Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 12, pp. 1271-1274, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00207-3

Photoactivatable Analogues of a Substance P Non-Peptidic Antagonist, for Probing the Antagonist Binding Site of the NK_1 receptor

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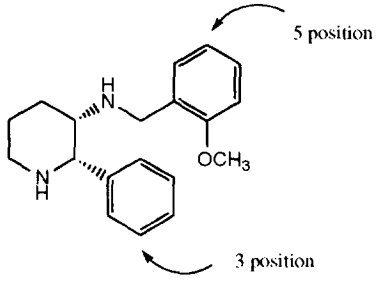
Abstract: (+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994) binds with high affinity (pK_i 9.6) to the human NK_1 receptor. Racemic analogues of CP-99,994 containing either benzoyl or diazirinyl moieties also bind with high affinity (pK_i 9.4 and 9.1 respectively). These analogues are of tremendous potential for mapping the antagonist binding site of the NK_1 receptor.

Substance P is a neuropeptide that has been shown to have an important biological role in a variety of human diseases.[1] Within the last few years several potent, competitive antagonists of Substance P have been synthesised.[2] One of these (+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994), has been shown to bind with high affinity to the human NK_1 receptor,[3] although the antagonist binding site has only partially been identified using other similar NK_1 antagonists.[4]

Photoaffinity labelling is an extremely powerful tool for probing the binding sites of many biological systems.[5] The major use of this technique has been by the use of peptides containing amino acids functionalised with photoactivatable groups, such as benzoyl, diazirinyl or azido. In order to probe the antagonist binding site of the human NK-1 receptor, we have prepared novel photoactivatable analogues of (±)-CP-99,994.

Structure-activity studies on CP-99,994 carried out in Glaxo (unpublished results) revealed that analogues containing substituents at positions shown in Figure 1, did not in general show a marked reduction in affinity for the NK_1 receptor. These sites were obvious starting points for the introduction of photoactivatable groups.



