New μ-Opioid Receptor Agonists with Piperazine Moiety

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New μ-opioid receptor (MOR) agonists containing piperazine and homopiperazine moieties in the structures were synthesized and their affinities to and agonist potencies on MOR were evaluated. Among the synthesized compounds, 4-[4-(2-methoxyphenyl)piperazin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide (20Aa) showed the highest affinity to the human MOR expressed in Chinese hamster ovary (CHO)-K1 cells, and the highest agonist potency on the MOR in isolated guinea-pig ileum preparation.

Key words μ-opioid receptor agonist; piperazine; Pd-catalyzed amination; cross-coupling

Recently, it has been reported that morphine, a representative μ-opioid receptor (MOR) agonist, showed an analgesic activity in the periphery in mice.[1] Additionally it has been recognized that tolerance to the peripheral analgesia was not developed after the repeated administration of morphine in mice.[2] Furthermore, it has been reported that the MORs were increased around the inflammatory tissues in animals and humans.[3,4] These reports indicate that the MOR agonists are useful analgesics in the periphery, especially against the inflammatory tissues.

On the other hand, there are nonsteroidal antiinflammatory drugs (NSAIDs) as peripheral analgesics but they do not have an analgesic efficacy toward some serious peripheral inflammations.[5] Therefore, we think that in the serious inflammation cases such as severe burns and grazes, MOR agonists can be effective medicines.

As MOR agonists not having the morphinane structures, fentanyl,[6] loperamide[7] and diphenoxylate[8,9] are known. As for fentanyl, to improve the quality of life for cancer patients with pain, the plaster,[10] which has an analgesic activity that lasts for far seventy-two hours, has been developed so the patients do not need to go frequently to the hospital. Although loperamide and diphenoxylate are MOR agonists, they do not easily pass through the blood–brain barrier (BBB). Therefore, they are mainly used as antidiarrheals now.

Recently, it has also been reported that when loperamide was administered to a burn on a rat as a peripheral percutaneous cream, it was effective and could not easily pass through the BBB so the manifestation of tolerance did not occur.[11]

Therefore, our final objective is the synthesis of MOR agonists having peripheral analgesic activity, and as the first step, we intended to synthesize compounds having more potent activities than loperamide in the two *in vitro* tests mentioned in the above summary. The three compounds mentioned above have a piperidine moiety in their structures. Currently there is no compound having a piperazine moiety in the structure as a MOR agonist on the market. Therefore, we started to synthesize compounds having a piperazine moiety in their structures. In this paper, we describe the synthesis of the compounds and their affinities to the human MOR and δ- and κ-opioid receptors (DOR and KOR) expressed in CHO cells and their agonist activities on MOR in guinea-pig ileum.

Chemistry

To develop our new MOR agonists instead of the already known MOR agonists not having morphinane structures, we thought that our compounds had to have a structural feature. Therefore, based on the knowledge already known, we designed the piperazine ($n=1$) or homopiperazine ($n=2$) derivatives, which have side chains of fentanyl, diphenoxylate and loperamide on a nitrogen with another nitrogen directly connected to various aromatic rings including the heteroaromatic rings.

Considering the novelty of our compounds, we first selected the heteroaryl groups as aryl groups. It has been well known that a halogen located in the α- or γ-position to the nitrogen in six-membered nitrogen heteroaromatic rings are so-called active halogens which easily react with amines. Therefore, we first synthesized the *N*-arylpiperazine or *N*-arylhomopiperazine derivatives by the nucleophilic displacement reaction. As it is difficult to synthesize the *N*-aryl derivatives from the halides located in the inactive site (β-position) by this method, we synthesized them by the cross-coupling method[12,13] using a palladium catalyst, a method that

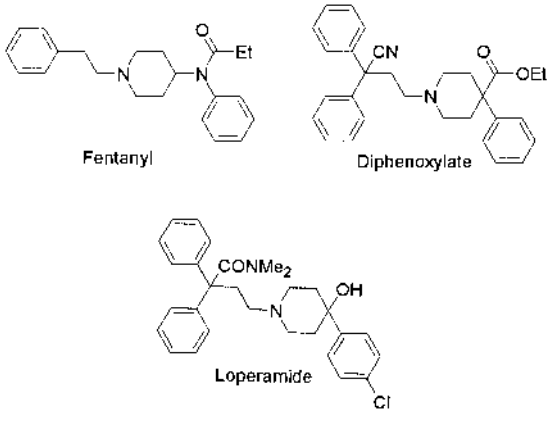

Fig. 1

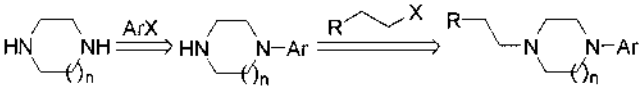

Chart 1

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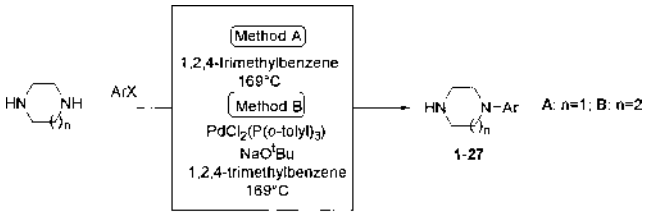
was recently developed by Buchwald. As a matter of course, this method is applicable to the synthesis of aromatic carbon ring derivatives represented by benzene.

The synthesis by nucleophilic displacement reaction (Method A): When 2-chloropyrazine, 2-bromopyridine, 2-chloroquinoline, 2-bromothiazole, 4-bromopyridine and 4-chloroquinoline were heated with 1.5 equivalents of piperazine or homopiperazine at 169 °C in 1,2,4-trimethylbenzene, mono-heteroaryl substituted piperazines and homopiperazines were selectively obtained in yields between 42% and 69%.

The synthesis of *N*-aryl derivatives using the palladium catalysts (Method B): When 3-bromopyridine, 5-bromopyrimidine, 4-bromoisoquinoline, iodobenzene, 1-bromonaphthalene and 2-bromonaphthalene were heated with piperazine or homopiperazine using 5% dichlorobis(tri-*o*-tolylphosphine)palladium (II) [$PdCl_2(P(o\text{-tolyl})_2)_2$] in the presence of NaO^tBu at 169 °C in 1,2,4-trimethybenzene, the corresponding *N*-aryl derivatives were easily obtained as the target intermediates.

