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ARYLACETAMIDE KAPPA-SELECTIVE OPIOID LIGANDS

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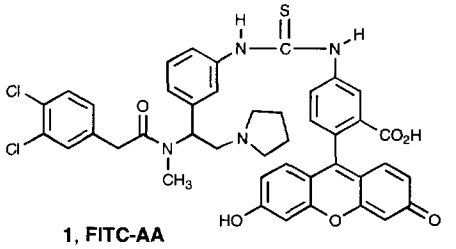
Abstract: A family of arylacetamide opioid ligands was prepared. Competition binding studies indicate that in each case the (*S*)-(+) series was two orders of magnitude more potent than the corresponding (*R*)-(−) series and that the amine (*S*)-(+)-**3** and the acetamide (*S*)-(+)-**4** were selective for the κ opioid binding site. Binding and flow cytometry experiments demonstrated that (*S*)-(+)-FITC-AA **5** was the enantiomer that bound to the κ opioid receptor. © 1997 Elsevier Science Ltd.

In 1995, we reported the synthesis of a fluorescein isothiocyanate-conjugated arylacetamide (FITC-AA), compound **1**.[3] This compound displayed high affinity for the κ opioid receptor and was used in indirect immunofluorescence experiments to specifically stain κ opioid receptors on lymphocytes. The syntheses of both stereoisomers of FITC-AA **1**, as well as various analogs, would allow us to determine the more potent form of the arylacetamides as well as the site selectivity. Here, we outline the syntheses of a variety of new analogs related to **1**, and report their opioid receptor binding properties.

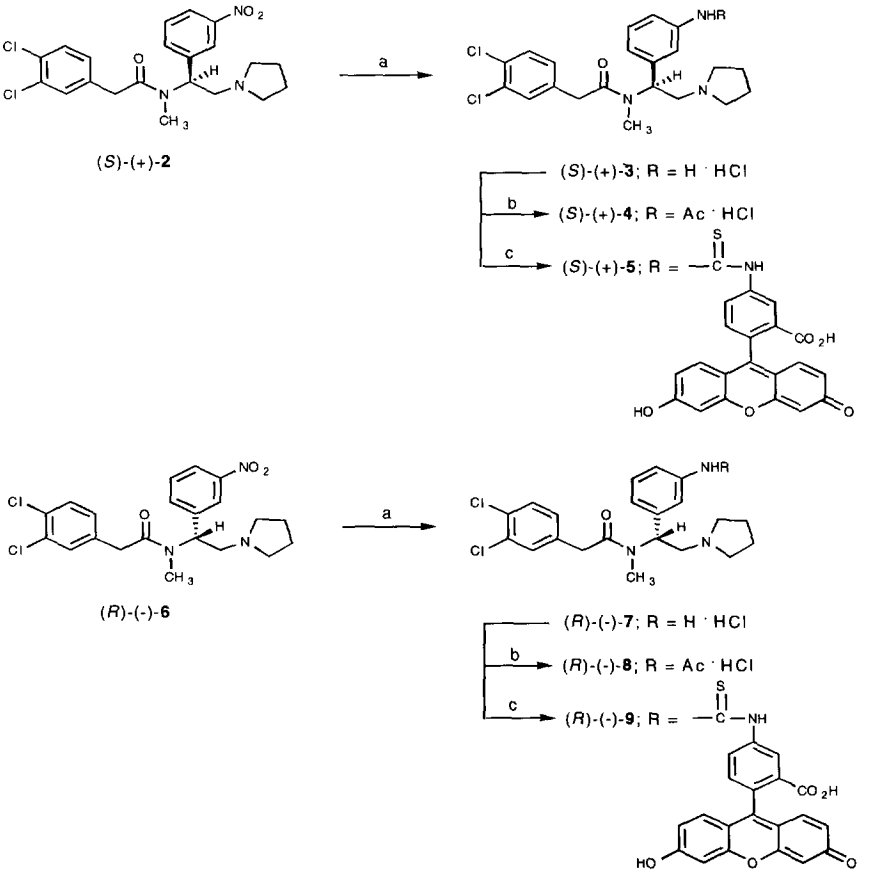


1, FITC-AA

CHEMISTRY

The nitro derivative (*S*)-(+)-**2** served as the starting point and was synthesized in five steps from (*S*)-(+)-phenylglycine as described by Chang et al.[4] Hydrogenation of compound (*S*)-(+)-**2** using 10% Pd/C in acetic acid provided a 71% yield of the amine (*S*)-(+)-**3**.[5] Compound (*S*)-(+)-**3** was then transformed into acetamide (*S*)-(+)-**4**[6,7] in 25% yield, or to the fluorescein isothiocyanate (FITC) derivative (*S*)-(+)-**5** in 80% yield.[8,9] The analogs in the (*R*)-(−) series,[10] specifically nitro derivative (*R*)-(−)-**6**, amine (*R*)-(−)-**7**,[11] acetamide (*R*)-(-)-**8**,[12] and FITC-AA derivative (*R*)-(−)-**9**[13] were synthesized from (*R*)-(−)-phenylglycine in the same fashion.