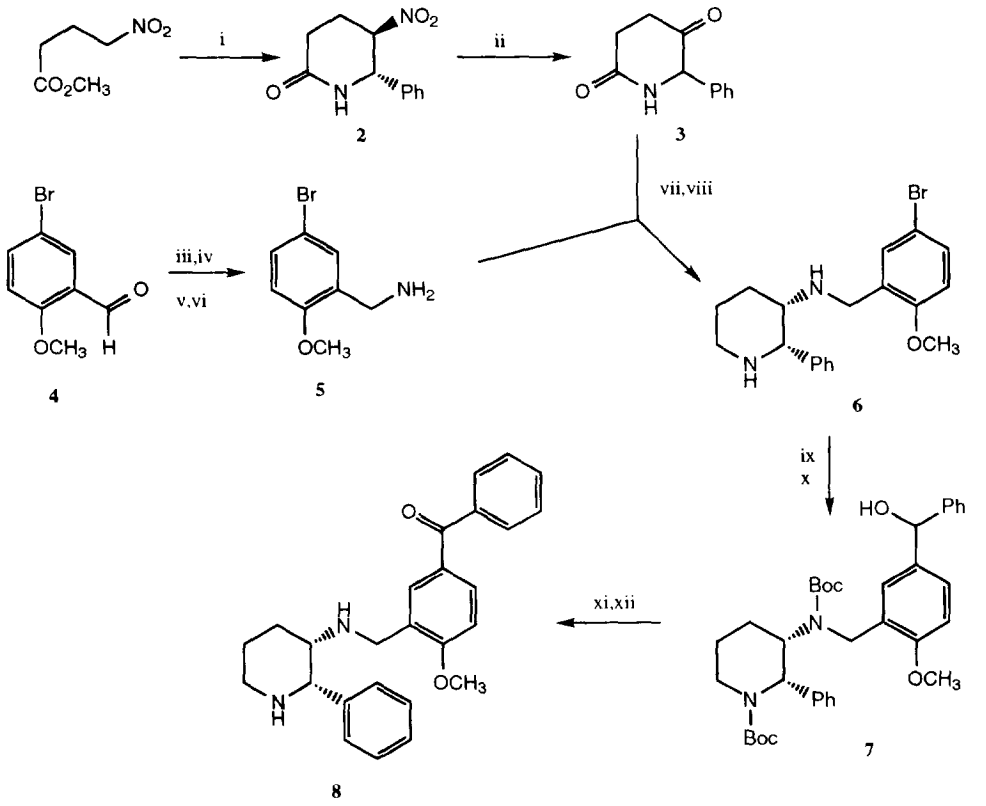
(+)-1

Figure 1

Our initial strategy for synthesising these photoactivatable analogues was mainly based on the work of Desai *et al.*[2d] This method allowed us facile entry into aryl halide analogues of CP-99,994, which were then derivatised with photoactivatable substituents using organolithium reagents. Our latter strategy for incorporating a photoactivatable moiety was designed so that a radiolabel such as tritium could be incorporated at a late stage in the synthesis, *via* reductive amination.

Synthesis of benzoyl analogue of (±)-CP-99,994:

Initially a Knoevenagel reaction of methyl-4-nitrobutyrate with benzaldehyde and ammonium acetate, followed by an intramolecular cyclisation gave the *trans* piperidone **2**.[2d,6] The nitro group was then converted to a carbonyl group using ozone and potassium t-butoxide followed by quenching with dimethylsulphide.[2d,7] The benzylamine **5** was prepared in high yield from commercially available 5-bromo-o-anisaldehyde **4** in four facile steps. Reductive amination of the oxopiperidone **3** and the benzylamine **5** using sodium triacetoxyborohydride resulted in the piperidone having the substituents in the corect *cis* stereochemistry.[8] Reduction of the piperidone with borane dimethylsulphide.THF complex afforded the bromo-derivative of CP-99,994 **6**.[2d] The secondary amino functions were protected using butoxycarbonyl groups, prior to treatment of the piperidine with nBuLi at -78°C and benzaldehyde. Flash column chromatography gave the alcohol **7** in 61% yield. Attempts to prepare the benzoyl substituted compound directly using nBuLi with inverse addition[9] to benzoyl chloride resulted in a mixture of compounds. The alcohol was then oxidised using pyridinium chlorochromate[10] followed by boc deprotection, giving benzoyl-(±)-CP-99,994 **8**, which was subsequently converted into the dihydrochloride salt using ethereal HCl (Scheme 1).