The phenolic derivatives **17A**, **18A** and **19A**, except for the previously mentioned intermediates, were synthesized by demethylation using 48% hydrobromic acid from the com-



1: 2-pyridinyl; **2**: 3-pyridinyl, **3**: 4-pyridinyl: **4**: 2-thiazolyl;
5: 2-pyradinyl; **6**: 2-pyrimidinyl; **7**: 5-pyrimidinyl; **8**: 2-quinalinyl;
9: 4-quinolinyl; **10**: 4-isoquinolinyl; **11**: phenyl; **12**: 1-naphthyl;
13: 2-naphthyl; **14**: 2-methylphenyl; **15**: 3-methylphenyl;
16: 4-methylphenyl; **17**: 2-hydroxyphenyl; **18**: 3-hydroxyphenyl;
19: 4-hydroxyphenyl;**20**: 2-methoxyphenyl; **21**: 3-methoxyphenyl;
22: 4-methoxyphenyl; **23**: 2-chlorophenyl;**24**: 3-chlorophenyl;
25: 4-chlorophenyl; **26**: 2-fluorophenyl; **27**. 4-fluorophenyl

Chart 2

Table 1. Methods, Yields, Properties (Melting Points) and Mass Spectral Data for *N*-Arylpiperazines and -Homopiperazines

Compound	Method	Yield (%)	Property [mp (°C)]	Formula	MS *m/z* M^+	HR-MS *m/z* M^+ Calcd (Found)
1B	A	42	Tan viscous liquid	$C_{10}H_{15}N_3$	177	177.1266 (177.1271)
2A	B	32	Tan viscous liquid	$C_9H_{13}N_3$	163	163.1110 (163.1111)
3A	A	52	Yellowish solid (138—139)	$C_9H_{13}N_3$	163	163.1110 (163.1111)
4A	A	66	Tan viscous liquid	$C_7H_{11}N_3S$	169	169.0674 (169.0682)
5A	A	68	Tan viscous liquid	$C_8H_{12}N_4$	164	164.1062 (164.1060)
7A	B	9	Tan viscous liquid	$C_8H_{12}N_4$	164	164.1062 (164.1080)
8A	A	61	Yellowish solid (74—75)	$C_{13}H_{15}N_3$	213	213.1266 (213.1254)
9A	A	69	Yellowish solid (69—70)	$C_{13}H_{15}N_3$	213	213.1266 (213.1247)
10A	B	33	Tan viscous liquid	$C_{13}H_{15}N_3$	213	213.1266 (213.1235)
11B	B	11	Tan viscous liquid	$C_{11}H_{16}N_2$	176	176.1314 (176.1315)
12A	B	29	Tan viscous liquid	$C_{14}H_{16}N_2$	212	212.1313 (212.1274)
12B	B	16	Tan viscous liquid	$C_{15}H_{18}N_2$	226	226.1470 (226.1495)
13A	B	52	Yellowish solid (123—125)	$C_{14}H_{16}N_2$	212	212.1313 (212.1338)
13B	B	42	Tan viscous liquid	$C_{15}H_{18}N_2$	226	226.1470 (226.1433)

Table 2. ^{1}H-NMR and IR Spectral Data for *N*-Arylpiperazines and -Homopiperazines

Compound	^{1}H-NMR ($CDCl_3$) δ	IR (neat) cm^{-1}
1B	1.84—1.96 (2H, m), 2.08—2.20 (1H, br), 2.86 (2H, t, *J*=5.8 Hz), 3.04 (2H, t, *J*=5.4 Hz), 3.66—3.79 (4H, m), 6.44—6.56 (2H, m), 7.36—7.48 (1H, m), 8.10—8.18 (1H, m)	3300
2A	1.74—1.86 (1H, br), 3.00—3.08 (4H, m), 3.14—3.22 (4H, m), 7.15—7.20 (2H, m), 8.07—8.13 (1H, m), 8.28—8.35 (1H, m)	3300
3A	2.16—2.34 (1H, br), 3.00 (4H, t, *J*=5.4 Hz), 3.31 (4H, t, *J*=5.4 Hz), 6.67 (2H, d, *J*=6.6 Hz), 8.27 (2H, d, *J*=6.6 Hz)	3360 (KBr)
4A	1.72—1.84 (1H, s), 2.99 (4H, t, *J*=5.2 Hz), 3.47 (4H, t, *J*=5.2 Hz), 6.57 (1H, d, *J*=3.7 Hz), 7.21 (1H, d, *J*=3.7 Hz)	3300
5A	1.92 (1H, s), 2.90—3.08 (4H, m), 3.48—3.65 (4H, m), 7.84 (1H, d, *J*=2.9 Hz), 8.06 (1H, dd, *J*=2.9, 1.5 Hz), 8.13 (1H, d, *J*=1.5 Hz)	3295
7A	1.85—1.98 (1H, br), 3.02—3.10 (4H, m), 3.17—3.27 (4H, m), 8.37 (1H, d, *J*=1.5 Hz), 8.70 (2H, d, *J*=1.5 Hz)	3380
8A	1.80—2.00 (1H, br), 2.96—3.06 (4H, m), 3.66—3.76 (4H, m), 6.97 (1H, d, *J*=9.2 Hz), 7.18—7.28 (1H. m), 7.48—7.64 (2H, m), 7.71 (1H, d, *J*=8.5 Hz), 7.89 (1H, d, *J*=9.2 Hz)	3570 (KBr)
9A	1.73—1.96 (1H, br), 3.05—3.36 (8H, m), 6.83 (1H, d, *J*=5.0 Hz), 7.47 (1H, t, *J*=8.5 Hz), 7.64 (1H, t, *J*=8.5 Hz), 7.96—8.10 (2H, m), 8.72 (1H, d, *J*=5.0 Hz)	3380 (KBr)
10A	1.96 (1H, s), 3.16 (8H, s), 7.60 (1H, t, *J*=8.2 Hz), 7.70 (1H, t, *J*=8.2 Hz), 7.96 (1H, d, *J*=8.2 Hz), 8.13 (1H, d, *J*=8.2 Hz), 8.20 (1H, s), 8.98 (1H, s)	3405
11B	1.88—2.06 (2H, m), 2.90 (2H, t, *J*=5.8 Hz), 3.08 (2H, t, *J*=5.2 Hz), 3.38—3.52 (1H, br), 3.58 (2H, t, *J*=5.8 Hz), 3.60 (2H, t, *J*=5.2 Hz), 6.63—6.74 (3H, m), 7.17—7.28 (2H, m)	3300
12A	2.01 (1H, s), 2.80—3.40 (8H, m), 7.08 (1H, d, *J*=7.4 Hz), 7.34—7.60 (4H, m), 7.78—7.87 (1H, m), 8.16—8.27 (1H, m)	3375
12B	1.91 (1H, s), 1.97—2.08 (2H, m), 3.10—3.25 (4H, m), 3.27—3.42 (4H, m), 7.17 (1H, d, *J*=7.4 Hz), 7.32—7.58 (4H, m), 7.76—7.86 (1H, m), 8.22—8.34 (1H, m)	3405
13A	1.99 (1H, s), 3.05—3.15 (4H, m), 3.21—3.32 (4H, m), 7.13 (1H, d, *J*=1.9 Hz), 7.20—7.35 (2H, m), 7.35—7.50 (1H, m), 7.60—7.80 (3H, m)	3420 (KBr)
13B	1.90—2.12 (2H, m), 2.50—2.80 (1H, br), 2.80—2.93 (2H, m), 3.02—3.20 (2H, m), 3.58—3.77 (4H, m), 6.88 (1H, s), 7.03—7.22 (2H, m), 7.28—7.40 (1H, m), 7.50—7.76 (3H, m)	3415

