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A Non-Peptidic Photoactivatable Antagonist for Mapping the Antagonist Binding Site of the Tachykinin NK_2 receptor

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Abstract: SR 48968, N-Methyl-N-[4-(4-acetamido-4-phenylpiperidinyl)-2S-(3,4-dichlorophenyl) butyl]benzamide is a potent and selective non-peptidic antagonist of the NK_2 receptor. A photoactivatable analogue containing a diazirine moiety also binds with high affinity. This compound is potentially useful for identifying residues at the antagonist binding site of the NK_2 receptor.

The neurokinins, comprising substance P, neurokinin A and neurokinin B, are a group of peptide neurotransmitters that have a direct pathogenic role in a number of human diseases.[1] Due to the enormous therapeutic potential, a great deal of research is underway to produce specific antagonists to block their actions.

The initial leads towards non-peptide antagonists have come principally from chemical file screening, however, improvements in compound affinity have been achieved through probing the ligand/receptor interaction using SAR analysis, mutagenesis and modelling (of receptors and ligands).[2] Unfortunately, site-directed mutagenesis can be misleading, since conformational changes can be introduced by changes distant from the ligand binding site, which nevertheless effect the binding affinity of the ligand. More precise information on the binding site can be achieved using photoaffinity labelling, which has been shown to enable identification of residues present at the binding sites of many biological systems.[3]

Only two potent non-peptidic antagonists of neurokinin A have been published in the literature, SR 48968 (**1**)[4] and GR159897 (**2**)[5] (Figure 1).

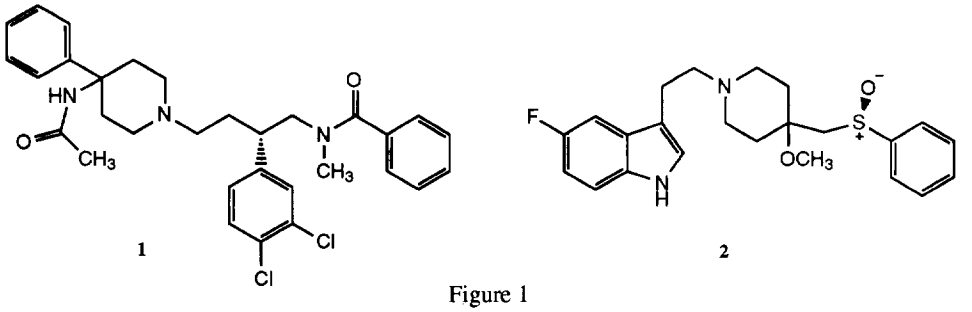
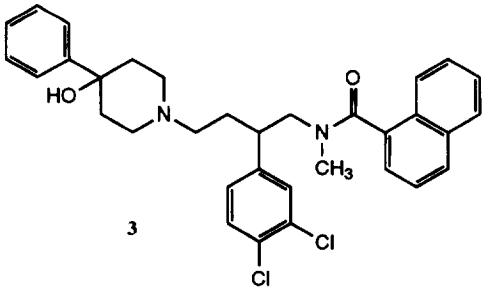


Figure 1

The Sanofi compound, SR 48968 has undergone extensive SAR analysis[4d] from which it was demonstrated that substitutions on the benzamide moiety can readily be tolerated, since replacement with naphthamide did not significantly reduce the binding affinity of the ligand. In addition, replacement of the acetamido moiety of the piperidine with a hydroxyl moiety also enabled retainment of affinity, as in compound **3**

(Figure 2).



3

Figure 2

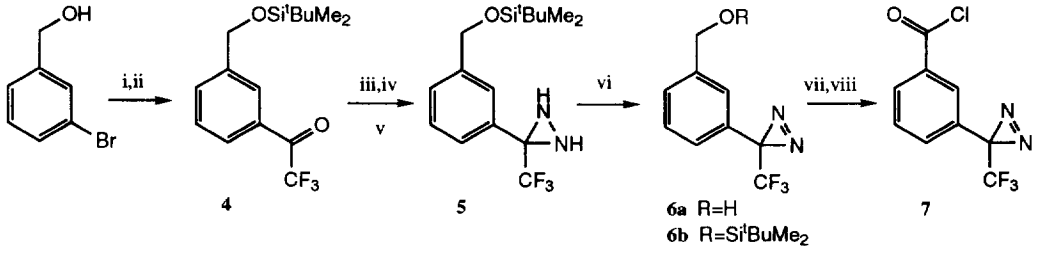
Therefore, the 3-position of the benzamide was an obvious site for incorporation of a photoactivatable moiety.

3-Trifluoromethyl-3-phenyl-3*H*-diazirines have been shown to be extremely useful photoaffinity labels. Upon photolysis, at wavelengths that do not cause damage to biological systems, they unleash highly reactive carbenes that can undergo both O-H and C-H insertion reactions.[3a,6]

Our strategy for synthesising this photoactivatable analogue of SR 48968, containing the 3-trifluoromethyl-3-phenyl-3*H*-diazirinyl moiety, was based on the published procedures for the preparation of SR 48968 by Emonds-Alt *et al.*[4d] and Hale *et al.*[7] Combining these methods, the synthesis was designed so that the final step could allow radiolabelling of the compound, *via* reductive amination using tritiated sodium cyanoborohydride.[8]

Synthesis of diazirinyl-analogue of SR 48968:

Initially, commercially available 3-bromobenzylalcohol was converted into 3-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoyl chloride **7** *via* a relatively facile and high yielding eight step synthesis. Protection of the alcohol using the *tert*-butyldimethylsilyl group[9] followed by reaction with nBuLi and N,N-diethyltrifluoro acetamide gave the trifluoromethyl ketone **4**.[10] Conversion to the diaziridine **5** was accomplished *via* formation of the oxime and the tosylate.[10] Oxidation of diaziridine **5** to the diazirine using *tert*-BuOCl,[10] resulted in a mixture of both silylated **6b** and non-silylated alcohol **6a**. This mixture was treated with TBAF to give the unprotected alcohol **6a**.[11] Oxidation of the alcohol to the carboxylic acid with $KMnO_4$,[12] followed by thionyl chloride gave 3-(3-trifluoromethyl-3*H*-diazirin-3yl)benzoyl chloride **7** in an overall yield of 59%, (Scheme 1).

