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# LOW MOLECULAR WEIGHT NEUROKININ $NK_2$ ANTAGONISTS

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A deletion - optimization strategy based on an initial heptapeptide lead structure **1** led to potent and selective neurokinin $NK_2$ antagonists **5** ($pK_B$=9.3) and **9** ($pK_B$=7.9) of substantially reduced molecular size. Tetrapeptide **5** (0.1 μmol/Kg i.v.) potently inhibits $NK_2$ agonist-induced bronchoconstriction in guinea-pigs. Whilst less potent than **5** *in vivo*, the dipeptoid **9** (5 μmol/Kg i.v.) had a significantly longer biological half-life (> 2 h), and provides a potential lead towards non-peptide analogues.

The mammalian tachykinins, substance P, neurokinin A and neurokinin B are the preferred endogenous agonists at three distinct neurokinin receptors designated $NK_1$, $NK_2$ and $NK_3$ respectively.[1] All three receptors have recently been cloned and shown to be homologous members of the G-protein coupled 7 transmembrane helix receptor superfamily.[2] Substance P and the neurokinins, which are widely distributed in the peripheral and central nervous systems are implicated in numerous physiological processes,[1,3,4] and there is much speculation on their pathophysiological roles.

Receptor selective neurokinin antagonists are essential tools for receptor characterisation *in vitro* and *in vivo*. Moreover, non-peptide antagonists with good oral bioavailability and/or CNS penetration are required for evaluation as potential therapeutic agents. Recent progress toward the latter objective is illustrated by the discovery of potent and selective $NK_1$ antagonists CP-96,345[5] and RP-67,580[6] using random screening approaches. $NK_2$ receptor selective antagonists have been described in the form of linear[7] and cyclic[8] peptides, but only one non-peptide $NK_2$ antagonist, SR-48968,[9] has been reported to date. The relatively weak anti-bronchoconstrictor activity of this high affinity antagonist ($pA_2$ 10.3) following intraduodenal (compared to intravenous) administration in the guinea pig indicates poor oral bioavailability and emphasizes the need to investigate alternative structural types. In this paper we describe key results from a deletion - optimization strategy, starting from a heptapeptide and resulting in moderately potent dipeptide $NK_2$ antagonists (**9** and **13**) as attractive leads towards non peptides. A similar minimal structure approach has previously enabled the rational design of "dipeptoid" gastrin and $CCK_B$ antagonists.[10]

Previous work in our laboratories[11] led to the highly potent and selective heptapeptide $NK_2$ antagonist GR94800 (**1**; $pK_B$[12] 9.56 +/- 0.07; MWt 904), and our next objective was to develop low molecular weight

(**1**) PhCO.Ala.Ala.(D)Trp.Phe.(D)Pro.Pro.Nle.$NH_2$

antagonists with comparable potency and reduced peptidic nature. Our strategy to achieve this goal was to

delete amino acid residues from the C-terminus of **1** to identify a minimal unit required for effective binding at the receptor. Optimization of the activity of the resulting shortened peptide was then investigated by modifications at the N and C terminus.

## Synthesis

All the compounds described below were prepared using conventional amide bond coupling reactions using DCC/HOBT in DMF with tBOC or Cbz protection of amino acids where appropriate. The imidazole (**9**) was prepared from 2-phenyl-imidazole-4-carboxylic acid,[13] and could also be prepared by a Claisen rearrangement from an appropriate amidoxime.[14]

## Tetrapeptides (Table 1)

Deletion of the terminal (D)Pro.Pro.Nle.$NH_2$ from **1** affords the tetrapeptide **2** with greatly reduced $NK_2$ antagonist potency. Most of the activity was restored by forming the C-terminal dimethylamide **3**. Further improvement was then accomplished by replacing the N-terminal alanine with a glycine, as in **4** and the equipotent cyclohexylcarbonyl analogue **5**. GR100679 (**5**) has activity only marginally less than **1**, but is considerably reduced in size.

**Table 1.** Tetrapeptide $NK_2$ Antagonists

| | Compound | $pK_B$ (rat colon) | Mol. Wt |
|---|---|---|---|
| (2) | PhCO.Ala.Ala.(D)Trp.Phe.OH | 6.6 (n=1) | 597.7 |
| (3) | PhCO.Ala.Ala.(D)Trp.Phe.$NMe_2$ | 8.56 +/- 0.15 | 624.8 |
| (4) | PhCO.Gly.Ala.(D)Trp.Phe.$NMe_2$ | 8.87 +/- 0.15 | 610.7 |
| (5) |  CO.Gly.Ala.(D)Trp.Phe.$NMe_2$ (GR100679) | 9.05 +/- 0.15 | 616.8 |

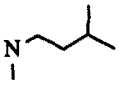
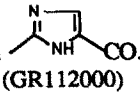
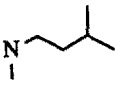
Furthermore, compound **5** maintained the high selectivity (> 1000 fold) with respect to $NK_1$ receptors of the original heptapeptide GR94800.[11] Thus in isolated guinea-pig trachea, **5** was a potent antagonist of the $NK_2$-selective agonist GR64349[15] ($NK_2$ $pK_B$ = 9.3 +/- 0.1), but antagonized the selective $NK_1$ agonist, substance P methyl ester, at high concentrations only ($NK_1$ $pK_B$ = 5.1 +/- 0.2).

