Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 9, pp. 1153-1156, 1994
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0960-894X/94 $6.00+0.00

0960-894X(94)E0118-X

N-Alkyl Quinuclidinium Substance P Antagonists

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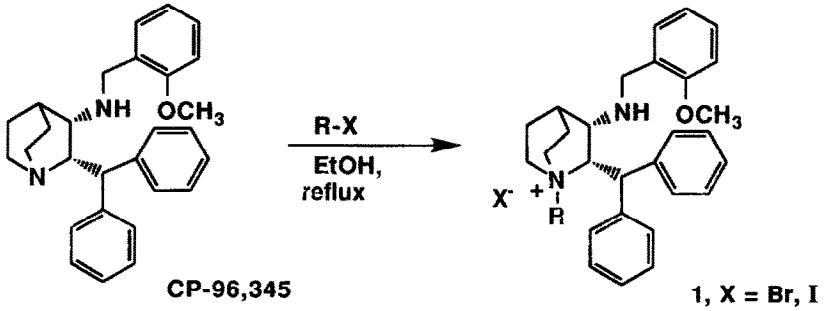
Abstract: The synthesis and SAR of bridgehead N-alkylated analogues of the nonpeptide substance P antagonist CP-96,345 are described. The results indicate that the bridgehead nitrogen may provide more of an anchoring function rather than being in intimate contact with the receptor.

Our recent studies on the synthesis[1] and SAR[2] of CP-96,345, the first nonpeptide substance P (SP) antagonist delineated the bridgehead nitrogen as an important structural feature for NK_1 receptor affinity. Since pK_a measurements indicate this strongly basic nitrogen is likely to be fully protonated under physiological conditions[2], we decided to investigate alkylation of this nitrogen to define its interactions with the receptor. The N-alkyl quinuclidinium salt **1** could also provide an NK_1 antagonist unable to penetrate the blood-brain barrier useful as a research tool to assess the contribution of NK_1 receptors in the central nervous system to biological activity.



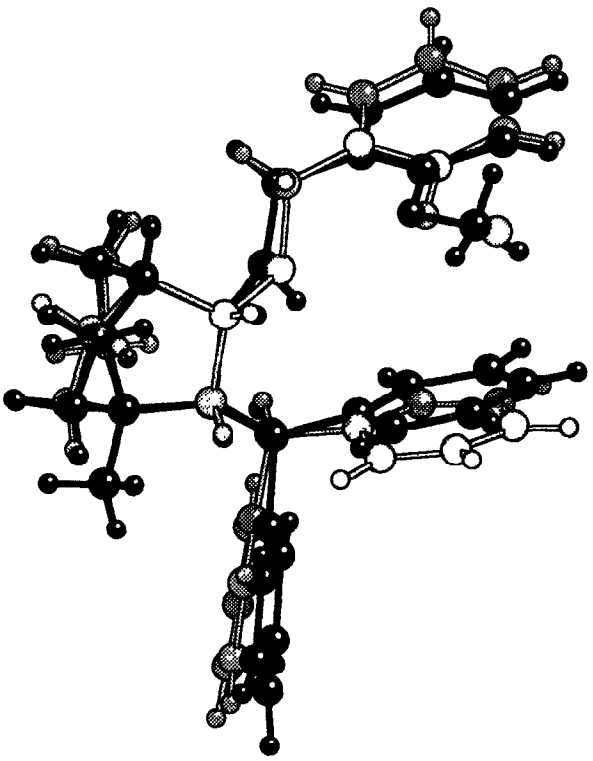
CP-96,345 **1, X = Br, I**

CPD	R	NK_1 IC_{50}, nM*
CP-96,345	-	0.52 ± 0.06
1a	CH_3	12.2 ± 2.9
1b	CH_2CONH_2	1.3 ± 0.15
1c	CH_2CO_2Et	32.7 ± 10.2
1d	$CH_2CH{=}CH_2$	22.3 ± 3.5
1e	CH_2Ph	332 ± 137

* *IC_{50} for displacement of ^{125}I-Bolton-Hunter Substance P in human IM-9 cells, ± s.e.m.*[3]

Even though the bridgehead nitrogen in CP-96,345 is hindered by the adjacent benzhydryl group, it proved possible to effect the desired alkylation in refluxing ethanol over several days with periodic addition of more alkylating agent. Products **1a** and **1b** were purified as the hydroiodide salt after crystallization from isopropanol to remove unreacted starting material.[5] As shown above, N-methylation of CP-96,345 reduces *in vitro* NK_1 receptor affinity measured in human IM-9 cells 19-fold. Addition of a carboxamide group *via* alkylation with iodoacetamide to give **1b** restores affinity almost to the level of CP-96,345, while compounds **1c** to **1e**, without the polarity of the carboxamide, show further decreases in affinity.

