratio based on AUC_{0-24} was 4.6 consistent with that observed in the intravenous study. There was less concern of brain retention of the compound because the brain half life ($t_{1/2}$ = 3.5 h, p.o.) was in the desired range.[14,15]

The behavioral safety of **8c** was evaluated orally in mice at 3, 10, and 30 mg/kg doses. Compound **8c** increased tail pinch response ($\geqslant$10 mg/kg) and slightly increased the body temperature (6 h, 30 mg/kg). At 2 h post dosing, tremors were observed for the 30 mg/kg group only; at 6 h post dosing, increased tail pinch response was demonstrated for the 30 mg/kg group. There was no effect on pupil size or body weight attributable to the administration of **8c**. There were no abnormal clinical signs observed during the one-week post-dose observation period.

In further profiling, it was found that all stereoisomers **8a**–**8d** displayed similar unwanted hERG affinities (Table 5). This led us

Table 4
Pharmacokinetic* parameters of **8c**

		C_{max} (ng/mL)	t_{max} (h)	$AUC_{(0-\infty)}$ (ng h/mL)	$t_{1/2}$ (h)	Cl (L/h/kg)	V_{dss} (L/kg)	Brain/plasma $AUC_{(0-24)}$ ratio
i.v.	Plasma	2000	0.083	560	2.6	3.6	5.2	
	Brain	4820	0.083	2600	1.6	—	—	4.7
p.o.	Plasma	1310	0.17	4000	3.6	—	—	
	Brain	4290	1.00	18,000	3.5	—	—	4.6

i.v. Dosing at 2 mg/kg in 50% 1-methyl-2-pyrrolidinone in saline (1 mg/mL).
p.o. Dosing at 10 mg/kg in 5% DMSO/0.5% MC/0.2% tween 80 (1 mg/mL).
* PK was run with male C57BL/6 mouse.

Table 5
Safety assessment of **8a**–**8d**

Compound		8a	8b	8c	8d
Stereochemistry		2*R*,3′*S*	2*S*,3′*R*	2*S*,3′*S*	2*R*,3′*R*
hERG affinity[a]	% Inhibition @ 1.0 μM	43.0	38.8	43.4	33.7
	% Inhibition @ 10 μM	84.3	88.6	84.3	77.5
	IC_{50} (μM)	ND	1.8	1.1	ND
Cyp inhibition[b]	IC_{50} (μM)	>30	>30	>30	>30
Ames II		Not mutagenic	Not mutagenic	Not mutagenic	Not mutagenic
MNT test		Negative	Negative	Negative	Negative

[a] hERG was determined using patch-clamp technique in the whole-cell configuration on Chinese hamster ovary (CHO) cells stably transfected with the human ERG gene. ND = not determined.
[b] Cytochrome P450 isoenzymes determined: 1A2, 2B6, 2C9, 2C19, 2D6, and 3A4.

to postulate that the hERG affinity of **1** may arise from the overly lipophilic nature of the molecule. Different stereochemistry at 2-methyl[1,3′]bipyrrolidinyl-1′-yl portion of the molecule could not provide enough differentiation for hERG channel affinity among stereoisomers. Therefore, a different strategy to address the hERG issue should be sought.

In conclusion, we have developed an asymmetric synthetic route which allows the syntheses of all four stereoisomers **8a**–**8d** of a potent and selective H_3R antagonist, 5-fluoro-2-methyl-*N*-[2-methyl-4-(2-methyl-[1,3′]bipyrrolidinyl-1′-yl)phenyl]benzamide (**1**) individually with high optical activity (optical purity >99% for all four stereoisomers). The physico-chemical properties, in vitro H_3R affinities and ADME of **8a**–**8d** were characterized. Stereoisomer **8c** (2*S*,3′*S*) was selected for further profiling in in vivo PK and drug safety. Compound **8c** displayed superior in vitro H_3R affinity and functional antagonism over other three stereoisomers and excellent PK properties with high exposure, desired brain to plasma ratio and reasonable brain half life. There was less concern on brain retention of this compound. However, all stereoisomers displayed similar unwanted hERG affinities. These results should direct the future optimization to focus on 2*S* configuration (as seen in **8c**) by introducing polar functional groups to lower log*P* and to increase total polar surface area (TPSA) in order to address hERG channel affinity issue. The continued optimization is currently underway and the progress will be communicated in due course.