## Tripeptides (Table 2)

Further deletion of the C-terminal Phe residue produced the tripeptide **6** which once again showed reduced

activity. Some potency could subsequently be regained by increasing the lipophilicity of the C-terminal amide, exemplified by compounds **7** and **8**.

**Table 2.** Tripeptide $NK_2$ Antagonists

| | Compound | $pK_B$ (rat colon) | Mol. Wt |
|---|---|---|---|
| (6) | PhCO.Ala.Ala.(D)Trp.$NMe_2$ | 6.45 +/- 0.01 | 477.6 |
| (7) | PhCO.Ala.Ala.(D)Trp.N(Me)$CH_2CH_2Ph$ | 7.40 +/- 0.15 | 567.7 |
| (8) | PhCO.Ala.Ala.(D)Trp.N  | 8.00 +/- 0.04 | 533.7 |
| (9) | Ph  CO.Ala.(D)Trp.N  (GR112000) | 7.64 +/- 0.01 | 528.7 |

Replacement of the terminal alanine with other amino acids in both the tri- and tetrapeptide series (data not shown) led us to speculate that in the bio-active conformation of these molecules the first residue would likely adopt a fully extended ($\phi$ = $\psi$ = 180°) conformation.We therefore sought to replace the alanine with an alternative non-peptide group which could bias the backbone conformation in this region of conformational space. The imidazole group was chosen as an amide isostere which fixes the $\phi$ torsional angle at 180° (Fig 1) and compound **9** was prepared.[16] Gratifyingly **9** retained a good level of $NK_2$ antagonist activity and ca. 1000 fold selectivity with respect to $NK_1$ receptors in guinea-pig trachea ($NK_2$ $pK_B$ = 7.9 +/- 0.1; $NK_1$ $pK_B$ = 4.9 +/- 0.4).

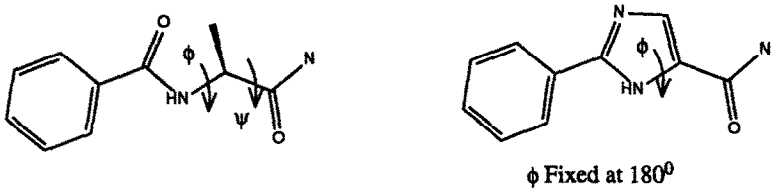
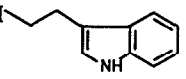
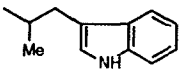
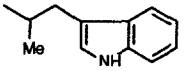
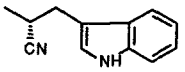



**Fig 1.** Imidazole as a replacement for the N-terminal alanine residue

**Dipeptides (Table 3)**

The dipeptide **10**, derived from **8** by deletion of the terminal carboxamide, showed only weak $NK_2$ antagonist activity. Some activity was recovered by $\alpha$ substitutions in the tryptamine sidechain, with most of the activity residing in the single diastereoisomer **12**. The cyano-analogue **13** was equipotent with **12** in rat colon, but being slightly more potent in guinea-pig trachea ($NK_2$ $pK_B$ = 7.4 +/- 0.1), it was selected for evaluation *in vivo*.

**Table 3.** Dipeptide $NK_2$ Antagonists

| | Compound | $pK_B$ (rat colon) | Mol. Wt |
|---|---|---|---|
| **(10)** | PhCO.Ala.Ala.NH  | 6.02 +/- 0.06 | 406.5 |
| **(11)** | PhCO.Ala.Ala.NH  Isomer 1 | < 5 | 420.5 |
| **(12)** | PhCO.Ala.Ala.NH  Isomer 2 | 6.66 +/- 0.18 | 420.5 |
| **(13)** | PhCO.Ala.Ala.NH  | 6.8 (n = 1) | 431.5 |

## $NK_2$ Antagonism *in vivo*

After intravenous administration to anaesthetised guinea-pigs, antagonists **5**, **9** and **13** blocked the transient increases in tracheal pressure induced by intravenous challenge with the $NK_2$ agonist GR64349. The inhibition of bronchoconstriction (measured as a dose-ratio), was monitored for time periods up to 2 hours using repeated agonist challenges (Fig. 2). Potency *in vivo* correlated with $NK_2$ receptor affinity *in vitro*. However, although less potent than tetrapeptide **5**, the dipeptoid **9** had a significantly longer biological half-life (140 min compared with 26 min for **5**), perhaps reflecting reduced peptidic character and metabolic clearance.

