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AN SAR STUDY FOR THE NON-PEPTIDE SUBSTANCE P RECEPTOR (NK_1) ANTAGONIST, CP-96,345.[1]

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Abstract : Results from an SAR study around the novel, non-peptide substance P receptor NK_1 antagonist, (±)CP-96,345 (1) are described. The importance of the 2° nitrogen and the aromatic moieties are clarified.

The mammalian tachykinin neuropeptides, substance P, neurokinin A, neurokinin B, neuropeptide K and neuropeptide γ are widely distributed throughout the central and peripheral nervous systems, and all contain a common C-terminal sequence, -Phe-xxx-Gly-Leu-MetNH_2.[2] Of these substance P, isolated in 1931 by von Euler and Gaddum,[3] but characterized only more recently in 1970 by Chang and Leeman,[4] has been the most extensively studied. There is good evidence to support the existence of at least three distinct tachykinin receptor types,[5] termed NK_1, NK_2 and NK_3, which bind preferentially substance P, neurokinin A and neurokinin B, respectively. These receptors mediate a variety of biological effects of the tachykinin neuropeptides, including smooth muscle contraction, vasodilation, bronchoconstriction, salivary secretion and neuronal excitation, and thus represent major targets for drug research in this area. To date, a detailed understanding of the physiological roles of tachykinin neuropeptides, both in the periphery and the central nervous system, has not been possible because of the lack of selective tachykinin receptor agonists and antagonists possessing high affinity and good *in vivo* activity. However, Snider *et al.* (Pfizer Inc.) have recently described a novel, high affinity, non-peptide antagonist of the NK_1 receptor, CP-96,345.[1] Lowe *et al* (Pfizer Inc.) have subsequently reported the preparation and radiolabelling of CP-96,345 along with its affinity and that of two analogues (the 2-Cl and unsubstituted benzyl derivatives, compounds **2** and **6** respectively in this study) for the NK_1 receptor in bovine caudate using [^{3}H]SP as the radioligand.[6] Presented here are the results of a study to determine the importance of various functional groups present in racemic ((±)CP-96,345 (**1**).

The compounds studied are shown in table 1 and their syntheses outlined in Scheme 1; all compounds are racemates. Compounds **1-8** were prepared by the method of Lowe,[7] briefly this was as follows. A base

catalysed condensation of 3-quinuclidinone with benzaldehyde gave the enone **9**. Regioselective addition of phenylmagnesium bromide to **9** with $CuCl_2$ catalysis gave the ketone **10**. This could be elaborated to the final compounds **1–8** by stereoselective reductive amination with the appropriate amine and 9-BBN. Compound **11** was prepared by a similar reductive amination of ketone **12**.[8]

Hydrogenolysis of **6** gave the 1° amine **13**,[9] treatment of this with 2-chlorobenzoic acid activated with DCC gave the amide **14**.

The ether **15** was obtained by stereoselective reduction of the ketone **10**,[9] followed by regiospecific alkylation of the alcohol **16** with 2-chlorobenzylbromide using powdered KOH in DMSO.

The trans isomer of **1**, compound **17** was prepared using the methodology of Warawa and Mueller.[9]