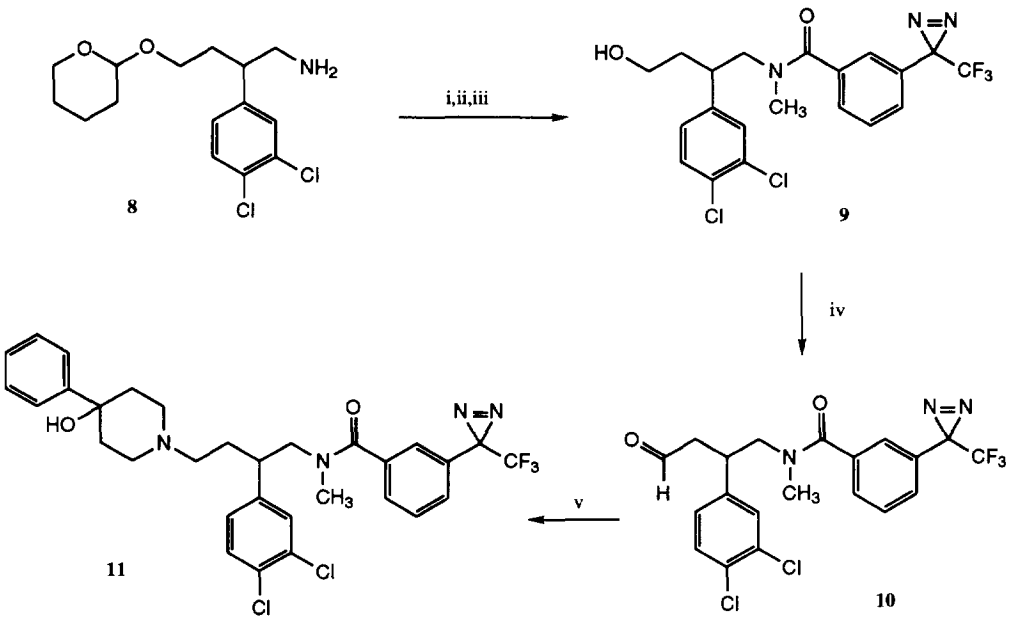


4 5 **6a** R=H **6b** R=Si^{t}BuMe$_2$ 7

Scheme 1

Reagents: i) TBDMSCl, DBU (96%); ii) nBuLi, THF at -78°C then $Et_2NCO.CF_3$ (94%); iii) $NH_2OH.HCl$, pyridine (91%); iv) NEt_3, DMAP, TsCl (98%); v) NH_3 (liq) (92%); vi) ^{t}BuOCl, then TBAF (94%); vii) $KMnO_4$ (85%); viii) $SOCl_2$ (100%)

Using an identical route to that published by Emonds-Alt *et al.*,[4d] except without the tartaric salt crystallographic resolution, we prepared the racemic amine **8**. Reaction with 3-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoyl chloride **7**, followed by N-methylation and removal of the tetrahydropyran moiety gave the alcohol **9**. Oxidation with either oxalyl chloride/dimethyl sulphoxide[13] or pyridinium chlorochromate[14] gave the aldehyde **10**, which decomposed upon column purification. The aldehyde was therefore used crude in the reductive amination with 4-hydroxy-4-phenylpiperidine perchlorate.[7,15] Flash column chromatography of the reaction mixture gave the diazirinyl-analogue of SR 48968 **11** as a colourless foam (58%), which upon addition of methanol yielded a colourless crystalline solid (Scheme 2). The perchlorate of the piperidine was used instead of the hydrochloride, since the perchlorate counterion has been reported to enable better conversion to the iminium salt, this being of fundamental importance in reductive aminations.[16] Conversion of the diazirinyl-analogue of SR 48968 **11** to the hydrochloride was carried out using ethereal HCl.[17]



Scheme 2

Reagents: i) 3-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoyl chloride **7** (74%); ii) NaH, MeI (94%); iii) *p*TsOH (81%); iv) PCC; v) 1.5 eq. 4-hydroxy-4-phenylpiperidine perchlorate, MeOH/THF (1/1), $NaCNBH_3$ (58%)

This analogue of SR 48968 retains good affinity for the human NK_2 receptor (Table 1).

Table 1. *In vitro* binding affinity of SR 48968 (**1**) and analogue (**11**) for the human NK_2 receptor in CHO cells using [^{125}I]-neurokinin A.

compound	IC_{50}(nM)
(±)-1	0.8
(±)-11	6.7

The NK_2 receptor is a G-protein coupled receptor, comprising seven transmembrane helices, with a structure based upon that of rhodopsin. Initial exploration of the binding region of SR 48968 using both chimeric receptors and site-directed mutagenesis has identified several regions, namely, the third extracellular loop, the sixth and seventh transmembrane segments as being important in binding the ligand.[18,19] However, no precise binding residues have been identified.

This photoactivatable analogue of SR 48968 has considerable potential for the identification of amino acid residues present at the binding site of the receptor, which in conjunction with further mutagenesis and modelling studies should increase our knowledge of the binding site of the NK_2 receptor, perhaps giving insight into the design of non-peptidic antagonists.

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