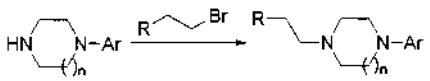
mercially available methoxy derivatives **20A**, **21A** and **22A**. Furthermore, in the other cases, the commercially available piperazine derivatives were used as the starting materials.

The *N*-arylpiperazines and *N*-arylhomopiperazines mentioned above were heated with 2-phenylethyl bromide, 3,3-diphenyl-3-cyanopropyl bromide or dimethyl (tetrahydro-3,3-diphenyl-2-furylidene) ammonium bromide[7] equivalent to 4-bromo-*N*,*N*-dimethyl-2,2-diphenylbutanamide in the presence of sodium carbonate in 1,3-dimethylbenzene or DMF to give the final target compounds.

Binding Assays and Agonist Activities

Each sample was transformed to the HCl salts to increase their solubility in water for the assays mentioned below and then converted into amorphous powder.

We selected the human MOR binding assay as the first screening. Next, to the some samples selected in this screening, we checked the human DOR and KOR binding assays to confirm the selectivity of the samples. And finally, we examined the potencies of the samples relative to that of [D-Ala^2, N-MePhe^4, Gly^5-ol]-enkephalin (DAMGO), which is high selective MOR agonist in guinea-pig ileum.



Ar=**1-27** A: n=1; B: n=2 a: R=$Ph_2C(CONMe_2)$; b: R=$Ph_2C(CN)$; c: R=Ph

Chart 3

First, according to our policy as mentioned above, we synthesized **1Aa**—**c**, fixed 2-pyridinylpiperazine, having a side chain of loperamide, diphenoxylate or fentanyl, and examined the affinity to the MOR. As the results, **1Aa** having the side chain of loperamide showed the highest affinity to the receptor among them (Table 6).

Next we examined the affinity of **1Aa** to the DOR and KOR to confirm the selectivity of that (Table 7). We confirmed that it had selectivity to the MOR because it showed extremely lower affinities to the DOR and KOR than to the MOR.

Furthermore, we confirmed the agonistic nature against MOR of **1Aa** using naloxone, but it showed a lower potency when compared to DAMGO (Table 8).

Although **2Aa** and **3Aa** had the 2-pyridinyl group of **1Aa** replaced with the 3-pyridinyl or 4-pyridinyl group, **1Aa** showed the highest affinity to the MOR among the tested compounds (Table 6).

1Ba had the piperazine of **1Aa** replaced by homopiperazine and it showed a lower affinity to the MOR than **1Aa** (Table 6).

Although several compounds (**4Aa**—**10Aa**) had the 2-pyridinyl group of **1Aa** replaced with other heteroaryl groups except for the pyridines, nothing showed a higher affinity to the MOR than **1Aa** (Table 6).

Among the compounds having heteroaryl groups, the 2-pyridinyl derivative (**1Aa**) showed the highest affinity to the MOR. Therefore, we synthesized **11Aa** having the 2-

Table 3. Yields, Properties (Melting Points) and Mass Spectral Data for the Final Products

Compound	Yield (%)	Property [mp (°C)]	Formula	MS *m/z* M^+	HR-MS *m/z* M^+ Calcd (Found)
1Aa	71	Yellowish viscous liquid	$C_{27}H_{32}N_4O$	428	428.2574 (428.2590)
1Ab	38	Yellowish viscous liquid	$C_{25}H_{26}N_4$	382	382.2156 (382.2150)
1Ac	73	Yellowish viscous liquid	$C_{17}H_{21}N_3$	267	267.1734 (267.1733)
1Ba	77	Yellowish viscous liquid	$C_{28}H_{34}N_4O$	442	442.2731 (442.2717)
2Aa	62	Yellowish viscous liquid	$C_{27}H_{32}N_4O$	428	428.2574 (428.2568)
3Aa	79	Yellowish viscous liquid	$C_{27}H_{32}N_4O$	428	428.2574 (428.2561)
4Aa	77	Yellowish viscous liquid	$C_{25}H_{30}N_4OS$	434	434.2139 (434.2175)
5Aa	80	Yellowish viscous liquid	$C_{25}H_{31}N_5O$	429	429.2529 (429.2510)
6Aa	85	White solid (149—150)	$C_{25}H_{31}N_5O$	429	429.2529 (429.2552)
7Aa	71	White solid (111—112)	$C_{25}H_{31}N_5O$	429	429.2529 (429.2510)
8Aa	88	Yellowish viscous liquid	$C_{31}H_{34}N_4O$	478	478.2731 (478.2711)
9Aa	96	Yellowish viscous liquid	$C_{31}H_{34}N_4O$	478	478.2731 (478.2739)
10Aa	79	Yellowish viscous liquid	$C_{31}H_{34}N_4O$	478	478.2731 (478.2729)
11Aa	97	Yellowish viscous liquid	$C_{28}H_{33}N_3O$	427	427.2624 (427.2626)
11Ba	65	Yellowish viscous liquid	$C_{29}H_{35}N_3O$	441	441.2780 (441.2754)
12Aa	72	Yellowish viscous liquid	$C_{32}H_{35}N_3O$	477	477.2778 (477.2759)
12Ba	66	Yellowish viscous liquid	$C_{33}H_{37}N_3O$	491	491.2937 (491.2923)
13Aa	quant.	Yellowish viscous liquid	$C_{32}H_{35}N_3O$	477	477.2778 (478.2755)
13Ba	82	Yellowish viscous liquid	$C_{33}H_{37}N_3O$	491	491.2937 (491.2947)
14Aa	quant.	Yellowish viscous liquid	$C_{29}H_{35}N_3O$	441	441.2780 (441.2797)
15Aa	93	Yellowish viscous liquid	$C_{29}H_{35}N_3O$	441	441.2780 (441.2797)
16Aa	95	Yellowish viscous liquid	$C_{29}H_{35}N_3O$	441	441.2780 (441.2780)
17Aa	99	Yellowish viscous liquid	$C_{28}H_{33}N_3O_2$	443	443.2573 (443.2555)
18Aa	94	Yellowish viscous liquid	$C_{28}H_{33}N_3O_2$	443	443.2573 (443.2572)
19Aa	93	Yellowish viscous liquid	$C_{28}H_{33}N_3O_2$	443	443.2573 (443.2584)
20Aa	97	Yellowish viscous liquid	$C_{29}H_{35}N_3O_2$	457	457.2729 (457.2716)
21Aa	89	Yellowish viscous liquid	$C_{29}H_{35}N_3O_2$	457	457.2729 (457.2710)
22Aa	90	White solid (139—140)	$C_{29}H_{35}N_3O_2$	457	457.2729 (457.2737)
23Aa	96	Yellowish viscous liquid	$C_{28}H_{32}ClN_3O$	461	461.2234 (461.2228)
24Aa	96	Yellowish viscous liquid	$C_{28}H_{32}ClN_3O$	461	461.2234 (461.2229)
25Aa	99	Yellowish viscous liquid	$C_{28}H_{32}ClN_3O$	461	461.2234 (461.2241)
26Aa	98	Yellowish viscous liquid	$C_{28}H_{32}FN_3O$	445	445.2529 (445.2515)
27Aa	98	Yellowish viscous liquid	$C_{28}H_{32}FN_3O$	445	445.2529 (445.2512)