Scheme 1

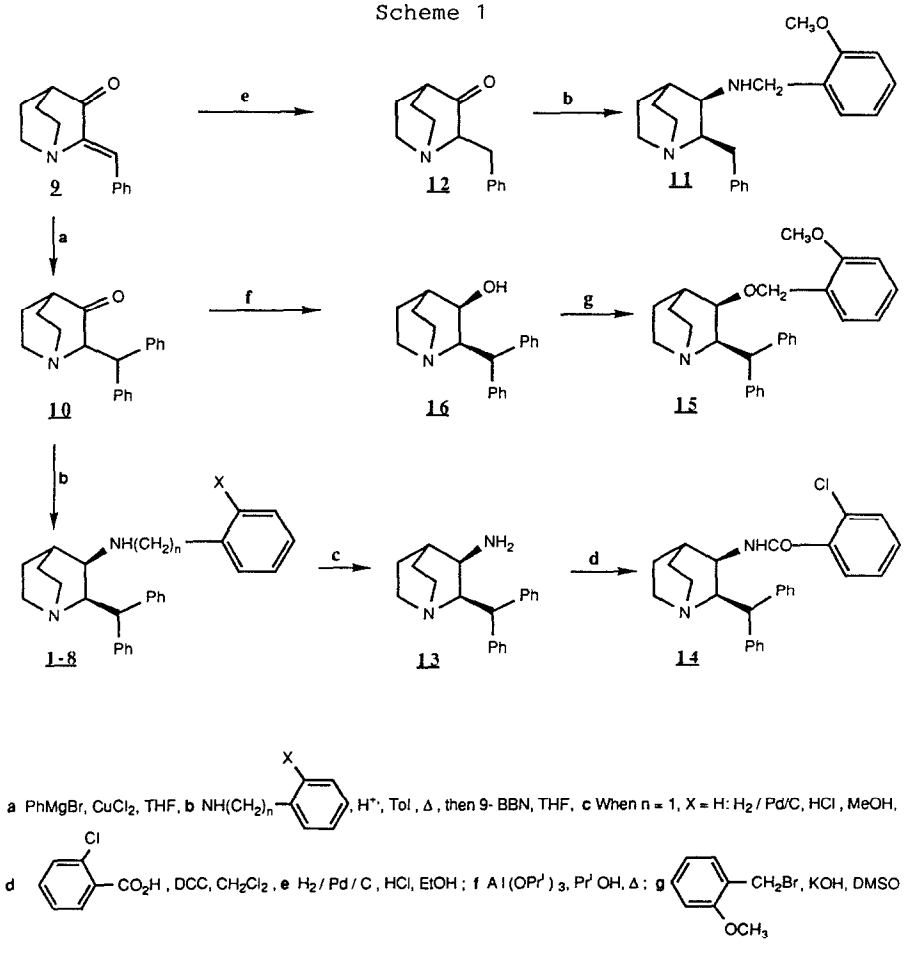
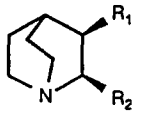


Table 1: Binding of(+/-) CP-96,345(1)analogues and standards to NK_1 sites in guinea pig and rat cerebral cortex membranes



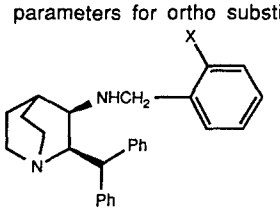
No.	R_1	R_2	NK_1 Binding IC_{50}(nM) Guinea Pig cerebral cortex	Rat cerebral cortex	IC_{50} ratio Rat / Guinea Pig
1	$NHCH_2$(2-CH_3OPh)	$CH(Ph)_2$	1.2(-0.2,+0.3)[a]	120(-15,+17)	100
2	$NHCH_2$(2-ClPh)	$CH(Ph)_2$	8.5(-1.7,+2.0)	110(-15,+17)	13
3	$NHCH_2$(2-CF_3Ph)	$CH(Ph)_2$	33(-2,+3)	740(-120,+150)	22
4	$NHCH_2$(2-FPh)	$CH(Ph)_2$	37(-12,+17)	990(-240,+310)	27
5	$NHCH_2$(2-CH_3Ph)	$CH(Ph)_2$	227(-30,+35)	700(-110,+130)	3
6	$NHCH_2$Ph	$CH(Ph)_2$	52(-10,+13)	2000(-500,+660)	38
7	$NH(CH_2)_2$(2-ClPh)	$CH(Ph)_2$	160(-25,+29)	7200(-1000,+1200)	45
8	$NH(CH_2)_3$(2-ClPh)	$CH(Ph)_2$	1100(-270,+360)	>10,000	>9
11	$NHCH_2$(2-CH_3OPh)	CH_2Ph	1510(-380,+580)	>10,000	>7
13	NH_2	$CH(Ph)_2$	>10,000	>10,000	-
14	NHCO(2-ClPh)	$CH(Ph)_2$	>10,000	>10,000	-
15	OCH_2(2-CH_3OPh)	$CH(Ph)_2$	150(-34,+44)	~10,000	67
17	trans isomer of 1		10(-1.3,+1.5)	800(-180,+230)	80
	Substance P		0.07(-0.01,+0.01)	0.12(-0.01,+0.01)	1.8
	Neurokinin A		3.5(-1.3,+2.0)	31(-9,+12)	9
	Neurokinin B		13(-3,+5)	69(-15,+19)	5
	Spantide		820(-190,+250)	650(-180,+250)	0.8

[a](geometric mean - , + s.e.m)

The affinities of the above compounds for NK_1 receptor binding sites present in either rat or guinea-pig cerebral cortex membranes were determined according to the method of Lee *et al*,[10] with minor modifications.[11] The results are collated in Table 1, together with those obtained for the endogenous tachykinins and the peptide based antagonist [D-Arg[1], D-Trp[7,9], Leu[11]]SP (spantide).

