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Bioorganic & Medicinal Chemistry Letters 14 (2004) 4779–4782
Bioorganic & Medicinal Chemistry Letters

Design and synthesis of substituted *N*-methylbenzamide analogues derived from SR 48,968 as neurokinin-2 receptor antagonists

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Received 7 May 2004; accepted 16 June 2004
Available online 19 July 2004

Abstract—A series of *N*-methylbenzamide analogues (**2**–**18**) that is structurally derived from SR 48,968, a potent neurokinin-2 (NK_2) receptor antagonist (pK_b 9.1), has been obtained using asymmetric synthesis. Isothiocyanato-*N*-methylbenzamide (**10**–**12**) and bromoacetamido-*N*-methylbenzamide derivatives (**16**–**18**) have been designed to serve as potential electrophilic affinity labels. Nitro-*N*-methylbenzamide (**4**–**6**) and acetamido-*N*-methylbenzamide (**13**–**15**) were designed to serve as the nonelectrophilic controls for these ligands. Functional assay results using guinea pig trachea indicate that electrophilic *N*-methylbenzamide analogues exhibit potent but surmountable NK_2 receptor antagonist activity. Several members of this series (**2**, **3**, **7**–**9**) exhibit potent NK_2 receptor antagonist potencies with pK_b values in the range of 9.1–9.7. *para*-Fluoro substituted analogue **3** was found to be highly potent with a pK_b of 9.7.

The mammalian neurokinins, namely, substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) are neuropeptides of 10-11 amino acid residues in length. They share a common C-terminal sequence Phe-X-Gly-Leu-Met, wherein X is typically an aromatic or branched aliphatic amino acid.[1] These peptides are associated with a variety of biological activities such as pain transmission, neurogenic inflammation, anxiety, smooth muscle contraction, salivary secretion, and activation of the immune system. The SP, NKA, and NKB bring about their effects through activation of the G-protein-coupled receptors neurokinin-1 (NK_1), neurokinin-2 (NK_2), and neurokinin-3 (NK_3), respectively.[2] The presence of NK_2 receptors in tissues throughout the body is well established, and, in recent years, evidence has been presented for the presence of NK_2 receptors in the central nervous system.[3] A number of potential therapeutic indications of NK_2 receptor antagonists have been proposed, which include the treatment of diseases such as asthma, inflammatory bowel disorders, rheumatoid arthritis, pain, emesis, and psychiatric disorders.[4]

Electrophilic affinity labels bind covalently to the receptors and are useful in the structural and functional characterization of receptors at the molecular level.[5,6] The availability of such ligands is helpful in complementing molecular biological studies where evidence for important amino acids in the binding site is obtained indirectly by site-directed mutagenesis studies. Potent and selective electrophilic affinity labels have demonstrated their usefulness as pharmacologic tools in studying a number of receptor systems including opioid, muscarinic, adrenergic, adenosine, serotonin, and benzodiazepine receptors.[5] A diazirinyl-analogue of SR 48,968 (IC_{50} of 6.7 nM) has been previously reported as a potent photoaffinity label for the NK_2 receptors, which upon photolysis is expected to generate a highly reactive carbene.[7] However, to our knowledge no NK_2 receptor selective electrophilic affinity labels have been reported to date.

SR 48,968 (**1a**) is a potent and selective NK_2 receptor antagonist (Fig. 1).[8] In this study, we have employed the racemic 4-hydroxy-4-phenyl piperidine analogue

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doi:10.1016/j.bmcl.2004.06.053

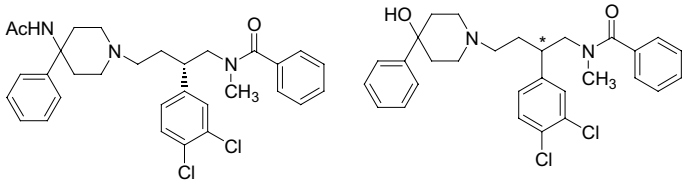


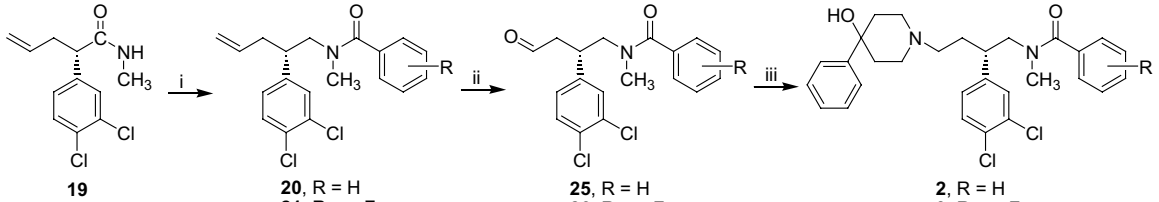
Figure 1. Potent neurokinin-2 antagonists: (**1a**) SR 48,968. (**1b**) Racemic 4-hydroxy-4-phenyl piperidine derivative of SR 48,968.