Scheme



a: 10% Pd/C, 30 psi H_2, HOAc. b: AcCl, Et_3N, CH_2Cl_2. c: fluorescein isothiocyanate, THF

BIOLOGICAL RESULTS

The K_i values for the inhibition of μ, δ, and κ opioid binding to guinea pig brain membranes by the amines, the acetamides, and the FITC-AA derivatives are listed in Table 1. For all analogs, the (*S*)-(+) isomers proved to be two orders of magnitude more potent than the corresponding (*R*)-(−) isomers for binding to the μ, δ, and κ sites. Derivatives **3** and **4** had very high affinity for the κ site. As expected, the racemic FITC-AA **1** had an activity which reflected those of the (*R*)-(−) and the (*S*)-(+) series. It should be noted further that in all cases the K_i values for the acetamides (*S*)-(+)-**4** and (*R*)-(−)-**8** are very similar to those of the corresponding amines (*S*)-(+)-**3** and (*R*)-(−)-**7**. This effect strongly implies that substitution on the amino group does not necessarily affect the activity of the ligand. However, (*S*)-(+)-FITC-AA **5** showed low site selectivity, perhaps resulting from the presence of the large fluorescein portion of the ligand.

Table 1. K_i Values for the Inhibition of μ, δ, and κ Opioid Binding to Guinea Pig Brain Membranes.

	K_i (nM ± S.E.)		
Ligand	μ site	δ site	κ site
(*S*)-(+) series			
3 (NH_2)	7.65 ± 1.2	7.78 ± 0.11	0.0592 ± 0.0019
4 (NHAc)	8.37 ± 0.24	13.9 ± 1.3	0.0855 ± 0.005
5 FITC-AA	98.0 ± 11.9	0.898 ± 0.14	1.40 ± 0.128
(*R*)-(-) series			
7 (NH_2)	1380 ± 61	1850 ± 120	14.9 ± 2.8
8 (NHAc)	1040 ± 24	3070 ± 660	17.5 ± 1.9
9 FITC-AA	1190 ± 6.9	85.7 ± 3.7	139 ± 22
Racemic series			
1 FITC-AA	176 ± 30	1.84 ± 0.14	8.2 ± 0.7

Guinea pig brain membranes were incubated with varying concentrations of the compounds in the presence of radiolabelled ligands that were selective for the μ, δ, or κ receptors. Specifically, 0.25 nM [^{3}H][D-Ala^2,N(Me)Gly-ol^5]enkephalin (DAMGO), 0.2 nM [^{3}H]naltrindole, or 1 nM [^{3}H]U69,593 were used to measure the affinity of the compounds for the μ, δ, or κ sites, respectively. After equilibrium was reached, membranes were filtered onto glass filter fibers. Data are expressed as the mean K_i value ± S.E. for three determinations performed in triplicate. K_i values were calculated as described by Cheng and Prusoff.[14]

Racemic FITC-AA **1** has been used to label κ opioid receptors expressed on a mouse thymoma and on mouse thymocytes.[3] While having high affinity for δ sites, the binding of the racemic FITC-AA **1** to mouse thymocytes was not displaced by δ-selective opioids.[15] As shown in Table 2, specific binding of (*S*)-(+)-FITC-AA **5** was obtained when R1EGO thymoma cells were incubated with (*S*)-(+)-FITC-AA **5** followed by an amplification procedure, using phycoerythrin as the fluorophore.[3] In contrast, no specific fluorescent labelling of the κ opioid receptor was obtained with the enantiomer, compound (*R*)-(−)-**9**. The binding and flow cytometry experiments demonstrate that (*S*)-(+)-FITC-AA **5** is the enantiomer that binds to the κ opioid receptor. These results are consistent with the results obtained with the amine and acetamide derivatives in the competition binding studies outlined in Table 1.

Table 2. Fluorescent Labelling of R1EGO Thymoma Cells with (*S*)-(+)-FITC-AA **5** and (*R*)-(−)-FITC-AA **9**.

