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STRUCTURE-ACTIVITY-RELATIONSHIP PERTAINING TO THE NORTH-WEST REGION OF THE KAPPA OPIOID AGONIST, U-50,488

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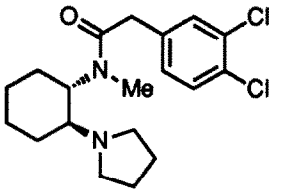
Abstract

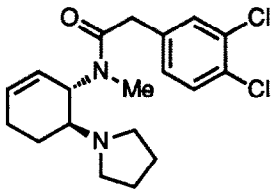
Compound **2**, an unsaturated analog of U-50,488, has been prepared and found to be an active analgesic. Modeling studies with U-50,488, compound **2** and four other unsaturated U-50,488 analogs (namely, **3**, **4**, **5**, **6**) have been performed and enable us to define the size and shape of the hydrophobic pocket of the kappa receptor with which the north-west part of the ligands interacts.

The discovery[1] of the selective kappa opiate agonist U-50,488 (**1**) has been followed by the preparation of many additional agonists based on this 1,2-aminophenylacetamide template[2].

We now report a synthesis of a new olefinic analog **2** which contains a double bond in the north-west part of the molecule of U-50,488. Compound **2** joins a group of biologically active analogs which contain unsaturation, with or without bulk, in the north-west part of the lead compound. This group includes known compounds **3**[3], **4**[4a,b], **5**[4a,b] and **6**[5].



U-50,488 (**1**)



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The new analog **2** was prepared from trans-3-methylamino-4-pyrrolidinylcyclohexene (**10**). Compound **10** was prepared by a regiospecific and stereospecific synthesis. First, 3,4-epoxycyclohexene (**8**)[6] was prepared by epoxidation of 1,3-cyclohexadiene (**7**) with peracetic acid. The subsequent reaction of epoxide **8** with pyrrolidine gave the hydroxy pyrrolidine derivative **9**. The regiospecific formation of **9** arose from the attack of pyrrolidine at the allylic carbon as observed before in the case of epoxide **8** and dimethylamine[7a]. Compound **9** was allowed to react with methanesulfonyl chloride to give a mesylate, which was converted to diamine **10** on reaction with methylamine. As expected,[7b] the pyrrolidine group underwent a 1, 2 transposition in going from compound **9** to **10**. The diamine compound **10** was allowed to react with 3,4-dichlorobenzoyl chloride in the presence of triethylamine to give the desired amide **2** (Scheme 1). In vivo testing of compound **2** showed that it is an active analgesic in mice.[8] Absence of Straub tail was consistent with the conclusion that the compound was acting through a kappa receptor.

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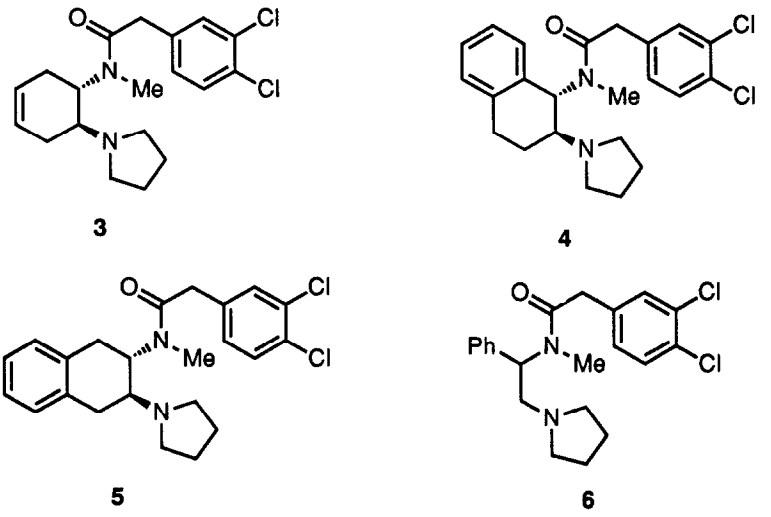
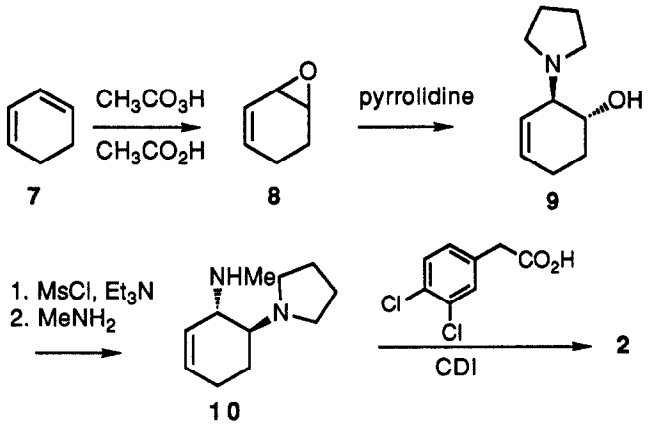


