

## Syntheses of 7-Substituted $\alpha$ -Cyperone Derivatives for Selective Sigma-1 Receptor over Cannabinoid-1 Receptor Binding Affinities

Ju-Young Park, Young-Gyun Shin, Kee-Won Kim,<sup>†</sup> Young-Bae Kwon,<sup>‡</sup> and Sung-Hwa Yoon\*

Department of Molecular Science and Technology, Ajou University, Suwon 443-749, Korea. \*E-mail: shyoon@ajou.ac.kr

<sup>†</sup>Department of Pharmacology, Institute for Medical Sciences, Chonbuk National University, Jeonju 561-180, Korea

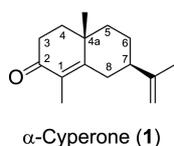
<sup>‡</sup>Department of Laboratory Animal Medicine, College of Veterinary Medicine, Chonbuk National University, Jeonju 561-180, Korea

Received July 5, 2013, Accepted August 28, 2013

**Key Words :**  $\alpha$ -Cyperone, CB<sub>1</sub> receptor, Sigma-1 receptor

A number of herbal products have been used for treating various psychiatric disorders including anxiety, depression and addiction.<sup>1-3</sup> Among these products, the extract of *Cyperus rotundus* has been used as a folk medicinal herb in Korea, China, India and Japan for the treatment of spasm and stomach disorders and the main ingredients of the extract are  $\alpha$ -cyperone,  $\beta$ -selinene, cyperene, cyperol and nootkatene.<sup>4,5</sup> Recent reports revealed that the extract of *Cyperus rotundus* possesses analgesic, nootropic, sedative, anti-inflammatory, antipyretic and antifungal activity.<sup>6,7</sup> In addition, the neuroprotective effects of the herb were reported in Parkinson's disease and cerebral ischemia model.<sup>7,8</sup>

As part of our program to develop new natural materials to treat various psychiatric disorders, we have investigated a number of herbal products to test their usefulness and therapeutic benefits for the treatment of depression, anxiety and drug dependence. We found that, among the various ingredients of *Cyperus rotundus* extract,  $\alpha$ -cyperone (Figure 1.) has a binding affinity *in vitro* to cannabinoid-1 (CB<sub>1</sub>) and sigma-1 receptor (IC<sub>50</sub> = 1.9  $\mu$ M and 22.1  $\mu$ M, respectively).

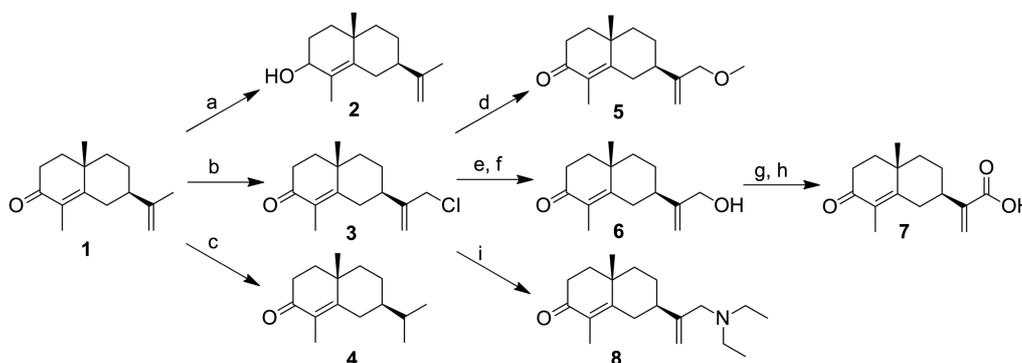


**Figure 1.** Structure of  $\alpha$ -cyperone (1).

Since sigma-1 and CB<sub>1</sub> receptor binding affinity are closely related to the physiological effects from drug addiction, depression and anxiety in receptors throughout our body,<sup>9-14</sup> we have synthesized various  $\alpha$ -cyperone derivatives with the aim to improve both the selectivity of CB<sub>1</sub> receptor binding affinity versus sigma-1 receptor binding affinity and the potency simultaneously. Here, we report the syntheses of some  $\alpha$ -cyperone derivatives containing a heteroatom moiety at the C7 position and their selective binding affinities *in vitro* for the sigma-1 and CB<sub>1</sub> receptors.

