SYNTHESIS AND CHARACTERIZATION OF NOVEL PHOTOREACTIVE NALTREXONE ANALOGS AS ISOMERIC CARBENE-GENERATING PROBES FOR OPIOID RECEPTORS

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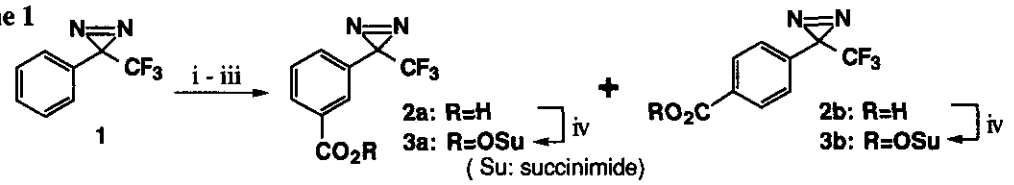
Abstract- A convenient synthesis of *m*- and *p*-CF_3-diazirinylbenzoic acid was developed. A pair of novel photoaffinity probes bearing these diazirines on a naltrexyl framework bind reversibly with high affinity at μ-, δ-, and κ- receptors.

The three major types of opioid receptors, termed μ, δ, and κ, have been cloned from different species and sequenced.[1] Recent success in the construction of chimeric opioid receptors demonstrated the presence of several extracellular regions which could be responsible for the distinct ligand-binding profiles of the each type of opioid receptors.[2] However, it is generally impossible to exclude potential conformational changes which may result in alterations of the ligand binding. The method of photoaffinity labeling is an alternative approach to verify the genetically determined ligand binding sites.[3] Probes with the highest possible reactivity are required for topographical mapping which defines full environment of a ligand within a binding pocket. Various photoaffinity probes bearing nitrene-yielding aryl azides were already developed to label the opioid receptors.[4] However, we have demonstrated that the cross-link introduced by carbene-generating aryl diazirines was more stable compared to the corresponding azide probes.[5] By using ligands carrying a diazirine (**2b**) (Scheme 1), we have successfully determined the ligand binding regions of the sodium channel and the calcium channel. Now, we describe here the synthesis and characterization of the first example of diazirine bearing photoaffinity probes for opioid receptors. A convenient method for the synthesis of photoreactive moiety (**2b**) and its regioisomer (**2a**) was also developed.

Results and discussion

A simple method for the preparation of 3- and 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acids (2a and 2b). In the previous synthesis, it is a major drawback that the many steps are required for the construction of the diazirine ring of **2b**.[6] We have developed the new approach of derivatization without the repetition of all the steps of diazirine synthesis from the beginning.[7] According to the method we developed, **1** was subjected to thallation followed by subsequent palladium-catalyzed carboxylation to result in a mixture of methyl ester of **2** (53 %) which was hydrolyzed to give a 1 : 2 mixture of carboxylic acids (**2a**) and (**2b**) in 96 % yield. The *para* isomer (**2b**) was easily isolated by recrystallization from ether-hexane and hplc purification of the mother liquor gave the *meta* isomer (**2a**).[8] The photolysis of 0.5 mM ethanol solution of **2a** was examined with a 30 w black light lamp and a half life of decomposition was determined to be 3.4 min whose value is comparable to that of **2b** (2.8 min). Both acids were converted to the succinimede esters (**3a**) (97 %)[8] and (**3b**) (95 %)[6], respectively.

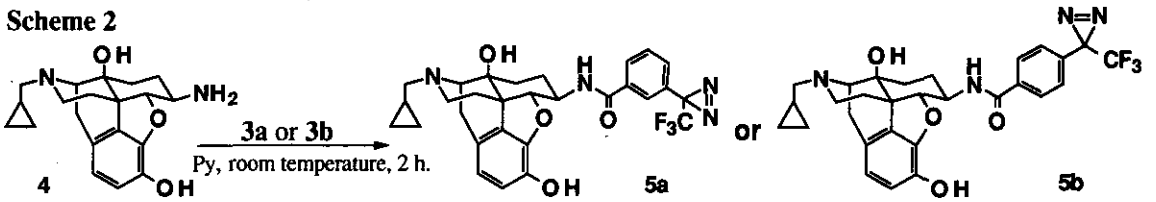
Scheme 1



i) $Tl(CO_2CF_3)_3$, TFA, 75 °C, 24 h. ii) CO, $PdCl_2$, MgO, LiCl, MeOH, room temperature, 24 h.
iii) 1N NaOH-MeOH(1:1), room temperature, 2 h. iv) DCC, HOSu, CH_2Cl_2, room temperature, 2 h.