Scheme 1



The kappa opioid receptor was cloned in 1993[9], but crystals are not yet available for an X-ray structure determination and receptor-ligand co-crystallization study. Therefore, indirect determination of receptor-ligand interactions by molecular modeling studies are of interest.

As described in the Experimental Section, we have constructed molecular models of compounds **1-6** and have performed a least-squares rigid overlay of the atoms common to all six to examine the relative placement of the compounds' north-west regions. The results are shown in Fig. 1. It is evident that the atoms not common to the six compounds form three ring clusters, consisting of (a) the cyclohexyl and cyclohexenyl rings of compounds **1**, **2**, **3**, **4**, and **5**, (b) the phenyl rings of compounds **4** and **6**, and (c) the phenyl ring of compound **5**. The union of these three clusters defines a complementary hydrophobic binding pocket in the kappa receptor, of dimensions 4.5-5 Å top to bottom (in the orientation of Fig. 1), 5 Å side to side, and 2-3 Å back to front.

These dimensions are atom-centered distances in the ligands and do not include an additional two van der Waals radii in each dimension.

During the energy minimization and overlay steps, no conformational searching was performed to take into account the flexibility of the acyclic central chain. Therefore, the construct shown in Fig. 1 is not intended to model the full binding conformation of the molecules. The dichlorophenyls, for example, may bind in a completely different location. Yet if we make the reasonable assumption that the atoms in common to all six molecules bind to the receptor in the same way from case to case, then the north-western regions must line up substantially as shown in Fig. 1, regardless of where the dichlorophenyl binds relative to the hydrophobic binding pocket.