Table 4. ^{1}H-NMR and IR Spectral Data for the Final Products

Compound	^{1}H-NMR ($CDCl_3$) δ	IR (neat) cm^{-1}
1Aa	2.04—2.18 (2H, m), 2.20—2.41 (3H, br), 2.41—2.56 (6H, m), 2.84—3.10 (3H, br), 3.44 (4H, t, *J*=5.1 Hz), 6.52—6.62 (2H, m), 7.22—7.48 (11H, m), 8.11—8.17 (1H, m)	1630
1Ab	2.44—2.60 (6H, m), 2.60—2.71 (2H, m), 3.52 (4H, t, *J*=5.1 Hz), 6.57—6.66 (2H, m), 7.22—7.52 (11H, m), 8.14—8.22 (1H, m)	2240
1Ac	2.58 (6H, m), 2.80—2.91 (2H, m), 3.58 (4H, t, *J*=5.1 Hz), 6.58—6.70 (2H, m), 7.15—7.35 (5H, m), 7.43—7.53 (1H, m), 8.16—8.24 (1H, m)	1600, 1490
1Ba	1.78—1.91 (2H, m), 2.14—2.48 (7H, m), 2.48—2.60 (2H, m), 2.60—2.72 (2H, m), 2.82—3.10 (3H, br), 3.49—3.59 (2H, m), 3.59—3.68 (2H, m), 6.40 (1H, d, *J*=8.8 Hz), 6.43—6.50 (1H, m), 7.18—7.44 (11H, m), 8.05—8.12 (1H, m)	1640
2Aa	2.07—2.17 (2H, m), 2.20—2.41 (3H, br), 2.41—2.56 (6H, m), 2.80—3.07 (3H, br), 3.13 (4H, t, *J*=5.0 Hz), 7.11 (1H, d, *J*=3.0 Hz), 7.12 (1H, d, *J*=3.0 Hz), 7.33—7.47 (8H, m), 8.05 (1H, t, *J*=3.0 Hz), 8.25 (1H, s)	1640
3Aa	2.06—2.16 (2H, m), 2.22—2.41 (3H, br), 2.41—2.52 (6H, m), 2.88—3.10 (3H, br), 3.23 (4H, t, *J*=5.0 Hz), 6.59 (2H, d, *J*=6.8 Hz), 7.22—7.47 (10 H, m), 8.22 (2H, d, *J*=6.8 Hz)	1635
4Aa	2.06—2.17 (2H, m), 2.22—2.41 (3H, br), 2.41—2.54 (6H, m), 2.88—3.08 (3H, br), 3.39 (4H, t, *J*=5.1 Hz), 6.52 (1H, d, *J*=3.7 Hz), 7.16 (1H, d, *J*=3.7 Hz), 7.16 (1H, d, *J*=3.7 Hz), 7.23—7.33 (2H, m), 7.33—7.46 (8H, m)	1640
5Aa	2.06—2.19 (2H, m), 2.22—2.40 (3H, br), 2.40—2.53 (6H, m), 2.86—3.08 (3H, br), 3.50 (4H, t, *J*=5.0 Hz), 7.24—7.33 (2H, m), 7.33—7.46 (8H, m), 7.80 (1H, d, *J*=2.6 Hz), 8.02 (1H, dd, *J*=2.6, 1.5 Hz), 8.07 (1H, d, *J*=1.5 Hz)	1640
6Aa	2.06—2.17 (2H, m), 2.24—2.43 (3H, br), 2.38 (4H, t, *J*=5.0 Hz), 2.44—2.53 (2H, m), 2.86—3.10 (3H, br), 3.72 (4H, t, *J*=5.0 Hz), 6.43 (1H, t, *J*=4.9 Hz), 7.22—7.32 (2H, m), 7.33—7.46 (8H, m), 8.26 (2H, d, *J*=4.9 Hz)	1630 (KBr)
7Aa	2.08—2.18 (2H, m), 2.22—2.40 (3H, br), 2.40—2.60 (6H, m), 2.86—3.12 (3H, br), 3.12—3.24 (4H, m), 7.24—7.34 (2H, m), 7.34—7.47 (8H, m), 8.30 (2H, d, *J*=2.9 Hz), 8.65 (1H, d, *J*=2.9 Hz)	1630 (KBr)
8Aa	2.07—2.19 (2H, m), 2.20—2.43 (3H, br), 2.43—2.57 (6H, m), 2.90—3.12 (3H, br), 3.66 (4H, t, *J*=5.1 Hz), 6.91 (1H, d, *J*=9.2 Hz), 7.18 (1H, t, *J*=7.7 Hz), 7.22—7.32 (2H, m), 7.32—7.46 (8H, m), 7.49 (1H, t, *J*=7.7 Hz), 7.55 (1H, d, *J*=7.7 Hz), 7.66 (1H, d, *J*=7.7 Hz), 7.83 (1H, d, *J*=9.2 Hz)	1630
9Aa	2.14—2.26 (2H, m), 2.26—2.46 (3H, br), 2.46—2.56 (2H, m), 2.60—2.72 (4H, br), 2.84—3.10 (3H, br), 3.10—3.24 (4H, br), 6.77 (1H, d, *J*=5.0 Hz), 7.24—7.34 (3H, m), 7.24—7.34 (8H, m), 7.63 (1H, t, *J*=8.5 Hz), 7.97 (1H, d, *J*=8.5 Hz), 8.02 (1H, d, *J*=8.5 Hz), 8.69 (1H, d, *J*=5.0 Hz)	1630
10Aa	2.14—2.26 (2H, m), 2.26—2.46 (3H, br), 2.46—2.58 (2H, m), 2.58—2.78 (4H, br), 2.82—3.08 (3H, br), 3.08—3.22 (4H, br), 7.23—7.34 (2H, m), 7.34—7.50 (8H, m), 7.56 (1H, t, *J*=8.0 Hz), 7.65 (1H, t, *J*=8.0 Hz), 7.92 (1H, d, *J*=8.0 Hz), 8.05 (1H, d, *J*=8.0 Hz), 8.14 (1H, s), 8.93 (1H, s)	1640
11Aa	2.07—2.18 (2H, m), 2.22—2.42 (3H, br), 2.42—2.58 (6H, m), 2.86—3.06 (3H, br), 3.10 (4H, t, *J*=5.0 Hz), 6.80 (1H, t, *J*=7.2 Hz), 6.87 (2H, d, *J*=8.0 Hz), 7.16—7.32 (4H, m), 7.32—7.47 (8H, m)	1630
11Ba	1.78—1.96 (2H, m), 2.14—2.48 (7H, m), 2.48—2.60 (2H, m), 2.63—2.76 (2H, m), 2.84—3.08 (3H, br), 2.32—3.50 (4H, m), 6.56—6.68 (3H, m), 7.10—7.22 (2H, m), 7.22—7.46 (10H, m)	1640