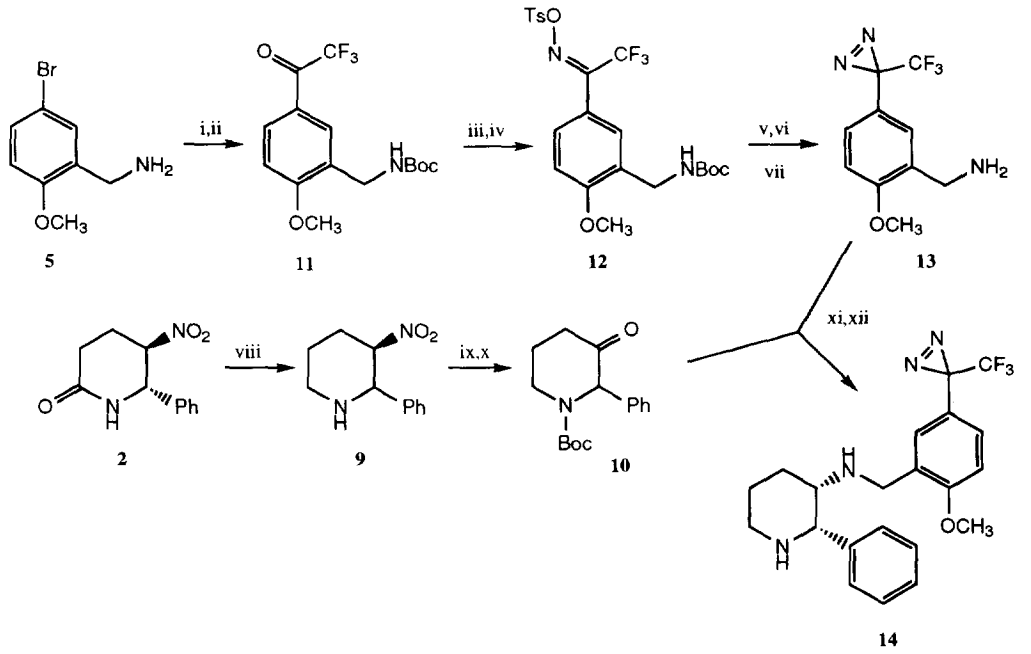


Scheme 1

Reagents: i) benzaldehyde, NH_4OAc, reflux (94%); ii) O_3, KO^tBu then Me_2S (72%); iii) $KMnO_4$ (76%); iv) $SOCl_2$, r.t. (100%); v) NH_3 soln (98%); vi) BH_3.THF (68%); vii) $NaBH(OAc)_3$ (48%); viii) $BH_3.Me_2S$ (88%); ix) Boc_2O, reflux (74%); x) nBuLi, THF at -78°C then benzaldehyde and allowed to warm to r.t. (61%); xi) PCC (84%); xii) TFA then ethereal HCl.

Synthesis of diazirinyl analogue of (±)-CP-99,994:

Initially, the carbonyl function of piperidone **2** was selectively reduced to the piperidine **9** using borane.THF complex.[11] The amino function was then protected using boc chemistry prior to conversion of the nitro group to a carbonyl group using the ozonolysis conditions described previously. The benzylamine **5** was boc protected and then treated with 2 equivalents of nBuLi at -78°C followed by addition of N,N-diethyltrifluoroacetamide.[12] After quenching at room temperature, flash column chromatography gave the trifluoromethyl ketone **11** in good yield (71%). The trifluoromethyl ketone **11** was then converted into the oxime using hydroxylamine hydrochloride and pyridine.[12] Treatment of the oxime with triethylamine and p-toluenesulphonyl chloride gave the tosylate **12** which upon reaction with liquid ammonia gave the diaziridine.[12] Oxidation using t-butyl hypochlorite[12] gave the diazirine which was then boc deprotected to give the benzylamine **13**. Reductive amination of ketone **10** and benzylamine **13** with sodium triacetoxyborohydride gave the desired boc protected piperidine.[8] Deprotection, followed by reaction with ethereal HCl gave diazirinyl-(±)-CP-99,994 **14** as the dihydrochloride salt (Scheme 2).



Scheme 2

Reagents: i) Boc_2O (95%); ii) nBuLi, THF at -78°C then $Et_2NCO.CF_3$ and allowed to warm to r.t. (71%); iii) $NH_2OH.HCl$, pyridine (94%); iv) NEt_3, DMAP, TsCl (93%); v) NH_3 (liq) (94%); vi) ^tBuOCl (72%); vii) TFA (92%); viii) $BH_3.THF$ (83%); ix) Boc_2O (76%); x) O_3, KO^tBu then Me_2S (73%); xi) $NaBH(OAc)_3$ (61%); xii) TFA then ethereal HCl.

Both these analogues of (±)-CP-99,994 show high affinity for the human NK_1 receptor (Table 1). Other analogues prepared with diazirinyl and acetyl moieties in position 3 of the C2 phenyl showed reduced affinity for the receptor (pK_i 7.9 and 6.6 respectively).

Table 1. *In vitro* binding affinity of (±)-CP-99,994 analogues for the NK_1 receptor in human CHO cells using [^{3}H]-Substance P.[3,13]

compound	pK_i (± 0.1)
(+)-1	9.6
(±)-8	9.4
(±)-14	9.1

These results clearly indicate that both these photoactivatable analogues of (±)-CP-99,994[14] have tremendous potential as radiolabelled compounds for mapping the antagonist binding site of the NK_1 receptor. In addition, the non-radiolabelled compounds could be useful in modelling and mutagenesis studies for identifying residues near the binding site.[2b,4]

Acknowledgements

We are extremely grateful to Tony Hawcock for the binding assays, to Martin Huscroft, Bernard Watson and Tanya Marinko-Covell for analytical and spectroscopic data and to the Biotechnology and Biological Sciences Research Council and Glaxo Research and Development for their financial support.

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13. pK_i = -Log K_i ; K_i is obtained from the IC_{50} value using the Cheng-Prusoff equation (K_i=IC_{50}/(1+[L]/K_D).
14. All new compounds gave satisfactory analytical and spectroscopic data.

(*Received in Belgium* 17 *March* 1995; *accepted* 27 *April* 1995)