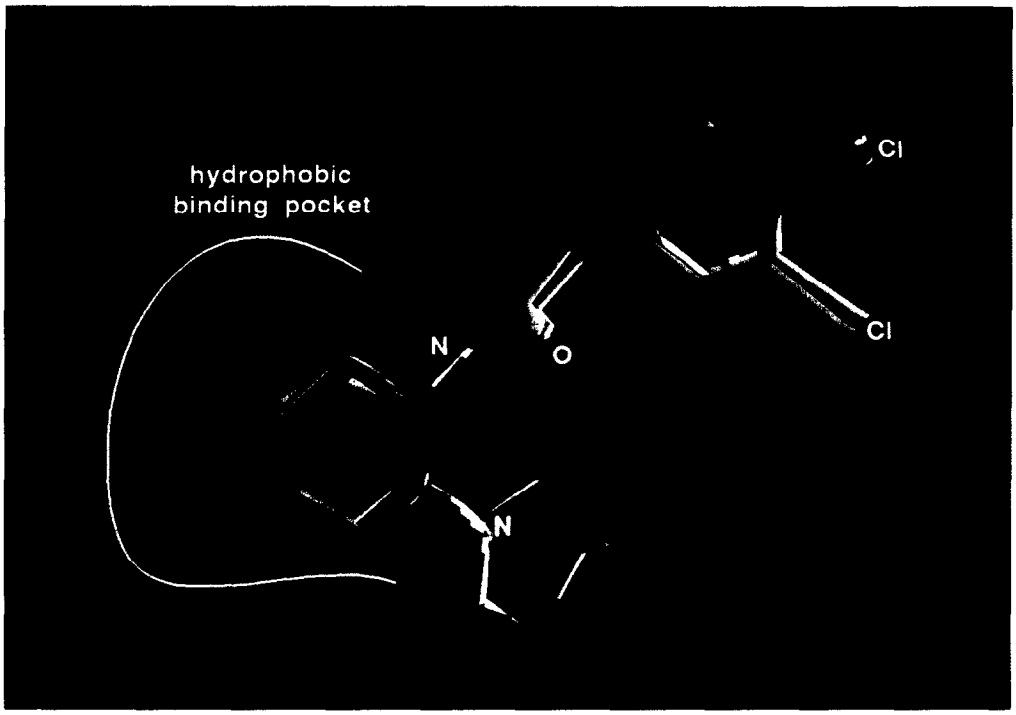


Figure 1
Rigid overlay of computer-based models of compound **1** (U-50,488)-pink; **2**-yellow; **3**-white; **4**-red; **5**-green; **6**-blue.

In summary, compound **2**, a new analog of U-50,488, was synthesized and its analgesic activity determined. This allows an extension of the structure-activity relationship in the north-west part of the template. Structural comparisions of six compounds in this series have also defined a complementary hydrophobic binding pocket in the receptor, to accommodate the north-western and western regions of the ligands.

Experimental Section

^{1}H NMR spectra were obtained at 300 MHz and ^{13}C NMR spectra were obtained at 75 MHz with $CDCl_3$ as a solvent unless otherwise stated.

***Trans*-2-pyrrolidinyl-3-hydroxy-cyclohexene (9):** A solution of peracetic acid in acetic acid (32% by weight, 24 mmol, 5. mL) was added dropwise to a mixture of 1,3-cyclohexadiene (2.4 mL, 25 mmol) and anhydrous Na_2CO_3 (10 g) in CH_2Cl_2 (25 mL) at 0 °C. The mixture was stirred at r.t. for 3 h, filtered and the solid was rinsed with CH_2Cl_2. The filtrate was concentrated in vacuo. The residue (2.4 g) was added to a flask containing pyrrolidine (2 mL) and the mixture was heated at 70 °C for 20 h. The product was diluted with ether (200 mL), washed with sat. Na_2CO_3 (20 mL) and brine (2 x 20 mL), dried over Na_2SO_4 and concentrated in vacuo to give a red oil (2.83 g, 71%): ^{1}H NMR δ 5.80(m, 1 H), 5.63 (dq, J = 10.2, 1.9 Hz, 1 H), 3.63 (ddd, J = 11.5, 8.8, 3.4 Hz, H-4), 3.23 (m, H-3), 2.57-2.77 (m, 4 H), 2.02-2.19 (m, 3 H), 1.75 (m, 4 H), 1.51 (m, 1 H); ^{13}C NMR δ 130.16, 123,37, 68.60, 63.86, 47.98, 28.93, 24.85, 23.90; MS (EI), m/e (rel intensity) 167 (4, M), 139 (3), 123 (100), 108 (56), 95 (30); HRMS (EI) *m/e* calcd for $C_{10}H_{17}NO$ 167.1310, found 167.1319.