We first synthesized  $\alpha$ -cyperone **1** by following the previously reported synthetic routes.<sup>15-19</sup> In brief, azeotropic imination of (+)-dihydrocarvone and (*R*)-(+)-1-phenylethylamine followed by alkylation with a slight excess of ethyl vinyl ketone (EVK) in THF at 40 °C produced the Micheal adduct. The resulting adduct was hydrolyzed and then treated with sodium methoxide at room temperature to give an easily separable mixture of  $\alpha$ -cyperone **1** and its side product. Flash chromatography resulted in pure  $\alpha$ -cyperone **1** in a 30% yield from (+)-dihydrocarvone.

The  $\alpha$ -cyperone derivatives were synthesized as illustrated in Scheme 1. Reduction of  $\alpha$ -cyperone with NaBH<sub>4</sub> in ethanol produced the allylic alcohol **2**.<sup>20</sup> The 7-substituted  $\alpha$ -cyperone derivatives were synthesized from nucleophilic substitution reaction of the chloride group in compound **3** with appropriate nucleophiles. Accordingly, after the allylic chloride derivative **3** was synthesized from compound **1** using hydro-



**Scheme 1.** (a) NaBH<sub>4</sub>, EtOH, rt, 1 h; (b) Vilsmeier reagent, 50% H<sub>2</sub>O<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, -15 °C, 1 h; (c) Pd-C, MeOH, rt, 5 h; (d) MeOH, TEA, reflux, 12 h; (e) NaI, acetone, rt, 3 h; (f) Cu<sub>2</sub>O, DMSO, 60 °C, 4 h; (g) MnO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 6 h; (h) AgNO<sub>3</sub>, KOH, EtOH, rt, 1 h; (i) diethylamine, CH<sub>2</sub>Cl<sub>2</sub>, TEA, reflux, 8 h.

gen peroxide and a Vilsmeier reagent, it was reacted with methanol under the basic condition to give the methoxy derivative **5**. Similarly, the reaction of **3** with a diethylamine afforded tertiary amino derivative **8**. For the synthesis of the allylic alcohol derivative **6**, the allylic chloride **3** was first transformed into the reactive iodine intermediate using sodium iodide, which was then hydrolyzed with Cu<sub>2</sub>O to produce the compound **6**.<sup>22</sup> The carboxylic acid derivative **7** was prepared by oxidation of the compound **6** with manganese oxide followed by silver nitrate in basic conditions. Hydrogenation of  $\alpha$ -cyperone **1** using Pd-C in methanol<sup>23</sup> gave compound **4** where the double bond at the C7 position was reduced. All  $\alpha$ -cyperone derivatives were characterized by NMR and MS.

The activities of the  $\alpha$ -cyperone derivatives for the CB<sub>1</sub> and sigma-1 receptors were measured by [<sup>3</sup>H]CP-55,940 and [<sup>3</sup>H]pentazocine competitive binding assay in rat cortex using previously described methods<sup>24-27</sup> and the results are presented in Table 1. The specific binding of [<sup>3</sup>H]CP-55,940 and [<sup>3</sup>H]pentazocine to its affinity receptor in rat cortex membrane was totally displaced by  $\alpha$ -cyperone derivatives in a concentration dependent manner.<sup>14</sup> We first investigated the effect of the carbonyl group at the C2 position of compound **1** on the binding affinities of CB<sub>1</sub> and sigma-1 receptor. When the carbonyl group of compound **1** was altered to a hydroxyl group, compound **2** had a similar binding affinity for the sigma-1 receptor but also showed a significantly decreased binding affinity for the CB<sub>1</sub> receptor in comparison to compound **1**. From this result, we assumed that the carbonyl group at the C2 position of compound **1** was needed to preserve the CB<sub>1</sub> receptor binding affinity.

