Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3609–3613

A highly selective κ-opioid receptor agonist with low addictive potential and dependence liability

Hee Sock Park,[a,b] Hee Yoon Lee,[a,*] Yong Hae Kim,[a,*] Jin Kyu Park,[b] Edwin E. Zvartau[c] and Heeseung Lee[d]

[a]*Department of Chemistry, KAIST, Daejeon 305-701, Republic of Korea*
[b]*Dong Kook Pharmaceutical Co. Ltd, Seoul 135-283, Republic of Korea*
[c]*Department of Psychopharmacology, Pavlov Medical University, St. Petersburg, Russia*
[d]*Department of Anesthesiology and Pain Medicine, Ewha Womans University, College of Medicine, Seoul, Republic of Korea*

Received 21 November 2005; revised 6 February 2006; accepted 8 February 2006
Available online 2 May 2006

Abstract—Buprenorphine analogs have been synthesized. In the studies of analgesic and addictive effects in mice and [^{35}S]GTPγS binding assay in human brain tissue, an analog of buprenorphine where the *tert*-butyl is replaced by a cyclobutyl moiety (**16**) has been identified as a selective κ-partial agonist which gives antinociceptive effects, but has low abuse potential. The results may lead to lower degrees of dysphoria than full κ-agonists.

Opioid analgesics are very important in human life. For example, unrelieved pain destroys a person's quality of life and is one of the driving forces behind interests in physician-assisted suicide and euthanasia.[1] However, opioids have several undesirable effects. Physician reluctance to prescribe opioids in the past has related to the associated risk of addiction and respiratory arrest.[2]

The importance of subtle structural differences between agonists and antagonists is evidenced far more exten-sively with opioids than any other class of drugs.[3] The effect by altering substituents can also be seen in the structurally related family of buprenorphine (BUP), etorphine, and diprenorphine having two-carbon bridge substituent not in morphine (Fig. 1).[4]

Although BUP may not be entirely devoid of abuse potential, its propensity to produce addiction appears to be much lower than that of other potent opioids.[5] However, it is still required that non-addictive opioids should be developed.

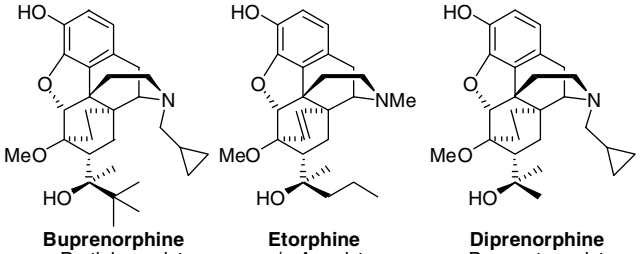


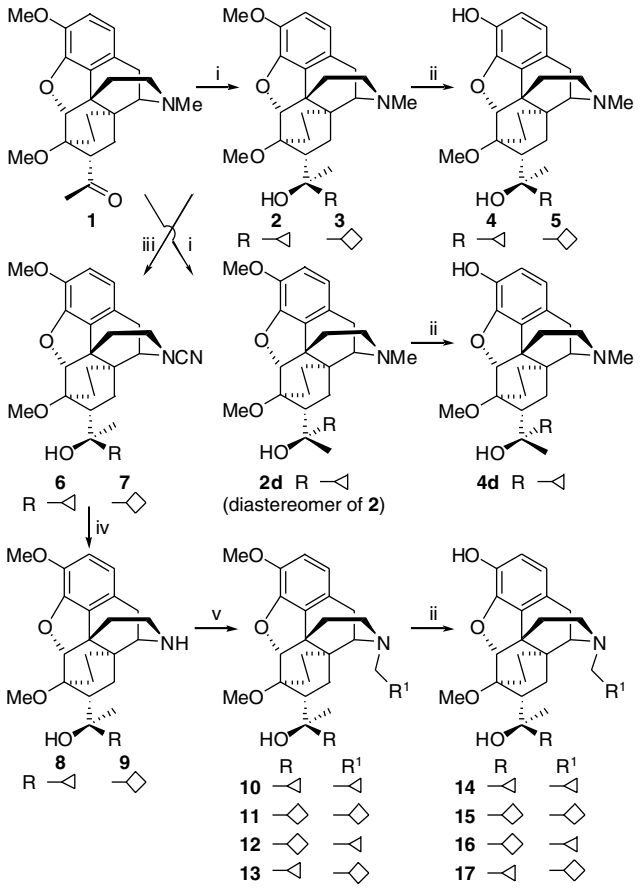
Figure 1. Buprenorphine and its analogs.