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References and notes

1. Passani, M. B.; Blandina, P.; Torrealba, F. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 24.
2. Lazewska, D.; Kiec-Kononowicz, K. *Expert Opin. Ther. Pat.* **2010**, *20*, 1147.
3. Yoshimoto, R.; Miyamoto, Y.; Shimamura, K.; Ishihara, A.; Takahashi, K.; Kotani, H.; Chen, A. S.; Chen, H. Y.; Macneil, D. J.; Kanatani, A.; Tokita, S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13866.
4. Tokita, S.; Takahashi, K.; Kotani, H. *J. Pharmacol. Sci.* **2006**, *101*, 12.
5. Esbenshade, T. A.; Fox, G. B.; Cowart, M. D. *Mol. Interv.* **2006**, *6*, 77.
6. Hancock, A. A.; Diehl, M. S.; Fey, T. A.; Bush, E. N.; Faghih, R.; Miller, T. R.; Krueger, K. M.; Pratt, J. K.; Cowart, M. D.; Dickinson, R. W.; Shapiro, R.; Knourek-Segel, V. E.; Droz, B. A.; McDowell, C. A.; Krishna, G.; Brune, M. E.; Esbenshade, T. A.; Jacobson, P. B. *Inflamm. Res.* **2005**, *54*, S27.
7. Nguyen, T.; Lau, D. C. *Can. J. Cardiol.* **2012**, *28*, 326.
8. Kang, J. G.; Park, C. Y. *Diabetes Metab. J.* **2012**, *36*, 13.
9. Gao, Z.; Hurst, W. J.; Guillot, E.; Czechtizky, W.; Lukasczyk, U.; Nagorny, R.; Pruniaux, M. P.; Schwink, L.; Sanchez, J. A.; Stengelin, S.; Tang, L.; Winkler, I.; Hendrix, J. A.; George, P. G. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3416.
10. Gao, Z.; Hurst, W. J.; Guillot, E.; Czechtizky, W.; Lukasczyk, U.; Nagorny, R.; Pruniaux, M. P.; Schwink, L.; Sanchez, J. A.; Stengelin, S.; Tang, L.; Winkler, I.; Hendrix, J. A.; George, P. G. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3421.
11. *Analytical data:* LC method: SYNERGI 2U HYDRO-RP 20 × 4.0 mm column, 0.1% TFA in water/acetonitrile 5–40% acetonitrile in 2 min. then, to 95% acetonitrile at 5 min at flow rate of 1.0 mL/min;
Compound **8a**: LC–MS: R_T = 1.61 min, MS: 396 ($M+H^+$).
^1H NMR (DMSO-d_6, 300 MHz), δ (ppm): 7.50 (d, 7.4 Hz, 1H), 7.25 (m, 1H), 7.05 (m, 2H), 6.40 (m, 2H), 3.51–3.20 (m, 5H), 3.00 (m, 1H), 2.78 (m, 1H), 2.58 (m, 1H), 2.50 (s, 3H), 2.28 (s, 3H), 2.05–1.95 (m, 3H), 1.95–1.65 (m, 3H), 1.45 (m, 1H), 1.08 (d, 6.4 Hz, 3H).
Compound **8b**: LC–MS: R_T = 1.61 min, MS: 396 ($M+H^+$).
^1H NMR (DMSO-d_6, 300 MHz), δ (ppm): 7.50 (d, 7.4 Hz, 1H), 7.25 (m, 1H), 7.05 (m, 2H), 6.40 (m, 2H), 3.51–3.20 (m, 5H), 3.00 (m, 1H), 2.78 (m, 1H), 2.58 (m, 1H), 2.50 (s, 3H), 2.28 (s, 3H), 2.05–1.95 (m, 3H), 1.95–1.65 (m, 3H), 1.45 (m, 1H), 1.08 (d, 6.4 Hz, 3H).
Compound **8c**: LC–MS: R_T = 2.14 min, MS: 396 ($M+H^+$).
^1H NMR (DMSO-d_6, 300 MHz), δ (ppm): 7.48 (d, 7.4 Hz, 1H), 7.22 (m, 1H), 7.04 (m, 2H), 6.42 (m, 2H), 3.53 (m, 1H), 3.42–3.18 (m, 4H), 3.05 (m, 1H), 2.81 (m, 1H), 2.54 (m, 1H), 2.49 (br s, 3H), 2.28 (br s, 3H), 2.20–1.95 (m, 3H), 1.9–1.9 (m, 2H), 1.60 (br, 1H), 1.48 (m, 1H), 1.15 (d, 6.0 Hz, 3H).
Compound **8d**: LC–MS: R_T = 2.13 min, MS: 396 ($M+H^+$).
^1H NMR (DMSO-d_6, 300 MHz), δ (ppm): 7.48 (d, 7.4 Hz, 1H), 7.22 (m, 1H), 7.04 (m, 2H), 6.42 (m, 2H), 3.53 (m, 1H), 3.42–3.18 (m, 4H), 3.05 (m, 1H), 2.81 (m, 1H), 2.54 (m, 1H), 2.49 (br s, 3H), 2.28 (br s, 3H), 2.20–1.95 (m, 3H), 1.9–1.9 (m, 2H), 1.60 (br, 1H), 1.48 (m, 1H), 1.15 (d, 6.0 Hz, 3H).
12. *Thermogravimetry analysis (TGA) and differential scanning calorimetry (DSC) conditions:* TGA data was acquired using a TA instrument TGA Q500 with thermal advantage (v4.9.3) and processed using universal analysis (v4.4A); atmosphere: nitrogen at 40 cc/min for balance and 60 cc/min for sample; rate: 10 °C/min. DSC data was acquired using a TA instruments DSC Q2000 with thermal advantage (v4.9.3) and processed using universal analysis (v4.4A); atmosphere: nitrogen at 50 cc/min; Crimped Tzero non-hermetic aluminum pan and lid with pinhole; rate: 10 °C/min.
13. Nersesian, D. L.; Black, L. A.; Miller, T. R.; Vortherms, T. A.; Esbenshade, T. A.; Hancock, A. A.; Cowart, M. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 355.
14. Barbier, A. J.; Berridge, C.; Dugovic, C.; Laposky, A. D.; Wilson, S. J.; Boggs, J.; Aluisio, L.; Lord, B.; Mazur, C.; Pudiak, C. M.; Langlois, X.; Xiao, W.; Apodaca, R.; Carruthers, N. I.; Lovenberg, T. W. *Br. J. Pharmacol.* **2004**, *143*, 649.
15. Zulli, A. L.; Aimone, L. D.; Mathiasen, J. R.; Gruner, J. A.; Raddatz, R.; Bacon, E. R.; Hudkins, R. L. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2807.