Ligand	Phycoerythrin Fluorescence (arbitrary units ± S.E.) Total Binding	Nonspecific Binding	Specific Binding	% Specific Binding
(*S*)-(+)-FITC-AA **5**	64 ± 12	7 ± 4	57 ± 10	91 ± 7
(*R*)-(-)-FITC-AA **9**	12 ± 4	-5 ± 9	---	---

R1EGO cells were incubated with 30 μM of the ligand in the absence or presence of 500 μM norbinaltorphimine, a κ-selective antagonist. The binding of the compound to the κ receptor was amplified as previously described.[3] Data are the mean median fluorescence values in arbitrary units after the subtraction of background fluorescence (amplification procedure without the addition of the FITC-AA ligand). Data are from seven experiments for (*S*)-(+)-FITC-AA **5** and three experiments for (*R*)-(−)-FITC-AA **9**, performed in triplicate.

In conclusion, we have synthesized a family of κ-selective opioid ligands. Also, it was shown using competition binding experiments and fluorescent labelling experiments that the active form of the arylacetamides based on structure **1** is the (*S*)-(+) form and that the ligands are selective for the κ site. The importance of κ-selective ligands is apparent in that physical dependence is not mediated by κ receptors.[16] Thus, κ-selective ligands represent potentially attractive therapeutic targets and may be used as probes to study the mechanisms of action of κ receptors.

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References and Notes

1. Current address: Albany Molecular Research, Albany, NY, 12203.
2. Dr. Sydney Archer passed away in August, 1996.
3. Lawrence, D. M. P.; El-Hamouly, W.; Archer, S.; Leary, J. F.; Bidlack, J. M. *Proc. Natl. Acad. Sci., U.S.A.* **1995**, *92*, 1062.
4. Chang, A. -C.; Takemori, A. E.; Ojala, W. H., Gleason, W. B.; Portoghese, P. S. *J. Med. Chem.* **1994**, *37*, 4490.
5. 2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-aminophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide, compound (*S*)-(+)-**3**, is also known (see ref 4). However, the known procedure differs significantly from our experimental procedure, outlined here: 2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-nitrophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide, compound (*S*)-(+)-**2** (0.878 g, 2.01 mmol), was dissolved in glacial

acetic acid (10 mL) in a Parr Hydrogenation bottle, and 10% Pd/C (40 mg) was added. The reaction mixture was hydrogenated at 30 psi for 1 h. The catalyst was filtered and the reaction solution made basic by careful addition of saturated aqueous Na_2CO_3 and solid NaOH. The resulting cloudy solution was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phases were washed with saturated aqueous NaCl (50 mL), dried over anhydrous Na_2SO_4, filtered, and the solvent removed in vacuo. The crude material was purified on a column of silica gel packed with $CH_2Cl_2/CH_3OH/NH_4OH$ 98:1:1. Evaporation of the appropriate fractions afforded compound (*S*)-(+)-**3** (650 mg, 1.60 mmol, 80%) which was crystallized using ethereal (*S*)-(+)-HCl as **3·HCl** salt. mp: softens at 195 °C, melts at 249 °C, lit[4]: softens at 149 °C and melts at 223–224 °C; $[\alpha]^{32}_D$ +132° (*c* 0.84, CH_3OH), lit[4]: $[\alpha]^{25}_D$ +143.7° (*c* 0.45, CH_3OH); IR (KBr) 3320, 2820, 1630 cm^{-1}; ^1H NMR data matched literature values.

6. Experimental procedure for the preparation of 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-acetamidophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide ((*S*)-(+)-**4**): To a solution of compound (*S*)-(+)-**3** (109 mg, 0.268 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C was added distilled triethylamine (0.120 mL, 1.34 mmol). After 15 minutes, acetyl chloride (0.061 mL, 1.34 mmol) was added rapidly. The reaction mixture was stirred and allowed to warm to room temperature over 7 h. The solvent was removed in vacuo and the residue was dissolved in EtOAc/3 M aqueous NaOH (30 mL each). The organic layer was washed with saturated aqueous NaCl, dried over anhydrous $MgSO_4$, filtered, and the solvent was removed in vacuo to provide a quantitative yield of the free amine which was pure by TLC and ^1H NMR. The material was crystallized using ethereal HCl as (*S*)-(+)-**4·HCl** (32 mg, 0.066 mmol, 25%). ^1H NMR ((*S*)-(+)-**4·HCl**, DMSO-d_6) δ 10.03 (s, 1H, NH), 7.57–7.56 (m, 3H, Ar), 7.47 (brs, 1H, Ar), 7.33–7.28 (m, 2H, Ar), 6.94 (d, *J* = 7.5 Hz, 1H, *m*-NAc Ar), 6.10 (d, *J* = 10.5 Hz, 1H, CH), 4.11–3.13 (complex, 8H, 3xNCH_2 & $ArCH_2CO$), 2.77 (s, 3H, NCH_3), 2.05 (s, 3H, CH_3CO), 2.04–1.95 (m, 4H, CH_2CH_2; mp: softens at 216, melts at 221–223 °C; $[\alpha]^{24}_D$ +108° (*c* 0.34, CH_3OH).