12Aa	2.12—2.25 (2H, m), 2.25—2.46 (3H, br), 2.46—2.58 (2H, m), 2.58—2.78 (4H, br), 2.86—3.20 (7H, m), 7.03 (1H, dd, *J*=7.4, 1.1 Hz), 7.22—7.56 (14H, m), 7.74—7.84 (1H, m), 8.08—8.18 (1H, m)	1625
12Ba	2.08—2.46 (5H, m), 2.60—2.80 (4H, m), 2.90—3.10 (3H, br), 3.10—3.62 (8H, m), 7.13 (1H, d, *J*=7.4 Hz), 7.16—7.52 (13H, m), 7.56 (1H, d, *J*=8.0 Hz), 7.74—7.86 (1H, m), 8.08—8.18 (1H, m)	1625
13Aa	2.09—2.22 (2H, m), 2.24—2.44 (3H, br), 2.44—2.70 (6H, m), 2.87—3.14 (3H, br), 3.14—3.34 (4H, br), 7.05 (1H, s), 7.10—7.55 (13H, m), 7.55—7.76 (3H, m)	1625
13Ba	1.95—2.37 (5H, m), 2.37—2.63 (4H, m), 2.63—3.05 (7H, m), 3.56 (2H, d, *J*=6.6 Hz), 3.60—3.80 (2H, m), 6.83 (1H, d, *J*=2.5 Hz), 7.01 (1H, dd, *J*=8.8, 2.5 Hz), 7.18 (1H, t, *J*=7.5 Hz), 7.22—7.50 (11H, m), 7.59 (1H, d, *J*=8.8 Hz), 7.62—7.70 (2H, m)	1625
14Aa	2.10—2.18 (2H, m), 2.23 (3H, s), 2.26—2.40 (3H, br), 2.43—2.58 (6H, m), 2.85 (4H, t, *J*=4.6 Hz), 2.91—3.05 (3H, br), 6.90—6.99 (2H, m), 7.07—7.15 (2H, m), 7.24—7.30 (2H, m), 7.33—7.46 (8H, m)	1635
15Aa	2.07—2.16 (2H, m), 2.23—2.40 (3H, br), 2.28 (3H, s), 2.42—2.55 (6H, m), 2.88—3.13 (7H, m), 6.60—6.71 (3H, m), 7.10 (1H, t, *J*=7.8 Hz), 7.23—7.45 (10H, m)	1635
16Aa	2.08—2.16 (2H, m), 2.23 (3H, s), 2.26—2.38 (3H, br), 2.43—2.55 (6H, m), 2.90—3.10 (7H, m), 6.78 (2H, d, *J*=8.8 Hz), 7.02 (2H, d, *J*=8.8 Hz), 7.23—7.29 (2H, m), 7.32—7.46 (8H, m)	1645
17Aa	2.09—2.17 (2H, m), 2.27—2.39 (3H, br), 2.43—2.58 (6H, m), 2.81 (4H, t, *J*=4.4 Hz), 2.93—3.05 (3H, br), 6.81 (1H, dt, *J*=7.8, 1.2 Hz), 6.89 (1H, dd, *J*=7.8, 1.2 Hz), 7.02 (1H, dt, *J*=7.8, 1.2 Hz), 7.11 (1H, dd, *J*=7.8, 1.2 Hz), 7.25—7.32 (2H, m), 7.34—7.46 (8H, m)	3330, 1645
18Aa	2.09—2.18 (2H, m), 2.27—2.37 (3H, br), 2.43—2.54 (6H, m), 2.91—3.06 (7H, m), 6.17—6.25 (2H, m), 6.34 (1H, d, *J*=8.0 Hz), 6.97 (1H, t, *J*=8.0 Hz), 7.21—7.43 (10H, m)	3285, 1620
19Aa	2.08—2.17 (2H, m), 2.28—2.36 (3H, br), 2.45—2.56 (6H, m), 2.93—3.02 (7H, br), 6.72 (4H, dd, *J*=9.3, 4.9 Hz), 7.24—7.30 (2H, m), 7.33—7.42 (8H, m)	3275, 1620
20Aa	2.10—2.19 (2H, m), 2.27—2.39 (3H, br), 2.44—2.60 (6H, m), 2.90—3.09 (7H, m), 3.80 (3H, s), 6.81 (1H, d, *J*=8.3 Hz), 6.84—6.90 (2H, m), 6.91—6.98 (1H, m), 7.22—7.30 (2H, m), 7.32—7.45 (8H, m)	1635
21Aa	2.08—2.14 (2H, m), 2.28—2.37 (3H, br), 2.43—2.52 (6H, m), 2.93—3.02 (3H, br), 3.10 (4H, t, *J*=4.9 Hz), 3.76 (3H, s), 6.37 (1H, dd, *J*=7.8, 2.0 Hz), 6.40 (1H, t, *J*=2.0 Hz), 6.47 (1H, dd, *J*=7.8, 2.0 Hz), 7.12 (1H, t, *J*=7.8 Hz), 7.24—7.30 (2H, m), 7.33—7.45 (8H, m)	1630
22Aa	2.08—2.15 (2H, m), 2.28—2.38 (3H, br), 2.43—2.55 (6H, m), 2.93—3.04 (7H, m), 3.74 (3H, s), 6.80 (2H, d, *J*=2.4 Hz), 6.84 (2H, d, *J*=2.4 Hz), 7.24—7.30 (2H, m), 7.33—7.45 (8H, m)	1645 (KBr)
23Aa	2.10—2.19 (2H, m), 2.26—2.40 (3H, br), 2.45—2.61 (6H, m), 2.88—3.07 (4H, m), 6.91 (1H, t, *J*=8.0 Hz), 6.99 (1H, d, *J*=8.0 Hz), 7.17 (1H, t, *J*=8.0 Hz), 7.23—7.46 (11H, m)	1635
24Aa	2.06—2.16 (2H, m), 2.24—2.39 (3H, br), 2.41—2.52 (6H, m), 2.88—3.02 (3H, br), 3.05—3.12 (4H, m), 6.70 (1H, d, *J*=8.5 Hz), 6.73 (1H, d, *J*=8.5 Hz), 6.79 (1H, s), 7.10 (1H, t, *J*=8.5 Hz), 7.23—7.30 (2H, m), 7.32—7.46 (8H, m)	1630
25Aa	2.07—2.16 (2H, m), 2.23—2.40 (3H, br), 2.41—2.54 (6H, m), 2.88—3.10 (7H, m), 6.76 (4H, d, *J*=9.0 Hz), 7.13 (4H, d, *J*=9.0 Hz), 7.22—7.30 (2H, m), 7.31—7.45 (8H, m)	1645
26Aa	2.10—2.18 (2H, m), 2.28—2.39 (3H, br), 2.43—2.58 (6H, m), 2.92—3.07 (7H, m), 7.24—7.31 (2H, m), 7.34—7.45 (8H, m)	1635
27Aa	2.08—2.17 (2H, m), 2.28—2.39 (3H, br), 2.42—2.57 (6H, m), 2.90—3.10 (7H, m), 6.78—6.85 (2H, m), 6.87—6.96 (2H, m), 7.24—7.31 (2H, m), 7.33—7.46 (8H, m)	1635