For the purpose of evaluating whether the binding affinities *in vitro* to the CB<sub>1</sub> and sigma-1 receptors were affected by the hydrogen bond donor and/or acceptor group, compounds which have different moieties at the C7 position of  $\alpha$ -cyperone **1** were investigated. Although compound **4**, revealed significantly decreased binding affinity for the CB<sub>1</sub> receptor but the binding affinity to sigma-1 receptor remained relatively unchanged. However, the methoxy derivative **5**, which has a moiety acting as a strong hydrogen bond acceptor, showed CB<sub>1</sub> receptor binding affinity (IC<sub>50</sub> = 0.4  $\mu$ M) 4.8 times higher than  $\alpha$ -cyperone **1** (IC<sub>50</sub> = 1.9  $\mu$ M) and did not bind at all to the sigma-1 receptor. On the contrary, the diethyl amino derivative **8** showed complete loss of CB<sub>1</sub> receptor binding affinity but had about 220 times higher sigma-1 receptor affinity (IC<sub>50</sub> = 0.1  $\mu$ M) than the parent compound (IC<sub>50</sub> = 22.1  $\mu$ M). This result indicated that different hetero atomic moiety at the C7 position of  $\alpha$ -cyperone **1** can dramatically affect the selectivity of the CB<sub>1</sub> receptor binding affinity over sigma-1 receptor binding affinity. The hydroxyl derivative **6**, which has a hydrogen bond donor capability, completely lost CB<sub>1</sub> receptor binding affinity and had a weaker sigma-1 receptor binding activity (IC<sub>50</sub> = 55.4  $\mu$ M) than the parent compound (IC<sub>50</sub> = 1.9  $\mu$ M) while the compound **7** containing a carboxylic acid group, which is usually involved in ionic bond binding with negative charge, resulted in the complete loss of sigma-1 receptor

**Table 1.** CB<sub>1</sub> and sigma-1 receptor binding affinities of  $\alpha$ -cyperone derivatives

Compound Number	IC <sub>50</sub> ( $\mu$ M) <sup>a</sup>	
	Sigma-1 receptor binding affinity	CB <sub>1</sub> receptor binding affinity
<b>1</b> ( $\alpha$ -Cyperone)	22.1	1.9
<b>2</b>	28.5	11.8
<b>4</b>	30.9	16.4
<b>5</b>	>100	0.4
<b>6</b>	55.4	> 100
<b>7</b>	>100	5.5
<b>8</b>	0.1	> 100

<sup>a</sup>The value of IC<sub>50</sub> was calculated using a non-linear regression analysis by the software SigmaPlot. 10.0

binding activity and 2.9 times less potent CB<sub>1</sub> receptor binding affinity (IC<sub>50</sub> = 5.5  $\mu$ M) than that of the parent compound (IC<sub>50</sub> = 1.9  $\mu$ M). These results clearly indicated that the presence of a hydrogen bond acceptor group at the C7 position of  $\alpha$ -cyperone **1** is beneficial for a high binding affinity to CB<sub>1</sub> receptor and specificity for the selective binding over sigma-1 receptor which had a much lower binding activity.

It is interesting to note that, unlike compound **5**, the compound **8** containing a diethyl amino moiety acted as a positive ionic bond binder after protonation under physiological condition (pH = 7.4) showed complete loss of CB<sub>1</sub> receptor binding affinity in spite of a high potency of sigma-1 receptor binding affinity. These results suggested that the positively charged ionic binder at the C7 position of  $\alpha$ -cyperone is favorable for binding to the sigma-1 receptor, but not for CB<sub>1</sub> receptor binding affinity.

In summary, we have successfully synthesized seven  $\alpha$ -cyperone derivatives and found that the presence of a hydrogen bond donor/acceptor groups at the C7 position of  $\alpha$ -cyperone significantly affects specificity and potency of CB<sub>1</sub> receptor binding affinity over sigma-1 receptor binding affinity. In particular, the presence of the amino moiety at the C7 position of  $\alpha$ -cyperone is beneficial for binding to sigma-1 receptor. The molecular mechanism of compound **8** involved in the high binding affinity to sigma-1 receptor is under investigation.