New photoaffinity probes having a naltrexyl framework. Photoaffinity labeling of recombinant receptors is the ideal answer to overcome the heterogeneity problems of opioid receptors. The labeling of recombinant receptors with common photoprobe could be useful to define the structural feature which is related to the ligand specificity of respective receptors. Thus, the diazirines (**2a,b**) were introduced on a naltrexyl frame-work which could bind μ-, δ-, and κ-opioid receptors.[4c] The primary amino group of naltrexamine (**4**) was readily acylated with **3a** or **3b** to give the desired probes (**5a**) (70 %) or (**5b**) (87 %), respectively.[8] For the detection of trace amount of photolabeled products, the radioactive analog of these photoprobes could be prepared by using [^{3}H]-$NaBH_3CN$ at the reductive alkylation step in the reported synthesis of **4**[9] or by replacing the photoreactive group with alkoxy-substituted phenyl diazirines.[7]

Scheme 2



Receptor Binding Assays. Binding affinity constants (IC_{50} value) of **5** were determined from competition experiments using a μ- ([^{3}H]DAGO), δ- ([^{3}H]DADLE), and κ-selective ([^{3}H]U69,593) ligands with crude membrane fractions from rat brain and guinea pig cerebellum[4c] (Figures 1, 2 and 3).

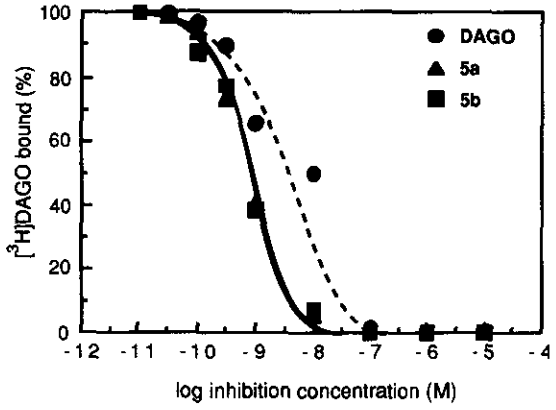


Figure 1. Competition by naltrexyl derivatives (**5**) for the binding of [^{3}H]DAGO to rat brain.

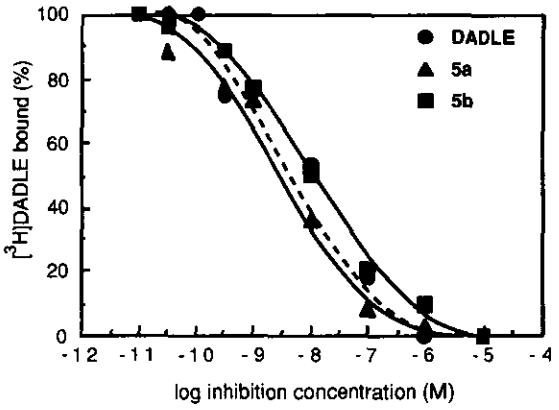


Figure 2. Competition by 5 for the binding of [^{3}H]DADLE to rat brain.

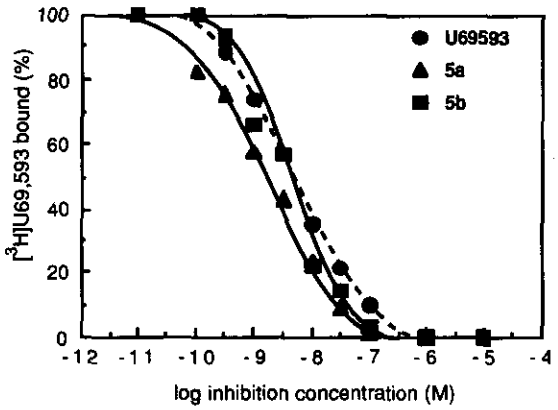


Figure 3. Competition by **5** for the binding of [^{3}H]U69,593 to guinea pig cerebellum.

Table 1. IC_{50} and *Ki* values for compound (**5**) at the μ-, δ-, and κ- receptors.

	5a		**5b**	
[^{3}H]ligand	IC_{50} (nM)	*Ki* (nM)	IC_{50} (nM)	*Ki* (nM)
DAGO	0.93	0.19	0.93	0.19
DADLE	3.2	1.5	11	5.2
U69,593	1.8	0.63	4.0	1.4

The *Ki* values of probes were calculated from their IC_{50} values according to the literature[10] and are listed in Table 1. The probe (**5a** and **5b**) exhibit high affinity binding to μ- and κ-receptors and slightly lower affinity for δ sites. The both reagent retain a sufficient affinity for the labeling study of these receptors.

In conclusion, we have developed a novel pair of photoprobes which only differ in the carbene generating sites. This structural feature of **5** is potentially useful to map the different sites within the ligand binding pocket and to elucidate the characteristic features of the each type of opioid receptors. The photoaffinity labeling experiments of recombinant receptor proteins are now under investigation with these photoprobes.