The present study aims to provide BUP analogs having better analgesia, but with lower side effects. Analgesia and self-administration (SA) assays[6] in mice are the primary bioassays used in this study and provide an initial model to assess the relative benefit/risk ratio of therapy and dependence.

BUP analogs, **4**, **4d**, **5**, **14**, **15**, **16**, and **17**, were prepared by modification of the known procedures (Scheme 1).[7,8] The compound **1**[9] was reacted with cyclopropyl magnesium bromide to give the mixture of **2** and **2d** in the ratio of about 7:3 (HPLC data). Compounds **2** and **2d** were separated by the silica gel column chromatography to

Keywords: κ-partial agonist; Opioid; Addiction; Buprenorphine.
* Corresponding authors. Tel.: +82 42 869 2818; fax: +82 42 869 5818; e-mail: kimyh@kaist.ac.kr

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.02.017



Scheme 1. Synthetic pathway for **1**–**17**. Reagents and conditions: (i) RMgBr, THF, reflux, 1.5 h; (ii) KOH, diethyleneglycol, 220 °C, 2 h; (iii) CNBr, CH_2Cl_2, reflux, 4 h; (iv) KOH, diethyleneglycol, 175 °C, 1.5 h; (v) R^1CH_2Br, $Mg(OAc)_2$, DMF, 100 °C, 2 h.

give 62% and 26% yields, respectively. The compound **3** was separated by the fractional crystallization in 60% yields. These compounds, **2**, **2d**, and **3**, were identified by comparing their ^1H NMR spectra with those of the known tetrahydrothebaine analogs.[10] O-Demethylated **4**, **4d**, and **5**, were obtained by hydrolysis of methoxy moiety of **2**, **2d**, and **3**.

Compounds **14**–**17** were prepared in several steps from **2** and **3**. Compounds **2** and **3** were converted to **6** and **7** with CNBr in 95% yields. Compounds **6** and **7** were hydrolyzed to free amines **8** and **9** in 89–90% yields. Amines **8** and **9** were alkylated to give **10**–**13** in 78–80% yields. Hydrolysis of **10**–**13** afforded compounds **14**–**17** in 68–79% yields. Finally, diastereomerically pure free bases **4**, **4d**, **5**, and **14**–**17** were converted to the corresponding HCl salts in 94–97% yields. HCl salts of **4**, **4d**, **5**, and **14**–**17** were prepared for the biological evaluations.

Compounds, **4**, **4d**, **5**, **14**, and BUP (1st set: Tables 1–5), were screened for analgesic and addictive activity[6] in male DBA mice after intravenous injection.

Rank order of full agonists in their analgesic potencies was as follows: **5** > **4** > **4d** (Table 1). The potency of **5** was 100–544 times higher than that of BUP. The full agonists, **4**, **4d**, and **5**, showed higher analgesic effects than those of **14** and BUP (Table 2).

Comparisons of additive properties of the compounds are summarized in Table 3. Analysis showed that there are different SA potencies in the following order: **5** > **4** > **4d** ⩾ **14** ⩾ BUP. Comparisons of efficacy criteria (Table 4) showed no difference between samples.

The important results have been obtained from the analysis of benefit/risk ratio. In comparing the analgesic and reinforcing effect (Table 5), the highest safety index was found in **4**. Addictive safety of the compounds when assessed together with analgesic potential decreased in the following order: **4** > **5** > BUP = **4d** ⩾ **14**. Addiction liability of BUP is demonstrated in the clinical study.[5]

Compounds, **15**, **16**, **17**, and BUP (2nd set: Tables 6–10), were also screened for analgesic and addictive activity[6] in male SHR mice.

On the basis of analgesic effect analysis, **15** and **17** demonstrated lower potencies when compared to BUP (Tables 6, 7, and 10). Their efficacies were either a bit lower (**17**) or similar (**15**) to that of BUP. While **16** was 33–299% more potent than BUP depending on the test (Table 6). It was more efficient in terms of maximum analgesic action across analgesic tests (Table 7).