These results indicate that the bridgehead nitrogen atom in CP-96,345 does not form an intimate ion-pair interaction at the receptor binding site, since the rigidity of the quinuclidine nucleus requires the bridgehead nitrogen lone pair to accept a hydrogen-bonding interaction in the space occupied by the N-alkyl substituent. The potent affinity of acetamido derivative **1b** indicates that these receptor interactions are nevertheless specific. The reduced affinity of **1a** is not a result of distortion of the quinuclidine framework and a concommitant change in the orientation of the benzylamino and benzhydryl groups, as shown by the following comparison of the X-ray structures of CP-96,345 (gray) and **1a** (black)[6]:



Overlap of X-ray structures of CP-96,345 (gray) and compound **1a** (black), depicted using Nemesis v2.1, © Oxford Molecular, Ltd.

Thus, although crucial for NK_1 receptor recognition[2], the bridgehead nitrogen does not seem to be in intimate contact with the receptor, but instead may provide an "anchor point" by virtue of the positive charge in its protonated form serving to orient the molecule for proper receptor interaction. This anchoring function may involve a diffuse ion-pair interaction with the negatively charged polar head groups of the phospholipid components of the membrane in which the receptor resides, since the binding site for CP-96,345 was recently shown to be located near the tops of transmembrane domains 5 and 6 where they emerge from the lipid environment of the membrane.[9] A basic nitrogen or ammonium group is a feature of many of the recently disclosed structurally novel NK_1 receptor antagonists[10], and hence this anchoring function may be a general strategy used by these compounds for proper orientation at their receptor binding site.

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3. The protocol for ^{125}I-BH-SP binding to human IM-9 cells which was used for SAR evaluation has been described previously[4]. Cells were harvested by centrifugation and washed twice in 50 mM TRIS HCL with 1 mM $MnCl_2$ pH 7.4 (wash buffer). The cells were resuspended in assay buffer (50 mM TRIS HCL, 1mM $MnCl_2$, 1 mM $MgCl_2$, 50 μg/ml chymostatin, 40 μg/ml leupeptin, 20 μg/ml phosporamidon, 10 μg/ml bacitracin and 0.02% BSA) at a final concentration of $6X10^6$ cells/ml. The assay was conducted in assay buffer in 96 well polypropylene plates with 25 μl of cells, 200 μl of buffer (containing 0.1 nM ^{125}I-BH-SP, final concentration) and 25 μl of blank, test compound or vehicle. Following incubation for 30 minutes at room temperature, the assay was terminated by filtration using a Skatron cell harvester onto Scheiler and Scheull # 30 filters which had been presoaked in 0.3% polyethylimine. The filters were washed with wash buffer, dried in a microwave and counted on a beta plate counter using liquid scintillation counting. Standard errors are indicated following the IC_{50} values for triplicate determinations using 8 concentrations.

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5. Analytical data, **1a**: mp 239-244°C. ^{13}C-NMR (δ, $CDCl_3$): 20.0, 22.4, 23.8, 46.1, 49.4, 53.9, 54.6, 55.3, 55.6, 61.2, 71.5, 110.2, 120.4, 126.9, 127.2, 127.9, 128.5, 129.6, 141.7, 143.5, 157.1. EIMS M

(%): 426 (parent, 1), 259 (31), 245 (46), 142 (45), 121 (100), 91 (62). Anal. Calc'd. for $C_{29}H_{35}N_2OI$: C 62.82, H 6.36, N 5.05. Found: C 62.74, H 6.14, N 5.02. α_D = +36.9 (c=1, CH_2Cl_2). **1b**: mp 150-158°C. IR ($cm.^{-1}$, KBr): 1661 (C=O). FAB MS (%): 470 (parent, 6), 413 (100), 167 (15), 121 (39), 91 (25). Anal. Calc'd. for $C_{30}H_{36}N_3O_2I \cdot HI \cdot 0.5H_2O$: C 49.06, H 5.21, N 5.72. Found: C 48.95, H 5.14, N 5.94. α_D = -9.9 (c=1, MeOH).

6. The X-ray crystal structure of **1a** was determined as follows: A representative crystal was surveyed and a 1 Å data set (maximum sin θ/λ=0.5) was collected on a Siemens R3RA/v diffractometer. Atomic scattering factors were taken from the International Tables for X-ray Crystallography[7]. All crystallographic calculations were facilitated by the SHELXTL[8] system. All diffractometer data were collected at room temperature. A trial structure was obtained by direct methods. This trial structure refined routinely. Hydrogen positions were calculated wherever possible. The hydrogens on nitrogen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least squares refinement were all less than 0.1 of their corresponding standard deviations. The final R-index was 0.072. A final difference Fourier revealed no missing or misplaced electron density. The refined structure was plotted using the SHELXTL plotting package. Coordinates, anisotropic temperature factors, distances and angles are available.

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(*Received in USA* 23 *March* 1994; *accepted* 31 *March* 1994)