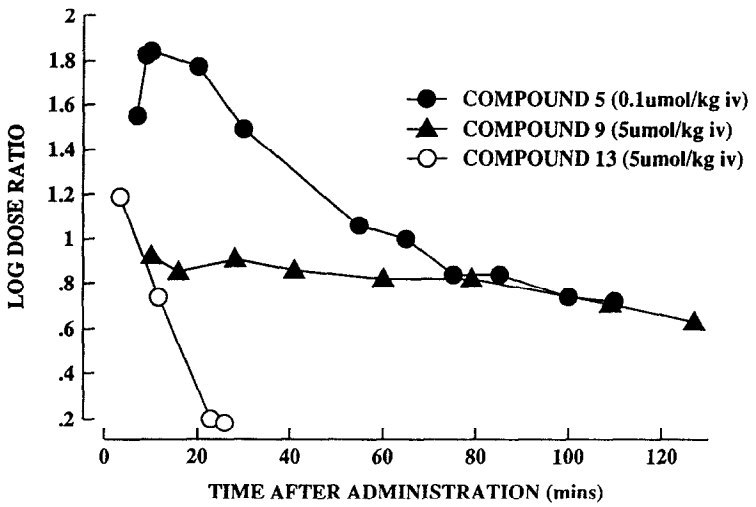



**Fig 2.** Antagonism of GR64349-induced bronchoconstriction in anaesthetized guinea-pigs by $NK_2$ receptor antagonists **5**, **9**, and **13**

## Conclusions

From a heptapeptide starting point we have developed high affinity selective tetrapeptide $NK_2$ antagonists **4** and **5**. However, the dipeptoid **9** represents an optimum compromise between molecular size and receptor affinity, and thus provides an attractive potential lead towards novel structural classes of non-peptide $NK_2$ antagonists. Comparing the structures of dipeptoid **9** and the non-peptide $NK_2$ antagonist SR-48968[9] (**14**, Fig 3; $pA_2$ = 10.3 in endothelium-deprived rabbit pulmonary artery), the receptor binding of both compounds appears to depend on interactions at three hydrophobic receptor sub-sites. However, as both molecules contain a considerable degree of torsional flexibility, any correspondence between receptor binding modes for the two antagonists remains unclear (eg. by molecular graphics overlays). Alternatively, the less potent dipeptides **12** and **13** may offer a more useful entry into a novel non-peptide analogue series. The latter antagonists apparently interact at only two hydrophobic receptor sub-sites, and have the advantage of lower molecular weight and lipophilicity.

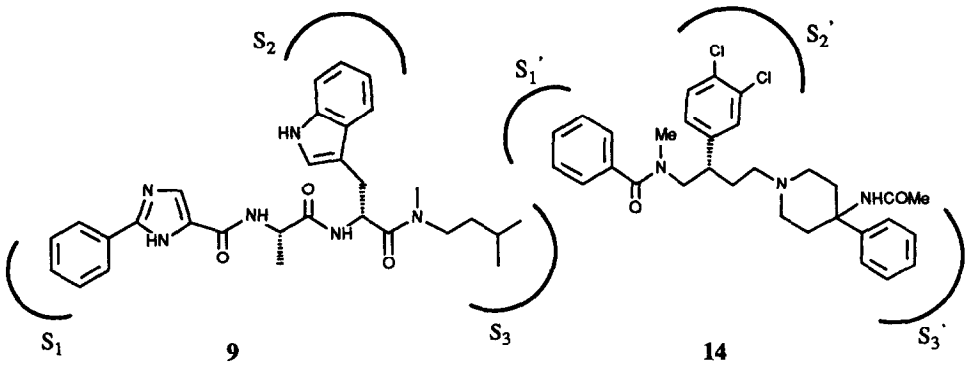



**Fig 3.** Schematic receptor binding modes for **9** and **14** (SR-48968)

## References and Notes

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11. McElroy AB, Clegg SP, Deal MJ, Ewan GB, Hagan RM, Ireland SJ, Jordan CC, Porter B, Ross BC, Ward P and Whittington AR, *J Med Chem (in press).*

12. Compounds were tested as agonists and as antagonists *in vitro* at $NK_2$ receptors in rat colon muscularis mucosae (RC) and at $NK_1$ and $NK_2$ receptors in guinea-pig trachea (GPT) using methods described previously.[12a,15] None of the compounds showed agonist activity (contraction). Antagonist affinities were determined from the parallel displacement of standard agonist concentration-response curves, and expressed as $pK_B$ values, calculated from the equation: $pK_B = \log_{10}(\text{concentration-ratio} - 1) - \log_{10}(\text{molar concentration of antagonist})$, where the concentration-ratio is the ratio of equiactive molar concentrations of the agonist in the presence and absence of the antagonist. $pK_B$ values are quoted as mean +/- s.e. mean of 3 - 8 replicate determinations, except where indicated (n = 1). Standard agonists were NKA in RC and GR64349[15] in GPT ($NK_2$ receptors), and SP-methyl ester in GPT ($NK_1$ receptors). a) Bailey SJ and Jordan CC, *Br J Pharmacol,* **1984,** *82,* 441.

13. Prepared from 2-phenylimidazole using the methodology of Lipshutz *et al., Tetrahedron Letters,* **1988,** *28,* 3411.

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16. The imidazole moiety in compound 9 is potentially amphoteric. Low aqueous solubility prevented $pK_a$ measurements in water. However, empirically estimated $pK_a$ values are 5.3 (basic) and 10.5 (acidic). Compound 9 may therefore be assumed to be essentially unionised at physiological pH.