(**1b**) of SR 48,968 (K_i 1.0 nM)[8] as the lead compound in order to develop electrophilic affinity labels with NK_2 receptor selectivity.

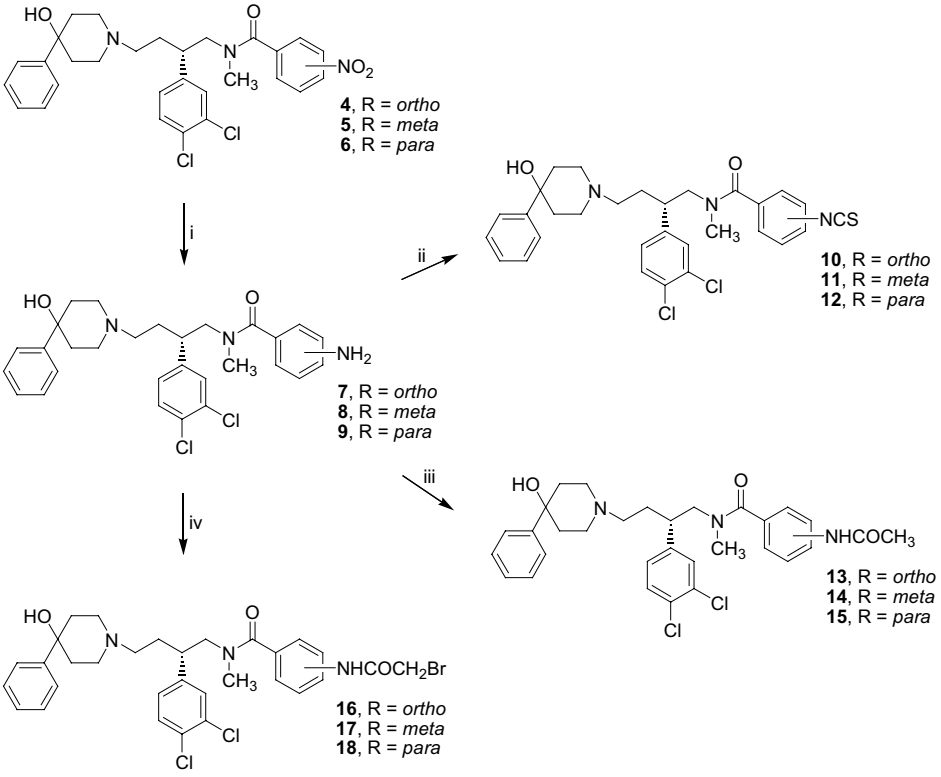
Since SR 48,968, the *S*-stereoisomer, was found to exhibit a nearly 2000-fold higher binding affinity for NK_2 receptors from rat duodenum membranes than its *R*-enantiomer SR 48,965,[8] all the target compounds have been designed to have *S*-configuration at the chiral center. Chimeric receptor studies and site-directed mutagenesis studies have been utilized in an attempt to delineate the binding site of SR 48,968 on the NK_2 receptors. The NK_1/NK_2 chimeric receptor studies suggest the involvement of transmembrane VI, extracellular loop three, and part of transmembrane VII in the binding of this ligand to the NK_2 receptors.[9] Furthermore, site-directed mutagenesis studies have provided information regarding the important amino acid residues for high affinity binding of SR 48,968. According to various studies, His198 plays an important role in the binding of SR 48,968 to NK_2 receptor,[10,11] although discrepant results have been reported regarding the role of His198 by one group.[12] Based on the hypothetical models proposed for SR 48,968 binding mode on the human NK_2 receptor, the benzamide moiety of SR 48,968 is close to His198 region.[13] In addition, other nucleophilic amino acids such as Tyr197, Tyr206, Met213, Tyr217, Ser218 are present in the vicinity.[13] Therefore, the benzamide moiety of the lead compound was chosen for substituting the electrophilic groups. Moreover, previous structure–activity relationship studies have shown that a wide variety of substituents are tolerated in the benzamide region of SR 48,968,[14] and of YM-35375, which is a spiro-substituted piperidine analogue of SR 48,968.[15] Isothiocyanate and bromoacetamide groups were chosen for attachment to the benzamide group to cover a range of reactivities and chemical selectivities. *ortho*, *meta* and *para* analogues were synthesized to map the receptor for the nucleophilic groups. The synthetic intermediates, nitro analogues **4**–**6**, and the acetamide analogues **13**–**15** were chosen to serve as the nonelectrophilic controls for the isothiocyanate **10**–**12** and bromoacetamide **16**–**18** analogues. Due to the similarity in sizes of fluorine and hydrogen atoms, *para*-fluoro analogue **3** was also synthesized.