***Trans*-3-methylamino-4-pyrrolidinylcyclohexene (10):** Triethylamine (1.69 mL, 12.1 mmol) and methanesulfonyl chloride (0.93 mL, 12.1 mmol) was added to a solution of compound **9** (1.83 g, 11.0 mmol) in CH_2Cl_2 (30 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at 25 °C for 1.5 h. The solvent was removed in vacuo and the residue was transferred to a bomb. Methylamine (5 mL, 33% in absolute ethanol) was added, and the bomb was sealed and heated to 70 °C for 20 h. The reaction mixture was cooled to r.t. Water (20 mL) was added and the solution was basified by the addition of solid KOH. It was extracted with CH_2Cl_2 (30 mL x 4). The combined extract was dried over sodium sulfate and concentrated in vacuo to give **10** as a red oil (1.43 g, 72%). Part of the product (0.68 g) was distilled to give a pale yellow oil (0.65 g, 54-56 °C/0.2 torr): ^{1}H NMR δ 5.74 (m, 2 H), 3.08 (m, 1 H), 2.52 (m, 5 H), 2.44 (s, CH_3), 1.50-2.22 (m, 9 H); ^{13}C NMR δ 128.09, 127.64, 60.33, 58.18, 48.86, 33.80, 24.61, 23.57, 19.82; MS (FAB), m/e (rel intensity) 181 (100, M + H), 150 (61), 110 (41), 98 (63), 83 (52), 70 (73); HRMS (FAB) *m/e* calcd for ($C_{11}H_{20}N_2$ + H) 181.1705, found 181.1713.

***Trans*-3,4-Dichloro-N-methyl-N-[6-(1-pyrrolidinyl)-4-cyclohexenyl]benzeneacetamide (2):** A solution of 3,4-dichlorophenylacetic acid (326 mg, 1.59 mmol) and carbonyldiimidazole (258 mg, 1.59 mmol) in CH_2Cl_2 (8 mL) was stirred at r.t. for 2 h. A solution of compound **10** (260 mg, 1.44 mmol) in CH_2Cl_2 (5 mL) was added. The reaction mixture was stirred at r.t. for 18 h. A solution of saturated Na_2SO_4 (10 mL) was added and the mixture was extracted with CH_2Cl_2 (2 x 20 mL). The combined extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed on silica gel eluting with EtOAc/MeOH (10/1) to give **2** as a yellow oil (380 mg, 72%). The hydrochloride of compound **2** was prepared with ethereal HCl and recrystalized from 2-propanol/ether: mp 196-197 °C; ^{1}H NMR (D_2O) δ 7.51 (d, J = 8.3 Hz, 1 H), 7.41 (d, J = 1.6 Hz, 1 H), 7.15 (dd, J = 8.33, 1.5 Hz, 1 H), 6.10 (m, 1 H), 5.36 (m, 2 H), 3.85 (m, 2 H), 3.65 (td, J = 8.7, 2.7 Hz, 1 H), 3.48 (m, 2 H), 3.25 (m, 2 H), 2.98 (s, CH_3), 1.80-2.30 (m, 9 H); MS (FAB), m/e (rel intensity) 367 (100, M + H), 296 (28), 159 (8), 150 (12), 97 (17), 70 (30); HRMS (FAB) *m/e* calcd for

($C_{19}H_{24}Cl_2N_2O$ + H) 367.1344, found 367.1355; Anal. Calcd for $C_{19}H_{24}Cl_2N_2O$·HCl: C, 56.52; H, 6.24; Cl, 26.34; N, 6.94. Found: C, 56.30; H, 6.31; Cl, 25.97; N, 6.78.

Molecular Modelling: Models of compounds **1-6** were constructed and studied with the Mosaic modelling system[10], in the following way. An approximate 3D model of compound **3** was retrieved from the Upjohn Company's proprietary database, having previously been generated from 2D form by the Concord program[11]. This approximate model was then converted to the other five compounds using Mosaic's model building facilities. Each was subjected to interactive energy minimization using the MM2* forcefield[12] and PRCG minimizer, as implemented in BatchMin 3.5[13]. Structures were considered fully converged at an rms gradient of less than 0.01 kcal/mol-Å.

The resulting models of the six compounds were overlaid by an iterative least-squares fitting (rigid body) which minimized the deviations of a user-selected set of corresponding atoms in each molecule. The atoms selected for the fitting were the atoms in common to all six molecules, including the dichlorophenyl ring, the methylamide chain, the two carbon atoms between that chain and the pyrrolidine, and the pyrrolidine ring itself. This provided the overlay shown in Fig. 1 and discussed in the text.

Acknowledgment. We thank the Upjohn Company for partial support of this research.

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