Table 2. Efficacies (max analgesic effects, %) of **4**, **4d**, **5**, **14**, and BUP

Test	4	4d	5	14	BUP
Tail clip	100	100	100	No effect	46.7
Tail flick	100	100	100	18.6	65.3
Hot plate	100	100	100	10.4	58.5
Writhing	100	100	100	100	100

Table 1. Potencies of **4**, **4d**, **5**, **14**, and BUP in analgesic tests

Test	ED_{50}[a] (μg/kg)				
	4	4d	5	14	BUP
Tail clip	27.5 (14.0–55.0)	122.0 (62.0–241)	3.7 (1.8–7.8)	ND	ND
Tail flick	19.7 (12.5–31.0)	197.0 (106–365)	2.4 (1.6–3.8)	ND	312.0 (200–600)
Hot plate	11.0 (7.8–15.0)	77.0 (47.0–126)	1.5 (0.8–3.1)	ND	195.0 (120–310)
Writhing	0.59 (0.4–0.9)	13.0 (7.0–24.0)	0.055 (0.04–0.08)	235.9 (131–424)	29.5 (16.0–53.0)

ND, not determined.

[a] According to Litchfield and Wilcoxon procedure.[11]

Table 3. Potencies of **4**, **4d**, **5**, **14**, and BUP in SA test

Parameter	**4**	**4d**	**5**	**14**	BUP
Unit ED_{50}[a]	0.62 (0.37–1.04)	1.28 (0.72–2.28)	0.028 (0.021–0.036)	1.35 (0.62–2.9)	2.04 (1.0–3.9)
Optimal unit dose[b]	1.54	2.56[d]	0.04	3.2	7.68
Dose[c]	28.5	65.3[d]	1.25	70.9	143.54

[a] μg/kg/injection, calculated by the aid of *N*+ values, taken from the ascending part of dose–response curve. A unit dose supporting an initiation of SA in 50% of subjects.
[b] μg/kg/injection, unit dose corresponding to the maximum values of reinforcing criteria (*delta* and *R*). A unit dose supporting an initiation of SA in maximum number of subjects.
[c] Obtained μg/kg, dose consumed during SA session at the concentration corresponding to the highest *R* value.
[d] Corresponding to maximum *N*+ criterion.

Table 4. Efficacies of **4**, **4d**, **5**, **14**, and BUP in SA test

Parameter (max)	**4**	**4d**	**5**	**14**	BUP
delta	13.75 ± 3.15	2.0 ± 18.76	25.38 ± 4.68	13.00 ± 4.80	13.00 ± 2.36
R	0.23 ± 0.06	0.21 ± 0.13	0.30 ± 0.04	0.19 ± 0.06	0.24 ± 0.06
N+	87.5	75	100	75	87.5

Table 5. Safety indices of **4**, **4d**, **5**, **14**, and BUP

Compound	Writhing test ED_{50} (μg/kg)	Safety index for unit ED_{50} dose	Safety index for optimal unit dose	Ratio of dose consumed for the SA session to analgesic ED_{50}
BUP	29.5	0.07	0.26	4.9
4	0.59	1.05	2.6	48.3
4d	13.0	0.1	0.2	5.0
5	0.055	0.51	0.73	22.7
14	235.9	0.006	0.01	0.3

Table 6. Potencies of **15**, **16**, **17**, and BUP in analgesic tests

Test	ED_{50} (μg/kg)			
	15	**16**	**17**	BUP
Tail clip	633.0 (357–1122)	28.4 (15.4–52.0)	ND	46.6 (22.5–96.7)
Tail flick	358.7 (208.7–617.7)	10.1 (5.3–19.3)	80.3 (53.5–120.5)	40.4 (24.9–65.5)
Hot plate	186.7 (117.8–295.8)	17.6 (9.4–33.0)	190.3 (89.7–404.0)	23.3 (14.5–37.5)
Writhing	22.4 (11.5–43.6)	11.2 (5.0–26.8)	47.4 (24.1–93.3)	22.3 (10.5–47.3)