Synthesis of amide **19** was accomplished using asymmetric synthesis according to steps described by Hale and co-workers.[16] Diisobutylaluminum hydride reduction of amide **19** to the corresponding secondary amine followed by acylation with benzoyl chloride, *p*-fluorobenzoyl chloride, or *o*-, *m*-, *p*-nitrobenzoyl chloride led to the formation of benzamide **20** and substituted benzamides **21**–**24**, respectively. Aldehydes **25**–**29** were obtained by the oxidative cleavage of the olefinic bond of **20**–**24** using osmium tetroxide and sodium periodate.[17] Reductive amination of **25**–**29** with 4-hydroxy-4-phenylpiperidine using sodium cyanoborohydride afforded compound **2**, the *S*-stereoisomer corresponding to the lead compound **1b**, the fluoro analogue **3**, and the nitro analogues **4**–**6** as illustrated in Scheme 1.

The synthetic intermediate aminobenzamide derivatives **7**–**9** were prepared by reduction of the corresponding nitro derivatives **4**–**6** with stannous chloride (Scheme 2),[15] which were then reacted with different reagents to provide target compounds **10**–**18**. The isothiocyanates **10**–**12** were obtained by reacting the amines with di-(2-pyridyl)thionocarbonate.[18] The acetamides **13**–**15** were synthesized from the amines by addition of acetic anhy-



Scheme 1. Reagents and conditions: (i) DIBALH, CH_2Cl_2/toluene, rt, then substituted or unsubstituted benzoyl chloride, toluene/satd $NaHCO_3$ solution; (ii) cat OsO_4, *N*-methylmorpholine *N*-oxide 2:1:1 v/v/v acetone/*t*-butanol/water, then $NaIO_4$, 4:1 v/v THF/water; (iii) 1.5 equiv 4-hydroxy-4-phenylpiperidine, $Na(CN)BH_3$, 1:1 v/v THF/methanol.



Scheme 2. Reagents and conditions: (i) $SnCl_2 \cdot 2H_2O$, ethyl acetate, reflux; (ii) di-2-pyridyl-thionocarbonate, CH_2Cl_2, rt; (iii) acetic anhydride, $CHCl_3$, rt; (iv) bromoacetyl bromide, THF, 0 °C.

dride at room temperature. The bromoacetamides **16**–**18** were obtained from the amines by the controlled addition of bromoacetyl bromide in THF at 0 °C.[19]

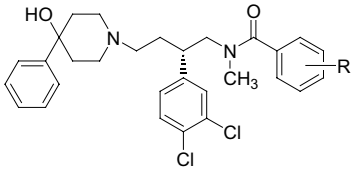
N-Methylbenzamide substituted analogues derived from SR 48,968 were synthesized and evaluated for their NK_2 receptor antagonist activity using isolated guinea pig trachea according to experimental protocols described previously.[20] The results are presented in Table 1. The unsubstituted analogue **2**, fluoro substituted analogue **3**, and the amino substituted analogues **7**–**9** were found to be the most potent compounds in this series of target compounds with pK_b values similar to or greater than that for the prototypical NK_2 receptor antagonist SR 48,968.

The unsubstituted *N*-methylbenzamide analogue **2**, which is the *S*-stereoisomer corresponding to the racemic lead compound **1b**, exhibited high NK_2 receptor antagonist potency with a pK_b value of 9.5. In comparison with the previously reported NK_2 receptor antagonist activity of SR 48,968 (**1a**) using [β-Ala[8]]NKA(4-10) in isolated guinea pig trachea in a similar assay,[14] compound **2** was found to be more potent suggesting that replacement of the 4-acetamide group on the piperidine ring of SR 48,968 with a hydroxyl group is a favorable modification. Additional modifications focused on substituting the *N*-methylbenzamide moiety. Modification of compound **2** by substituting a *para* fluoro group on the *N*-methylbenzamide moiety, which is sterically similar to hydrogen, led to compound **3**, which is the most potent analogue in this series with a pK_b of 9.7. Substitution of the *N*-methylbenzamide moiety with amino group at *ortho*, *meta*, or *para* positions led to analogues that retained high NK_2 receptor antagonist potencies. Thus, both electron-withdrawing fluoro group and the electron-releasing amino group maintained high NK_2 receptor antagonist potencies.