## Experimental

**Instruments and Chemicals.** Melting points were obtained on a Fisher-Johns melting point apparatus. <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained on a Varian Gemini NMR spectrometer at 400 and 100 MHz, respectively. <sup>1</sup>H chemical shifts were recorded relative to TMS (trimethylsilane, 0 ppm) and <sup>13</sup>C chemical shifts relative to CDCl<sub>3</sub> (77.0 ppm) and coupling constants *J* in Hz. Abbreviations for signal multiplicities are as follows: s (singlet), d (doublet), t (triplet) and m (multiplet). ESI-MS spectra were obtained by Shimadzu LCMS-2010EV.

**(2R,4aS,7R)-1,4a-Dimethyl-7-(prop-1-en-2-yl)2,3,4,4a,**

**5,6,7,8-octahydronaphthalen-2-ol (2):** Compound **2** was prepared by following the previously reported procedure,<sup>15</sup> and obtained as a yellow oil (3.21 g, 56% yield). The spectral data of the product were consistent with the previously reported data.

**(4aS,7R)-7-(3-Chloroprop-1-en-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one (3):** To a solution of  $\alpha$ -cyperone **1** (50 mg, 0.4 mmol) in ethanol (10 mL) at  $-18\text{ }^{\circ}\text{C}$  was added  $\text{NaBH}_4$  (50 mg, 1.5 mmol). The reaction mixture was stirred at room temperature for 1 h. After the reaction mixture was quenched with 1 *N* aqueous HCl solution at  $0\text{ }^{\circ}\text{C}$ , the solution was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The residue was purified with silica gel chromatography to afford the titled compound as a yellow oil (35 mg, 57% yield). The spectral data of the product were consistent with the previously reported data.<sup>20</sup>

**(4aS,7R)-7-Isopropyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one (4):** Compound **4** was prepared by following the previously reported procedure,<sup>21</sup> and obtained as a yellow oil (120 mg, 61% yield). The spectral data of the product were consistent with the previously reported data.

**(4aS,7R)-7-(3-Methoxyprop-1-en-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one (5):** A solution of (4aS,7R)-7-(3-chloroprop-1-en-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one **3** (100 mg, 0.45 mmol) and TEA (45 mg, 0.45 mmol) in MeOH (10 mL) was refluxed for 12 h. After being cooled to room temperature, the mixture was washed with water and brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The residue was purified with silica gel chromatography to afford the product as a colorless oil (37 mg, 40% yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.16 (s, 3H, 4aC- $\text{CH}_3$ ), 1.70 (s, 3H, 1C- $\text{CH}_3$ ), 3.27 (s, 3H,  $\text{OCH}_3$ ), 3.87 (s, 2H,  $\text{CH}_2\text{OCH}_3$ ), 4.96 (s, 1H,  $\text{C}(\text{CH}_2\text{OCH}_3)=\text{CHH}$ ), 5.03 (s, 1H,  $\text{C}(\text{CH}_2\text{OCH}_3)=\text{CHH}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  198.75, 161.60, 149.34, 128.66, 110.94, 74.92, 58.00, 42.00, 41.67, 37.47, 35.90, 33.85, 33.21, 27.29, 22.58, 11.05.

**(4aS,7R)-7-(3-Hydroxyprop-1-en-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one (6):** Compound **6** was prepared by following the previously reported procedure,<sup>21</sup> and obtained as a colorless oil (60 mg, 82% yield). The spectral data of the product were consistent with the previously reported data.

**2-((2R,4aS)-4a,8-Dimethyl-7-oxo-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl)acrylic acid (7):** Compound **7** was prepared by following the previously reported procedure,<sup>21</sup> and obtained as a colorless oil (45 mg, 51% yield). The spectral data of the product were consistent with the previously reported data.