In membranes prepared from **guinea-pig** cerebral cortex comparison of the affinities of compounds **1-6** highlight the importance of the ortho aromatic substituent, the 2-CH_3O analogue **1** binding 50-fold better than the unsubstituted compound **6**. Attempts to correlate a range of physicochemical properties[12] of the ortho substituents with the affinities of the compounds were unsuccessful (see Table 2).

Table 2 : NK_1 Binding data and physicochemical parameters for ortho substituents X



No.	X	Guinea Pig NK_1 binding (IC_{50}, nM)	Π	MR	E_s	$\mathcal{F}$	$\mathcal{R}$
1	CH_3O	1.2	-0.02	7.87	-0.55	0.26	-0.51
2	Cl	8.5	0.71	6.03	-0.97	0.41	-0.15
3	CF_3	33	0.88	5.02	-2.40	0.38	0.19
4	F	37	0.14	0.92	-0.46	0.43	-0.34
6	H	52	0	1.03	0	0	0
5	CH_3	227	0.56	5.65	-1.24	-0.04	-0.13

The benzyl group itself was shown to be highly significant for binding, its removal leading to the inactive 1° amine **13**.

The distance between the secondary nitrogen and ortho substituted phenyl was also found to have a considerable influence on binding. As this distance increased in compounds **2**, **7**, and **8** there was a gradual decrease in affinity.

The significance of the basic secondary nitrogen of the benzylamine moiety was investigated by replacing it with a neutral oxygen atom (**15**) or reducing its basic character by replacing the adjacent CH_2 with CO (**14**).

Both of these analogues were considerably less active, the ether **15** some 100-fold less and the amide **14** inactive.

Removal of one of the aromatic rings of the benzylhydryl moiety led to the very weakly active derivative **11** suggesting that either both rings of this group bind to the NK_1 receptor or that its effect on conformation is critical.

Finally, our results indicate that the stereochemistry is important, the trans analogue of **1**, compound **17** possessing only one tenth of the affinity.

When examined for their ability to displace the binding of [^{125}I]-BHSP to NK_1 sites present in rat cerebral cortex these compounds show a similar rank order of affinity, but all are less active. The decrease in affinity varies across the series, the largest difference being observed for the ortho methoxy analogue **1** (100-fold) and the smallest for the ortho methyl analogue **5** (3-fold). These data are in contrast to that observed for neurokinin A, neurokinin B, substance P and spantide which show similar binding affinities for both guinea-pig and rat NK_1 receptors (see Table 1).

Compounds **1-8**, **11**, **13-15** and **17** were also examined for their ability to displace the binding of [^{125}I-iodohistidyl]-NKA to NK_2 sites present in hamster urinary bladder membranes[13] and [^{3}H]-Senktide to NK_3 sites present in rat cerebral cortex membranes,[10] none of the compounds showed any significant affinities for these two sites (IC_{50} $\geq 10^5$ nM).

In conclusion, this study has highlighted the importance of the basic secondary nitrogen, ortho substituent and aromatic moieties for NK_1 receptor binding affinity in this series of compounds. In addition, differences in the rank order of affinities for this series of compounds between rat and guinea-pig NK_1 binding sites confirm and extend recent studies which suggest the existence of species-dependent NK_1 receptor subtypes.[5,14,15,16]

Acknowledgement

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11. Membranes (50–100 µg protein/tube) were incubated (60 min at room temperature) with 10^{-10} M [^{125}I]-Bolton Hunter labelled substance P ([^{125}I]-BHSP) in a final volume of 300 µl in the presence of 10^{-11}–10^{-5}M test compound, and then rapidly filtered through Whatman GF/C filters using a Brandel Cell Harvester. Substance P (10^{-6}M) and the selective NK_1 receptor agonist [Sar^9 Met$(O_2)^{11}$] substance P (10^{-6}M), were used to define non-specific binding in rat and guinea-pig tissue, respectively. Data from 3–5 individual displacement curves were analyzed using an iterative curve fitting programme on RS1 and expressed as IC_{50} values in nM (geometric mean –, + s.e.m.).
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