Nitro substituted analogues **4** and **6**, isothiocyanate analogue **10**, acetamide analogue **15**, and bromoacetamide analogue **18** showed somewhat reduced activities with pK_b values in the range of 8.3–8.55. The remaining analogues showed substantially reduced activities with pK_b values less than 8.0. Reduction in potency of nitro (**4**–**6**), isothiocyanate (**10**–**12**), acetamide (**13**–**15**), and bromoacetamide (**16**–**18**) analogues relative to unsubstituted analogue **2**, fluoro analogue **3**, and amino analogues **7**–**9** may be explained by a relative increase in the size of these substituents. Since both fluoro and amino analogues show good NK_2 receptor activity, electronic effect may not be playing a significant role in the activities of *N*-methylbenzamide substituted analogues.

The irreversibility of the target compounds was evaluated by their ability to inhibit the maximum obtained contraction of the NK_2 agonist [β-Ala[8]]-NKA(4-10). The maximum obtainable response of the agonist averaged approximately 96.7% and was not significantly ($P>0.05$) inhibited by any of the target compounds. Therefore, we conclude that antagonist activity of all the test compounds, including the electrophilic isothiocyanate derivatives **10**–**12** and bromoacetamide derivatives

Table 1. NK_2 receptor antagonist potency of substituted *N*-methylbenzamide analogues in guinea pig trachea



Compd	R	pK_b[a,b]	Maximum response in presence of antagonists (control = 96.7 ± 0.3%)
SR 48,968		9.1[c]	
2	H	9.5 ± 0.2	93.3 ± 2.9%
3	*p*-F	9.7 ± 0.2	94.7 ± 0.5%
4	*o*-NO_2	8.5 ± 0.4	92.7 ± 1.7%
5	*m*-NO_2	<8.0	98.6 ± 0.6%
6	*p*-NO_2	8.55 ± 0.4	98.0 ± 2.0%
7	*o*-NH_2	9.4 ± 0.3	98.2 ± 1.1%
8	*m*-NH_2	9.4 ± 0.4	97.4 ± 0.8%
9	*p*-NH_2	9.1 ± 0.5	95.9 ± 2.6%
10	*o*-NCS	8.5 ± 0.1	95.5 ± 0.3%
11	*m*-NCS	<8.0	95.8 ± 1.2%
12	*p*-NCS	<8.0	97.6 ± 1.5%
13	*o*-$NHCOCH_3$	<8.0	98.3 ± 0.7%
14	*m*-$NHCOCH_3$	<8.0	96.3 ± 0.8%
15	*p*-$NHCOCH_3$	8.4 ± 0.6	96.6 ± 3.2%
16	*o*-$NHCOCH_2Br$	<8.0	97.1 ± 0.7%
17	*m*-$NHCOCH_2Br$	<8.0	98.0 ± 0.7%
18	*p*-$NHCOCH_2Br$	8.3 ± 0.4	96.0 ± 3.1%

[a] pK_b are determined against [β-Ala[8]] NKA(4-10) in isolated guinea pig trachea; values are means ± SEM from four experiments.
[b] pK_b is the negative log of K_b where K_b = [antagonist]/(dose ratio-1).
[c] Data from Ref. 14.

16–**18**, is surmountable by the NK_2 agonist [β-Ala[8]]-NKA(4-10). This suggests that the electrophilic isothiocyanate and bromoacetamide groups in the *ortho*, *meta*, or *para* positions of the *N*-methylbenzamide group in the target compounds **10**–**12** and **16**–**18** are not within the covalent bonding distance of any nucleophilic group in the NK_2 receptor binding site.

In conclusion, compounds **2**, **3**, and **7**–**9**, which are obtained by structural modification of SR 48,968 exhibit potent NK_2 receptor antagonist activity with pK_b values in the range of 9.1–9.7. These ligands are potentially useful as pharmacologic tools when reversible NK_2 receptor antagonists are needed. *para*-Fluoro substituted analogue **3** was found to be highly potent with a pK_b of 9.7. It is approximately one-half of an order of magnitude more potent than the prototypical NK_2 receptor antagonist SR 48,968. The antagonist activity of the ligands with electrophilic substituents was surmountable by the standard NK_2 receptor agonist indicating the reversible nature of their binding.

Acknowledgements

This work was supported in part by NIH (Grant 1 R15 NS36367-01A1).

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