**(4aS,7R)-7-(3-(Diethylamino)prop-1-en-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one (8):** To a solution of (4aS,7R)-7-(3-chloroprop-1-en-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one **3** (40.0 mg, 0.41 mmol) and TEA (0.20 mL, 0.50 mmol) in

dichloromethane (10 mL) was added diethylamine (0.30 mL, 0.61 mmol) and refluxed for 8 h. After being cooled to room temperature, the mixture was washed with water and brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The residue was purified with silica gel chromatography to afford the title compound as colorless oil (20 mg, 39% yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.90-0.98 (t, 6H,  $J = 7.2\text{ Hz}$ ,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 1.16 (s, 3H, 4aC- $\text{CH}_3$ ), 2.36-2.46 (q, 4 H,  $\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 2.95 (s, 2H,  $\text{C}(\text{CH}_2\text{CH}_2\text{N})$ ), 4.87 (s, 1H,  $\text{C}(\text{CHH})\text{CH}_2\text{N}$ ), 4.94 (s, 1H,  $\text{C}(\text{CHH})\text{CH}_2\text{N}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  198.85, 162.37, 151.51, 128.38, 110.57, 58.53, 46.63, 42.07, 41.85, 37.42, 35.97, 33.84, 33.53, 27.26, 22.55, 11.79, 11.02; ESI-MS:  $m/z$  290.35 ( $\text{M} + \text{H}$ )<sup>+</sup>, 331.40 ( $\text{M} + \text{ACN} + \text{H}$ )<sup>+</sup>.

**CB<sub>1</sub> Receptor Binding Assay.** Preparation of rat cerebral cortex membrane and binding assays for the sigma-1 receptor were performed using methods published previously in detail.<sup>24,25</sup> Each tube contained 40  $\mu\text{g}$  membrane protein, TME buffer and 0.1 nM of [<sup>3</sup>H] CP-55,940 was incubated in total reaction volume of 500  $\mu\text{L}$  for 60 min at  $30\text{ }^{\circ}\text{C}$ . Non-specific binding was determined in the presence of 10  $\mu\text{M}$  CP-55,940. The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After being washed three times with the ice-cold Tris-HCl buffer, the filter was transferred to a liquid scintillation vial. After addition of EtOH and counting cocktail, the quantity of radioactivity was determined by liquid scintillation spectrometry.

**Sigma-1 Receptor Binding Assay.** Preparation of rat cerebral cortex membrane and binding assays for the CB<sub>1</sub> receptor were performed using methods published previously in detail.<sup>26,27</sup> In brief, Each tube contained 300  $\mu\text{g}$  membrane protein, Tris-HCl buffer (50 mM, pH 8.0) and 5 nM of [<sup>3</sup>H]pentazocine was incubated in total reaction volume of 500  $\mu\text{L}$  for 60 min at  $25\text{ }^{\circ}\text{C}$ . The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After being washed three times with the ice-cold Tris-HCl buffer, the filter was transferred to a liquid scintillation vial. After addition of EtOH and counting cocktail, the quantity of radioactivity was determined by liquid scintillation spectrometry.

**Acknowledgments.** This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (Grant No. 2011-0009050), and was partially supported by the Post Brain Korea 21 program.

## References

1. Witte, S.; Loew, D.; Gaus, W. *Phytother. Res.* **2005**, *19*, 183.
2. Fava, M.; Alpert, J.; Nierenberg, A. A.; Mischoulon, D.; Otto, M. W.; Zajecka, J.; Murck, H.; Rosenbaum, J. F. *J. Clin. Psychopharmacol.* **2005**, *25*, 441.
3. Thongsaard, W.; Marsden, C. A.; Morris, P.; Prior, M.; Shah, Y. B. *Psychopharmacology* **2005**, *180*, 752.
4. Yu, H. H.; Lee, D. H.; Seo, S. J.; You, Y. O. *Am. J. Chin. Med.* **2007**, *35*, 497.