Table 7. Efficacies (max analgesic effects, %) of **15**, **16**, **17**, and BUP

Test	**15**	**16**	**17**	BUP
Tail clip	88.9	100	44.4	100
Tail flick	84.5	100	81.0	100
Hot plate	100	100	100	100
Writhing	100	100	100	100

Table 8. Potencies of **15**, **16**, **17**, and BUP in SA test

Parameter	**15**	**16**	**17**	BUP
Unit ED_{50}	3.6 (1.9–6.9)	4.1 (2.6–6.3)	3.78 (2.5–5.6)	1.15 (0.6–0.21)
Optimal unit dose	4.29	8.7	4.34	1.92
Dose	180	1,240	200	80

In experiments with initiation of iv self-administration in drug-naive mice, **16** demonstrated much lower potency ratios (0.06–0.28) when compared to BUP (Table 8). Efficacy values of reinforcing actions of BUP and **17** were close to each other (Table 9).

Compound **16** appears to be one of candidates in the safe drugs (Table 10). Compound **16** exhibits very interesting pharmacological properties: it is more potent and effective as an analgesic when compared to BUP and at the same time it is less potent as a reinforcing agent. Compound **16** may not exhibit drug dependence liability at the doses tested in cancer and non-cancer pain compared with BUP. The comparatively long analgesic effect of **16** must be noted.[12]

In addition, the [^{35}S]GTPγS assay of **16** was carried out using human membranes which was initially documented in human neuroblastoma SH-SY5Y cells[13] as a valuable approach to demonstrate and quantify affinity of different opioid receptor agonists and partial agonists.[14]

The assays of specific receptor binding, defined with naloxone, were conducted, as described previously.[14a] The concentration of protein in human brain cortical membranes ($n = 4$) was 1.50 mg/mL ± 0.03 (SEM),

Table 9. Efficacies of **15**, **16**, **17**, and BUP in SA test

Parameter (max)	**15**	**16**	**17**	BUP
delta	11.56 ± 10.14	103.5 ± 41.65	21.56 ± 8.35	16.22 ± 4.78
R	0.21 ± 0.14	0.45 ± 0.15	0.24 ± 0.07	0.28 ± 0.05
N+	56	83	56	67

Table 10. Safety Indices of **15**, **16**, **17**, and BUP

Compound	Writhing test ED_{50} (μg /kg)	Safety index for unit ED_{50} dose	Safety index for optimal unit dose	Ratio of dose consumed for the SA session to analgesic ED_{50}
BUP	22.31	0.05	0.086	3.59
15	22.4	0.16	0.19	8.04
16	11.2	0.37	0.78	110.7
17	47.4	0.08	0.09	4.2

determined by the method of Bradford[15] with bovine serum albumin as the standard. The values of K_d and B_{max} are 0.83 (nM) and 64.4 (fmol/mg protein) for [^{3}H]DAMGO, 0.70 (nM) and 50.2 (fmol/mg protein) for [^{3}H]U69593, and 4.98 (nM) and 70.3 (fmol/mg protein) for [^{3}H]DPDPE, respectively.

Second, the agonist stimulation of [^{35}S]GTPγS to human brain cortical membranes was studied with **16** and selective reference agonists at opioid receptors. Compound **16** increased the in vitro binding of [^{35}S]GTPγS to human brain cortical membranes dependent upon concentration but with a maximum stimulation of 7.2% over baseline. U69593 was defined in this assay as a full κ-agonist and increased stimulation of [^{35}S]GTPγS by ca. 13.4% over baseline. EC_{50} values of U69593 and **16** were 64.3 and 1.84 nM, respectively.