Table 5. Analytical Data for **6Aa**, **7Aa** and **22Aa**

Compound	*Anal.* Calcd (Found)		
	C	H	N
6Aa	72.70 (72.43)	7.27 (7.29)	16.30 (16.11)
7Aa	72.70 (72.72)	7.27 (7.07)	16.30 (16.29)
22Aa	76.12 (76.17)	7.71 (7.71)	9.18 (9.18)

6Aa was recrystallized from ethyl acetate. **7Aa** and **22Aa** were recrystallized from diethyl ether.

Table 6. Binding Assays to Human Opiate μ-Receptor

Compound	IC_{50} (nM)	Compound	IC_{50} (nM)
1Aa	5.8	**13Aa**	27
1Ab	230	**13Ba**	220
1Ac	6400	**14Aa**	0.42
1Ba	35	**15Aa**	0.86
2Aa	30	**16Aa**	11
3Aa	55	**17Aa**	0.61
4Aa	11	**18Aa**	2.4
5Aa	28	**19Aa**	9.4
6Aa	9.0	**20Aa**	0.28
7Aa	200	**21Aa**	1.6
8Aa	73	**22Aa**	19
9Aa	17	**23Aa**	0.69
10Aa	7.0	**24Aa**	0.67
11Aa	1.8	**25Aa**	11
11Ba	12	**26Aa**	1.0
12Aa	1.1	**27Aa**	4.4
12Ba	11	Loperamide	0.99

Quantitative follow-up: IC_{50} assessed in three independent experiments over a range of 5—6 concentrations: total 34 tubes. This assay measures binding of the [^{3}H]Diprenorphine (DPN) to human opiate μ-receptor.

pyridinyl group of **1Aa** replaced with a phenyl group and then examined its affinity. To our surprise, **11Aa** showed a higher affinity to the MOR than **1Aa** (Table 6).

Next, we examined the affinity of **11Aa** to the ODR and KOR to confirm its selectivity (Table 7). We could then confirm that it had selectivity to the MOR because it showed extremely lower affinities to the DOR and KOR than to the MOR.

Furthermore, we confirmed the agonistic nature against MOR of **11Aa** using naloxone but then it showed almost the same potency when compared to **1Aa** so less than DAMGO (Table 8).

Although **11Ba** had the piperazine of **11Aa** replaced with homopiperazine, **11B** showed a lower affinity to the MOR than **11Aa** (Table 6). This result was similar to the case of the 2-pyridine derivatives, therefore we could easily accept this result.

Among the 1- and 2-naphthyl derivatives (**12Aa**, **12Ba**, **13Aa**, **13Ba**) having piperazine or homopiperazine, only **12Aa** showed a higher affinity to the MOR than **11Aa** (Table 6). Therefore, we checked the agonist activity against MOR of **12Aa**, but it had a lower potency than **11Aa**, so this compound was dropped.

Among the compounds (**14Aa**—**27Aa**) having the substituted phenyl groups, the 2-methoxyphenyl derivative **20Aa** showed the highest affinity to the MOR among all our compounds (Table 6).

We next examined the affinities of **20Aa** to the DOR and KOR to confirm its selectivity (Table 7). We confirmed that it had selectivity to the MOR because it showed extremely lower affinities to the DOR and KOR than to the MOR.

Table 7. Binding Assays to Human Opiate δ- and κ-Receptors

Compound	δ, IC_{50} (nM)[a]	κ, IC_{50} (nM)[b]
1Aa	6200	>10000
11Aa	1800	5100
20Aa	180	2000
Loperamide	880	640

Quantitative follow-up: IC_{50} assessed in three independent experiments over a range of 5—6 concentrations: total 34 tubes. *a*) This assay measures binding of [^{3}H]Naltrindole to human opiate δ-receptors. *b*) This assay measures binding of [^{3}H]DPN to human opiate κ-receptors.