5. Kumar, R. P.; Rajesh, K.; Yogender, M.; Dharmesh, S.; Karthiyagini, T. *International Journal of Research in Ayurveda & Pharmacology* **2010**, *1*, 536.
  6. Gupta, M. B.; Palit, T. K.; Singh, N.; Bhargava, K. P. *Indian J. Med. Res.* **1971**, *59*, 76.
  7. Sunil, A. G.; Kesavanarayanan, K. S.; Kalaivani, P.; Sathiya, S.; Ranju, V.; Priya, R. J.; Pramila, B.; Paul, F. D.; Venkatesh, J.; Babu, C. S. *Brain Res. Bull.* **2011**, *84*, 394.
  8. Lee, C. H.; Hwang, D. S.; Kim, H. G.; Oh, H.; Park, H.; Cho, J. H.; Lee, J. M.; Jang, J. B.; Lee, K. S.; Oh, M. S. *J. Med. Food* **2010**, *13*, 564.
  9. Horan, B.; Gardner, E. L.; Dewey, S. L.; Brodie, J. D.; Ashby, C. R., Jr. *Eur. J. Pharmacol.* **2001**, *426*, R1.
  10. Brammer, M. K.; Gilmore, D. L.; Matsumoto, R. R. *Eur. J. Pharmacol.* **2006**, *553*, 141.
  11. Hayashi, T.; Su, T. P. *Life Sciences* **2005**, *77*, 1612.
  12. Pacher, P.; Batkai, S.; Kunos, G. *Pharmacol. Rev.* **2006**, *58*, 389.
  13. Padgett, L. W. *Life Sci.* **2005**, *77*, 1767.
  14. Jeong, M. S. Ph.D. Thesis Chonbuk National University, February 2006.
  15. Zhabinskii, V. N.; Minnaard, A. J.; Wijnberg, J. B.; de Groot, A. J. *Org. Chem.* **1996**, *61*, 4022.
  16. Barbetti, P.; Chiappini, I.; Fardella, G.; Menghini, A. *Planta Med.* **1985**, *51*, 471.
  17. Yeo, H.; Kim, K.; Kim, J.; Choi, Y. *Phytochemistry* **1998**, *27*, 1129.
  18. Bohlmann, F.; Jakupovic, J.; Lonitz, M. *Chemische Berichte* **1977**, *110*, 301.
  19. Ahmed, A. A.; Jakupovic, J.; Bohlmann, F.; Regaila, H. A.; Ahmed, A. M. *Phytochemistry* **1990**, *29*, 2211.
  20. Barrett, H. C.; Buechi, G. *J. Am. Chem. Soc.* **1967**, *89*, 5665.
  21. Jean Rodriguez, J.-P. D. **1991**, *1991*, 477.
  22. Xiong, Z.; Yang, J.; Li, Y. *Tetrahedron-Asymmetry* **1996**, *7*, 2607.
  23. Effenberger, F.; Mueller, W.; Keller, R.; Wild, W.; Ziegler, T. *J. Org. Chem.* **1990**, *55*, 3064.
  24. Nam, Y.; Shin, E. J.; Yang, B. K.; Bach, J. H.; Jeong, J. H.; Chung, Y. H.; Park, E. S.; Li, Z.; Kim, K. W.; Kwon, Y. B.; Nabeshima, T.; Kim, H. C. *Neurochem. Int.* **2012**, *61*, 913.
  25. Yarim, M.; Koksal, M.; Schepmann, D.; Wunsch, B. *Chem. Biol. Drug Des.* **2011**, *78*, 869.
  26. Wiley, J. L.; Compton, D. R.; Dai, D.; Lainton, J. A.; Phillips, M.; Huffman, J. W.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 995.
  27. Lange, J. H.; van Stuijvenberg, H. H.; Coolen, H. K.; Adolfs, T. J.; McCreary, A. C.; Keizer, H. G.; Wals, H. C.; Veerman, W.; Borst, A. J.; de Looft, W.; Verveer, P. C.; Kruse, C. G. *J. Med. Chem.* **2005**, *48*, 1823.
-