ACKNOWLEDGMENTS

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REFERENCES AND NOTES

1 G. R. Uhl, S. Childers, and G. Pasternak, *Trends Neurosci.*, 1994, **17,** 89.

2 H. Kong, K. Raynor, H. Yano, J. Takeda, and G. I. Bell, *Proc. Natl. Acad. Sci. USA*, 1994, **91,** 8042; J.-C. Xue, C. Chen, J. Zhu, S. P. Kunapuli, J. K. de Riel, L. Yu, and L.-Y. Liu-Chen, *J. Biol. Chem.*, 1995, **270,** 12977; T. Onogi, M. Minami, Y. Katao, T. Nakagawa, Y. Aoki, T. Toya, S. Katsumata, and M. Satoh, *FEBS Lett.*, 1995, **357,** 93; M. Minami, T. Onogi, T. Nakagawa, Y. Katao, Y. Aoki, S. Katsumata, and M. Satoh, *FEBS Lett.*, 1995, **364,** 23.

3 J. Brunner, *Annu. Rev. Biochem.*, 1993, **62,** 483.

4 a) P. W. Schiller, 'Handbook of Experimental Pharmacology', Vol. 104-I ed. by A. Herz, Springer-Verlag, 1993, pp. 699-701. b) K. Okumura, Y. Hatanaka, H. Nakayama, and Y. Kanaoka, *Chem. Pharm. Bull.*, 1987, **35,** 256. c) A. Mejan, J. Hawkinson, R. Guthapfel, M. Goeldner, and C. Hirth, *Biochemistry*, 1992, **31,** 9685.

5 H. Nakayama, Y. Hatanaka, M. Taki, E. Yoshida, and Y. Kanaoka, *Ann. N. Y. Acad. Sci.*, 1993, **707,** 349.

6 Y. Hatanaka, H. Nakayama, and Y. Kanaoka, *Heterocycles*, 1993, **35,** 997.

7 Y. Hatanaka, M. Hashimoto, H. Kurihara, H. Nakayama, and Y. Kanaoka, *J. Org. Chem.*, 1994, **59,** 383.

8 **2a**; Colorless needles (hexane), mp 85-87 °C (decomp.). Uv (EtOH) λ_{max} (ε): 351 nm (300); ir: 1695 cm^{-1}. ^{1}H-Nmr: 7.51 (1H, br d, *J*=7.5 Hz), 7.56 (1H, t, *J*=7.5 Hz), 7.93 (1H, br s), 8.17 (1H, d, *J*=7.5 Hz). *Anal.* Calcd for $C_9H_5N_2O_2F_3$: C 46.97; H 2.19; N 12.17. Found: C 47.07; H 2.19; N 12.05. **3a**; Colorless oil. ^{1}H-Nmr: 2.92 (4H, br s), 7.58 (1H, br d, *J*=9.0 Hz), 7.60 (1H, t, *J*=9.0 Hz), 7.91 (1H, br s), 8.20 (1H, m). **5a**; Colorless amorphous solid. Ir: 1650 cm^{-1}. ^{1}H-Nmr: 4.21 (1H, m, 6-H), 4.55 (1H, d, *J*=5.5 Hz), 6.56 (1H, d, *J*=8.5 Hz), 6.71 (1H, d, *J*=8.5 Hz), 7.39 (1H, br d, *J*=7.5 Hz), 7.46 (1H, t, *J*=7.5 Hz), 7.59 (1H, d, *J*=9.0 Hz), 7.63 (1H, br s), 7.86 (1H, d, *J*=7.5 Hz). FAB ms *m/z*: 555 $(M+H)^+$. HR-FAB ms: Calcd for $C_{29}H_{30}N_4O_4F_3$: 555.2219. Found 555.2266. **5b**; Colorless solid. Ir: 1650 cm^{-1}. ^{1}H-Nmr: 4.20 (1H, m, 6-H), 4.55 (1H, d, *J*=5.5 Hz), 6.55 (1H, d, *J*=8.5 Hz), 6.70 (1H, d, *J*=8.5 Hz), 7.21 (2H, d, *J*=8.5 Hz), 7.57 (1H, d, *J*=9.0 Hz), 7.86 (2H, d, *J*=8.5 Hz). FAB ms *m/z*: 555 $(M+H)^+$. HR-FAB ms: Found 555.2191.

9 L. M. Sayre and P. S. Portoghese, *J. Org. Chem.*, 1980, **45,** 3366.

10 Y.-C. Chen and W. H. Prusoff, *Biochem. Pharmacol.*, 1973, **22,** 3099.

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