7. The racemic form of acetamide **4** has been synthesized. See Barlow, J. J.; Blackburn, T. P.; Costello, G. F.; James, R.; Le Count, D. J.; Main B. G.; Pearce, R. J.; Russell, K.; Shaw, J. S. *J. Med. Chem.* **1991**, *34*, 3149.

8. Experimental procedure for the preparation of 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-{(1*S*)-1-[*N*-(fluoresceinyl-5-thioureido)-3-aminophenyl]-2-(1-pyrrolidinyl)ethyl}acetamide ((*S*)-(+)-**5**): Fluorescein isothiocyanate (201 mg, 0.514 mmol) was added rapidly to amine (*S*)-(+)-**3** (209 mg, 0.514 mmol) dissolved in dry, distilled THF (60 mL). The reaction mixture was stirred for 24 h at room temperature, then concentrated to 1/4 of the original volume, and cooled to 0 °C. Two crops of crystals provided compound (*S*)-(+)-**5** (300 mg, 0.377 mmol, 73%). ^1H NMR (DMSO-d_6) δ 10.22–10.14 (m, 3H, 2xNH & COOH or OH), 8.16–6.58 (complex, 16H, Ar), 5.85 (m, 1H, CH), 3.88–3.85 (m, 1H, CH_2Ar), 3.74-3.71 (m, 1H, CH_2Ar), 3.61–2.68 (complex, 6H, 3xNCH_2), 2.75 (s, 3H, NCH_3), 1.66 (brs, 4H, CH_2CH_2); mp

180°C (dec); $[\alpha]^{27.5}_{D}$ +69.2° (*c* 5.0, CH_3OH + 1 drop triethylamine); Anal. Calcd for $C_{42}H_{36}N_4O_6SCl_2H_2O$: C, 61.99; H, 4.71; N, 6.88; S, 3.94; Cl, 8.72. Found: C, 61.58; H, 4.69; N, 6.65; S, 3.66; Cl, 8.49.

9. Compound (*S*)-(+)-**5** has been synthesized using a different procedure, but was reported as containing triethylamine in a 1:1 molar ratio with the FITC derivative. See Chang, A. -C.; Chao, C. C.; Takemori, A. E.; Gekker, G.; Hu, S.; Peterson, P. K.; Portoghese, P. S. *J. Med. Chem.* **1996**, *39*, 1729.
10. It should be noted that, to our knowledge, none of the R-(-) analogs have been previously prepared.
11. The physical data for 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*R*)-1-(3-aminophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide, compound (*R*)-(−)-**7**, were identical to those of (*S*)-(+)-**3** with the following exceptions: Reaction yield 80%; $[\alpha]^{30}_{D}$ -142° (*c* 1.70, CH_3OH).
12. The physical data for 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*R*)-1-(3-acetamidophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide ((*R*)-(−)-**8**) were identical to those of the (*S*)-(+)-**4** with the following exception: $[\alpha]^{28}_{D}$ -108° (*c* 1.10, CH_3OH + 1 drop triethylamine).
13. The physical data for 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-{(1*R*)-1-[*N*-(fluoresceinyl-5-thioureido)-3-aminophenyl]-2-(1-pyrrolidinyl)ethyl}acetamide ((*R*)-(-)-**9**) were identical to those of the (*S*)(+) derivative (*R*)-(-)-**5** with the following exceptions: reaction yield 80%; $[\alpha]^{32}_{D}$ -69.4° (*c* 3.0, CH_3OH + 1 drop triethylamine).
14. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.*, **1973**, 22, 3099.
15. Ignatowski, T. A.; Bidlack, J. M. *J. Pharmacol. Exp. Ther.*, in press.
16. Julien, R. M. *A Primer of Drug Action, Sixth Edition*; W. H. Freeman and Company: New York, 1992; p 192.

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