The effects of agonist stimulation of **16** and U69593 were antagonized by norBNI, a selective κ-opioid receptor antagonist[16] as shown in Figure 2. The shift in EC_{50} caused by 3 nM norBNI for **16** and U69593

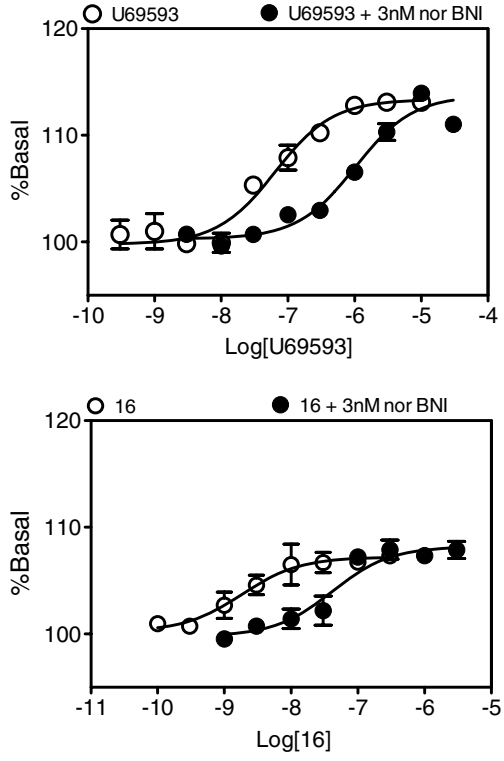


Figure 2. Stimulation of [^{35}S]GTPγS binding by U69593 and **16** in the presence of specific κ-antagonist (norBNI) in human brain cortical membranes. Shown are mean values ± SEM from 3 to 4 independent experiments. Each experiment was carried out in duplicate.

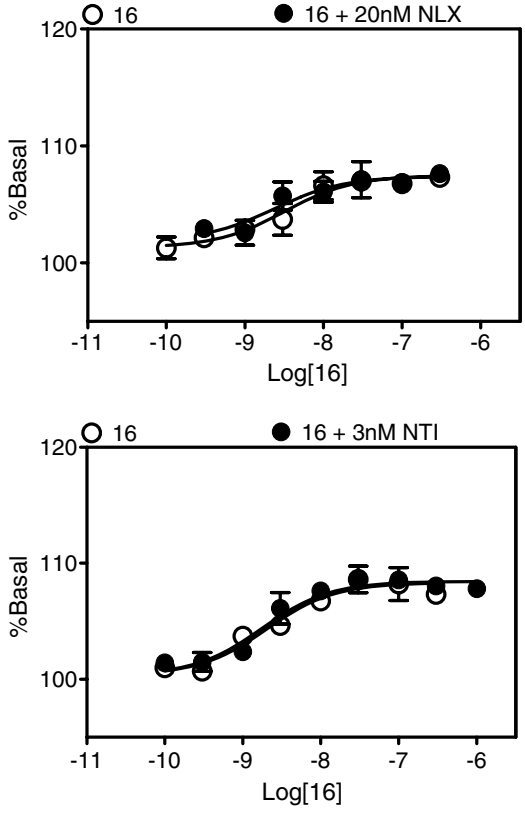


Figure 3. Stimulation of [^{35}S] GTPγS binding by **16** in the presence of specific μ-antagonist (naloxone, upper panel) and δ-antagonist (naltrindole, lower panel) in human brain cortical membranes. Shown are mean values ± SEM from two independent experiments. Each experiment was carried out in duplicate.

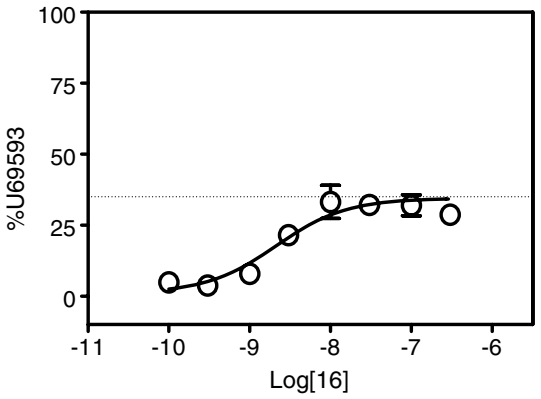


Figure 4. Relative efficacy of **16** [^{35}S]GTPγS stimulation compared with U69593. Shown are mean values ± SEM from three independent experiments. Each experiment was carried out in duplicate.

was 22.5-fold and 15.9-fold, respectively. The calculated K_e values of **16** and U69593 for norBNI inhibition were 0.14 nM (lower panel, Fig. 2) and 0.20 nM (upper panel, Fig. 2), respectively. Similar values for inhibition of two agonists indicate that these agonists activated the same receptor. Stimulation of [^{35}S]GTPγS by **16** was not antagonized by naloxone or naltrindole, a preferential antagonist of μ or *δ* receptors, respectively (Fig. 3). The results also showed that **16** behaved as a partial agonist at the κ-opioid receptor in human brain cortical membranes and with a relative efficacy of 35% when maximum stimulation of U69593, a known selective full κ-agonist, was set as 100% (Fig. 4).