Table 8. Potencies of Test Compounds Relative to That of DAMGO in Isolated Guinea-Pig Ileum Preparation

Compound	*n*	Relative Potency
1Aa	4	0.520±0.16
11Aa	4	0.470±0.096
12Aa	4	0.106±0.028
20Aa	6	12.0±1.1
DAMGO	22	1
Loperamide	4	5.43±0.68

The % inhibition of the stimulated muscle twitch produced by a compound was plotted against the log concentration of the compound to estimate the IC_{50} (concentration of the compound to produce 50% inhibition of the twitch). The relative potencies of the test compounds were calculated by the following formula: Relative Potency= DAMGO's IC_{50}/Compound's IC_{50}. The test compounds were confirmed to be an opioid agonist by antagonism of naloxone (sufficiently at 10^{-8} M). Values are the means± S.E.M. of *n* observations.

Furthermore, we confirmed the agonistic nature against MOR of **20Aa** using naloxone and then it finally showed a higher potency when compared to DAMGO and loperamide (Table 8).

The results mentioned above are summarized below: 1) The derivatives having a side chain of loperamide showed a high affinity to the MOR. 2) The piperazine derivatives showed a higher affinity to the MOR than the homopiperazine derivatives. 3) The derivatives having phenyl groups with substituent groups showed a high affinity to the MOR and a high agonist activity against MOR. 4) As the groups attached to the phenyl groups, both electron-donating groups and electron-withdrawing groups effectively increase the affinity. However, the *ortho*-substituted derivatives showed the highest affinity to the MOR, and in the order of *meta*- to *para*-substituted derivatives, the affinity decreased except for the two chlorine-substituted derivatives, **23Aa** and **24Aa**. 5) Finally, 4-[4-(2-methoxyphenyl)piperazin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide (**20Aa**) showed the highest affinity to the MOR and the highest potency on the MOR among our compounds.

As a result, we found **20Aa** showing a higher affinity to the MOR and the higher agonist activity than loperamide as a reference. Therefore we are now examining why it has these strong activities and to optimize its structure.

Conclusion

Novel MOR agonists with piperazine and homopiperazine moieties in their structures were synthesized. It was found that **20Aa** with the 2-methoxyphenyl moiety showed a higher

affinity to the human MOR expressed on CHO-K1 cells than loperamide and a higher potency on MOR in guinea-pig ileum than loperamide. Therefore, after confirming that **20Aa** has a peripheral analgesic activity *in vivo*, we will select **20Aa** as the lead compound for the peripheral μ-opioid analgesic mentioned above. Besides, we are now examining why it has these strong activities, and then optimize the structure of it followed by the pharmacological tests of the selected derivatives *in vivo*.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus without correction. IR spectra were measured with a Nihon-bunko IR-810 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini 2000 spectrometer in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6 using tetramethylsilane as the internal reference. The following abbreviations were used: s=singlet, d=doublet, dd=double doublet, dt=double triplet, t=triplet, q=quartet, m=multiplet and br=broad. MS or high-resolution mass spectra (HR-MS) were obtained using JEOL JMS-DX303 or JEOL JMS-AX500 mass spectrometer. TLC was performed by using Silica gel $60F_{254}$ (Merck). Column chromatography was performed with a Silica gel (BW-80S Fuji-sirial). Magnesium sulfate was employed as the drying agent. Dichlorobis(tri-*o*-tolylphosphine)palladium (II) and hydrogen chloride, 1.0 M solution in diethyl ether were obtained from Aldrich Chemical Company, Inc. The yields, physical and spectral data for the intermediates, **1B**, **2**—**5A**, **7**—**10A**, **11B**, **12A**, **B** and **13A**, **B** are shown in Tables 1 and 2. The yields, physical and spectral data for the final products, **1Aa**—**c**, **1Ba**, **2**—**11Aa**, **11Ba**, **12Aa**, **12Ba**, **13Aa**, **13Ba** and **14**—**27Aa** are shown in Tables 3 and 4. The analytical data for the solid compounds among the above compounds, **6Aa**, **7Aa** and **22Aa**, are shown in Table 5.

1-Heteroarylpiperazine or -Homopiperazine (General Procedure A)—Method A A mixture of piperazine (646 mg, 7.50 mmol) or homopiperazine (759 mg, 7.50 mmol), heteroarylhalide (5.00 mmol) and 1,2.4-trimethylbenzene (20 ml) was stirred at 169 °C for 8 h. The mixture was concentrated *in vacuo* and the residue was made alkaline with 1 N NaOH to pH 9 and extracted with $CHCl_3$. The extract was dried, concentrated *in vacuo* to give a brown oil which was purified by column chromatography on silica gel. Elution with 7% MeOH in $CHCl_3$ gave the target molecule.

1-Arylpiperazine or -Homopiperazine (General Procedure B)—Method B A mixture of piperazine (610 mg, 7.08 mmol) or homopiperazine (709 mg, 7.08 mmol), aryl halide (5.02 mmol), sodium *t*-butoxide (680 mg, 7.08 mmol), dichlorobis(tri-*o*-tolylphosphine)palladium (II) (194 mg, 0.247 mmol) and 1,2,4-trimethylbenzene (20 ml) was stirred at 169 °C for 24 h. After cooling, the mixture was diluted with THF and filtered through Celite®. The filtrate was concentrated *in vacuo* to give a tan oil which was purified by column chromatography on silica gel. Elution with 7% MeOH in $CHCl_3$ gave the target molecule.

1-(Hydroxyphenyl)piperazine (17—19A) (General Procedure C) A mixture of 1-(methoxyphenyl)piperazine (1.00 g, 5.20 mmol) and 48% hydrobromic acid (15.0 ml, 278 mmol) was stirred at 140 °C for 6 h. After cooling, the mixture was made alkaline with 3 N-NaOH to pH 9. The resulting precipitate was collected by filtration, washed with water, air-dried at room temperature to give **17**—**19A** as an yellowish solid.

1-(2-Hydroxyphenyl)piperazine (**17A**): mp 118—120 °C. Yield 54%. ^{1}H-NMR (DMSO-d_6) δ: 2.76—3.05 (8H, m), 4.40—6.20 (2H, br), 6.68—6.98 (4H, m). MS *m*/*z*: 178 (M^+). HR-MS *m*/*z*: 178.1071 (Calcd for $C_{10}H_{14}N_2O$: 178.1106). IR ν (KBr) cm^{-1}: 3300.

1-(3-Hydroxyphenyl)piperazine (**18A**): mp 210—212 °C. Yield 79%. ^{1}H-NMR (DMSO-d_6) δ: 2.70—2.90 (4H, m), 2.90—2.97 (4H, m), 2.97—3.43 (1H, br), 6.18 (1H, dd, *J*=7.8, 2.0 Hz), 6.27 (1H, s), 6.33 (1H, d, *J*=7.8 Hz), 6.96 (1H, t, *J*=7.8 Hz), 8.80—9.20 (1H, br). MS *m*/*z*: 178 (M^+). HR-MS *m*/*z*: 178.1075 (Calcd for $C_{10}H_{14}N_2O$: 178.1106). IR ν (KBr) cm^{-1}: 3260.