In summary, a variety of BUP analogs have been synthesized. In the studies of analgesic and addictive effects in mice and [^{35}S]GTPγS binding assay in human brain tissue, the new compound **16** has been identified as a selective κ-partial agonist which gives antinociceptive effects, but has low abuse potential. It is reported that the activation of κ-receptors leads to the suppression of unpleasant μ and δ mediated side effects such as the rewarding effect.[17] The fact that this compound has a profile (κ-partial agonist) may lead to lower degrees of dysphoria than full κ-agonists.[18] The compound may be valuable for the development of long-acting analgesic, as a requirement for protracted use in neuropathic pain and drug abuse medication.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.02.017.

References and notes

1. Joranson, D. E. *Symptom Control in Cancer Patients (Japan)* **1995**, *6*, 105.
2. Sapir, R.; Catane, R.; Strauss-Liviatan, N.; Cherny, N. I. *J. Pain Symptom Manage.* **1999**, *17*, 266.
3. Snyder, S. H.; Pasternak, G. W. *Trends Pharmacol. Sci.* **2003**, *24*, 198.
4. Deschamps, J. R.; Flippen-Anderson, J. L.; George, C. *Biopolymers* **2002**, *66*, 287.
5. Tzschentke, T. M. *Psychopharmacology* **2002**, *161*, 1.
6. (a) Semenova, S.; Kuzmin, A.; Zvartau, E. E. *Pharmacol. Biochem. Behav.* **1995**, *50*, 17; (b) Kuzmin, A. V.; Patkina, N. A.; Zvartau, E. E. *Brain Res.* **1994**, *652*, 1.
7. Compounds **2**, **4**, **6**, **8**, **10** ,and **14** were reported (Kim, Y. H.; Park, J. K.; Kim, K.; Park, H. S. U.S. Patent 5,849,915, 1998.
8. Bentley, K. W.; Hardy, D. G. *J. Am. Chem. Soc.* **1967**, *89*, 3281.
9. Bentley, K. W.; Hardy, D. G.; Meek, B. *J. Am. Chem. Soc.* **1967**, *89*, 3273.
10. (a) Fulmor, W.; Lancaster, J. E.; Morton, G. O.; Brown, J. J.; Howell, C. F.; Nora, C. T.; Hardy, R. A., Jr. *J. Am. Chem. Soc.* **1967**, *89*, 3322; (b) Soars, M. G.; Mattiuz, E. L.; Jackson, D. A.; Kulanthaivel, P.; Ehlhardt, W. J.; Wrighton, S. A. *J. Pharmacol. Toxicol. Methods* **2002**, *47*, 161.
11. (a) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99; (b) Schuster, C. R.; Johanson, C. E. *Neurosci. Biobehav. Rev.* **1981**, *5*, 315.
12. Compound **16** and BUP have a long duration of action compared to other compounds in analgesic tests; see Supporting information.
13. Traynor, J. R.; Nahorski, S. R. *Mol. Pharmacol.* **1995**, *47*, 848.
14. (a) Emmerson, P. J.; Liu, M.-R.; Woods, J. H.; Medzihradsky, F. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1630; (b) Ko, M. C. H.; Lee, H.; Harrison, C.; Clark, M. J.; Song, H. F.; Naughton, N. N.; Woods, J. H.; Traynor, J. R. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 179; (c) Remmers, A. E.; Clark, M. J.; Mansour, A.; Akil, H.; Woods, J. H.; Medzihradsky, F. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 827.
15. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
16. Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. *Life Sci.* **1987**, *4*, 1287.
17. Narita, M.; Funada, M.; Suzuki, T. *Pharmacol. Ther.* **2001**, *89*, 1.
18. Pfeiffer, A.; Brantl, V.; Herz, A.; Emrich, H. M. *Science* **1986**, *233*, 774.