1-(4-Hydroxyphenyl)piperazine (**19A**): mp 223—225 °C. Yield 73%. ^{1}H-NMR (DMSO-d_6) δ: 2.74—2.90 (8H, m), 2.97—3.50 (1H, br), 6.63 (2H, d, *J*=8.8 Hz), 6.74 (2H, d, *J*=8.8 Hz), 8.50—8.95 (1H, br). MS *m*/*z*: 178 (M^+). HR-MS *m*/*z*: 178.1075 (Calcd for $C_{10}H_{14}N_2O$: 178.1106). IR ν (KBr) cm^{-1}: 3280.

4-(4-Arylpiperazin-1-yl)-*N*,*N*-dimethyl-2,2-diphenylbutanamide (General Procedure D) A mixture of 1-arylpiperazine (1.25 mmol) or -homopiperazine (1.25 mmol), dimethyl (tetrahydro-3,3-diphenyl-2-furylidene) ammonium bromide (480 mg, 1.39 mmol), sodium carbonate (500 mg, 4.72 mmol) and DMF (15 ml) was stirred at 100 °C for 4 h. Then the mixture was concentrated *in vacuo.* After addition of water, the residure was extracted with $CHCl_3$. The extract was dried and concentrated *in vacuo* to give a tan oil which was purified by column chromatography on silica gel. Elution with 3% MeOH in $CHCl_3$ gave the final target molecule.

2,2-Diphenyl-4-[4-(2-pyridinyl)piperazin-1-yl]butanenitrile (1Ab) A mixture of 1-(2-pyridinyl)piperazine (240 mg, 1.47 mmol), 4-bromo-2,2-diphenylbutyronitrile (450 mg, 1.47 mmol), sodium carbonate (400 mg, 3.77 mmol) and 1,3-dimethylbenzene (15 ml) was stirred at 139 °C for 24 h. The reaction mixture was concentrated *in vacuo* and after addition of water, the residue was extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated *in vacuo* to give a tan oil which was purified by column chromatography on silica gel. Elution with 2% MeOH in $CHCl_3$ gave **1Ab** (213 mg, 38%) as yellowish viscous liquid.

1-(2-Phenylethyl)-4-(2-pyridinyl)piperazine (1Ac) A mixture of 1-(2-pyridinyl)piperazine (314 mg, 1.93 mmol), 2-phenylethyl bromide (400 mg, 2.16 mmol), sodium carbonate (400 mg, 3.77 mmol) and 1,3-dimethylbenzene (25 ml) was stirred at 139 °C for 24 h. The reaction mixture was concentrated *in vacuo.* After addition of water, the residue was extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated *in vacuo* to give a tan oil which was purified by column chromatography on silica gel. Elution with 3% MeOH in $CHCl_3$ gave **1Ac** (376 mg, 73%) as yellowish viscous liquid.

Preparation of HCl Salts (General Procedure E) A final compound (1.00 mmol) was dissolved in $CHCl_3$ (10 ml). 1.0 M Hydrogen chloride solution in diethyl ether (1.00 ml) was added into the solution, and the mixture was concentrated *in vacuo.* After addition of ethyl ether (15 ml) to the residue, the resulting solid was collected by filtration, washed with diethyl ether, air-dried at room temperature to give the HCl salts as a white amorphous powder.

Binding Assays to Human MORs Using [^{3}H]Diprenorphine (DPN) CHO-K1 cells stably transfected with a plasmid encoding the human MOR are used to prepare membranes in modified Tris–HCl pH 7.4 buffer using standard techniques. An 11 μg aliquot of membrane is incubated with 0.6 nM [^{3}H]DPN for 2.5 h at 25 °C. Nonspecific binding is estimated in the presence of 10 μM naloxone. Membranes are filtered and washed 3 times and the filters are counted to determine [^{3}H]DPN specifically bound.[14]

Binding Assays to Human DORs Using [^{3}H]Naltrindole CHO cells stably transfected with a plasmid encoding the human DOR are used to prepare membranes in modified Tris–HCl pH 7.4 buffer using standard techniques. A 0.9 μg aliquot of membrane is incubated with 0.9 nM [^{3}H]Naltrindole for 2 h at 25 °C. Nonspecific binding is estimated in the presence of 10 μM naloxone. Membranes are filtered and washed 3 times and the filters are counted to determine [^{3}H]Naltrindole specifically bound.[15]

Binding Assays to Human KORs Using [^{3}H]DPN CHO cells stably transfected with a plasmid encoding the human KOR are used to prepare membranes in modified Tris–HCl pH 7.4 buffer using standard techniques. A 30 μg aliquot of membrane is incubated with 0.6 nM [^{3}H]DPN for 1 h at 25 °C. Nonspecific binding is estimated in the presence of 10 μM naloxone. Membranes are filtered and washed 3 times and the filters are counted to determine [^{3}H]DPN specifically bound.[16]

Evaluation of MOR Agonist Activities *in Vitro*[17] Male Hartley guinea-pigs weighing 300—500 g were used for this study. The myenteric plexus-longitudinal muscle strip of guinea-pig ileum was prepared as described by Rang (1964).[18] The strip was suspended in a 4 ml organ bath, which contained Krebs solution (millimolar concentrations: NaCl 118; KCl 4.75; $CaCl_2$ 2.54; KH_2PO_4 1.19; $MgSO_4$ 1.2; $NaHCO_3$ 3.25; glucose 11) and 20 μM choline chloride, and was kept at 36 °C and bubbled with 95% O_2 and 5% CO_2. The resting tension of the myenteric plexus-longitudinal muscle strip was maintained at 250 mg. The intramural nerves were stimulated through two platinum electrodes with supramaximal rectangular pulses of 1.0 msec duration. The strip of guinea-pig ileum was stimulated at a frequency of 0.1 Hz. Compounds were added in 4—40 μl amounts and washed out with 4 ml portions of bath solution after their maximum effects had been noted. The % inhibition of the stimulated muscle twitch produced by a compound was plotted against the log concentration of the compound to estimate the IC_{50} (concentration of the compound to produce 50% inhibition of the twitch). The relative potencies of the test compounds were calculated by the following formula: Relative Potency=DAMGO's IC_{50}/Compound's IC_{50}. The test compounds were confirmed to be an opioid agonist by antagonism of naloxone (sufficiently at 10^{-8} M).

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