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Pharmacological Profile and Chemical Synthesis of SR 48968, a Non-Peptide Antagonist of the Neurokinin A (NK_2) Receptor

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Abstract : SR 48968 is a potent, competitive and selective non-peptide antagonist of the neurokinin A (NK_2) receptor.The synthesis of SR 48968 is described. Structure activity relationship is shown using binding and pharmacological results.

Tachykinins, namely neurokinin A (NKA), substance P (SP) and neurokinin B (NKB), are mammalian related peptides which are released from sensory nerves, including non-adrenergic non-cholinergic nerves. They exert various biological activities in the central nervous systems and in peripheral organs[1-3]. In particular, NKA induces the contraction of smooth muscles of the cardiovascular, gastrointestinal, respiratory and urinary systems[1,4] and it is implicated as a neurotransmitter in pain[5]. The biological effects of tachykinins are mediated through specific receptors and each tachykinin appears to activate a distinct receptor denoted NK_1, NK_2 or NK_3 for SP, NKA and NKB, respectively[1,2,4].

The first generation of antagonists include peptides derived from tachykinin structures[2]. However, these drugs have limited potency and metabolic stability. Non-peptide antagonists of the NK_1 receptor have been recently described, CP 96,345[6] and RP 67,580[7]. In the meantime, we have discovered the first potent non-peptide antagonist of the NK_2 receptor, SR 48968 [(S)-N-methyl-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide][8,9].

SR 48968 has been shown to selectively and competitively inhibit the binding of [^{125}I]-NKA to its receptor from rat duodenum or hamster urinary bladder membranes with an inhibition constant (K_i) of 0.51 ± 0.09 nM and 1.2 ± 0.1 nM, respectively[8-10]. In classical binding assays for NK_1 and NK_3 receptors, this compound did not significantly inhibit (K_i > 5000 nM) the binding of [^{125}I]-SP or [^{125}I]-eledoisin, respectively[9]. In in vitro pharmacological assays, SR 48968 has been shown to potently and competitively antagonize the contraction of smooth muscles induced by NKA or selective agonists for the NK_2 receptor; this antagonism has been observed in preparations of smooth muscles from various animal species and from different human tissues[9,11,12]. For example, SR 48968 antagonized the contraction of the endothelium-deprived rabbit pulmonary artery induced by [β Ala^8]-NKA (4-10) (a classical NK_2 receptor assay) with a pA_2 = 10.48 ± 0.06, whereas it was almost inactive in the classical NK_1 and NK_3 receptor assays[9]. In addition, SR 48968 potently antagonized the contraction of the guinea pig bronchus induced by the electric field stimulated release of endogenous NKA[13]. In vivo, SR 48968 has been shown to antagonize the NKA-induced bronchoconstriction in guinea pig with ID_{50} (inhibition dose 50%) of 37 μg/kg or 350 μg/kg for intravenous or intraduodenal route, respectively[9]. When administered at 1 mg/kg per os, SR 48968 showed a long acting inhibition of NKA-induced bronchoconstriction (Fig. 1). Moreover, as previously shown in vitro using the human isolated bronchus[12], SR 48968 did not significantly inhibit in the guinea pig the bronchoconstriction induced by various spasmogens (Table 1).

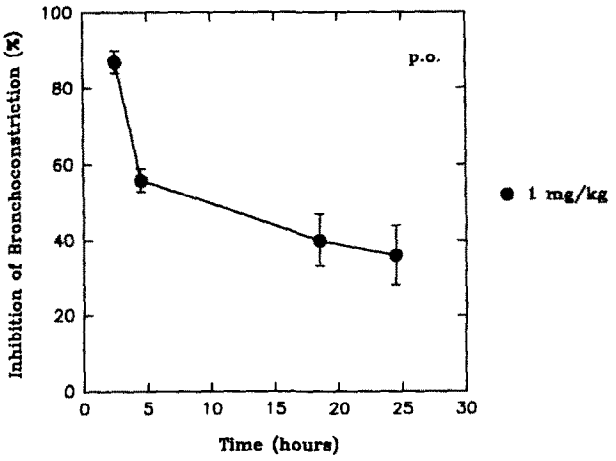


Fig. 1

Inhibition by SR 48968 of [Nle^{10}]-NKA(4-10)-induced bronchoconstriction in the guinea pig.

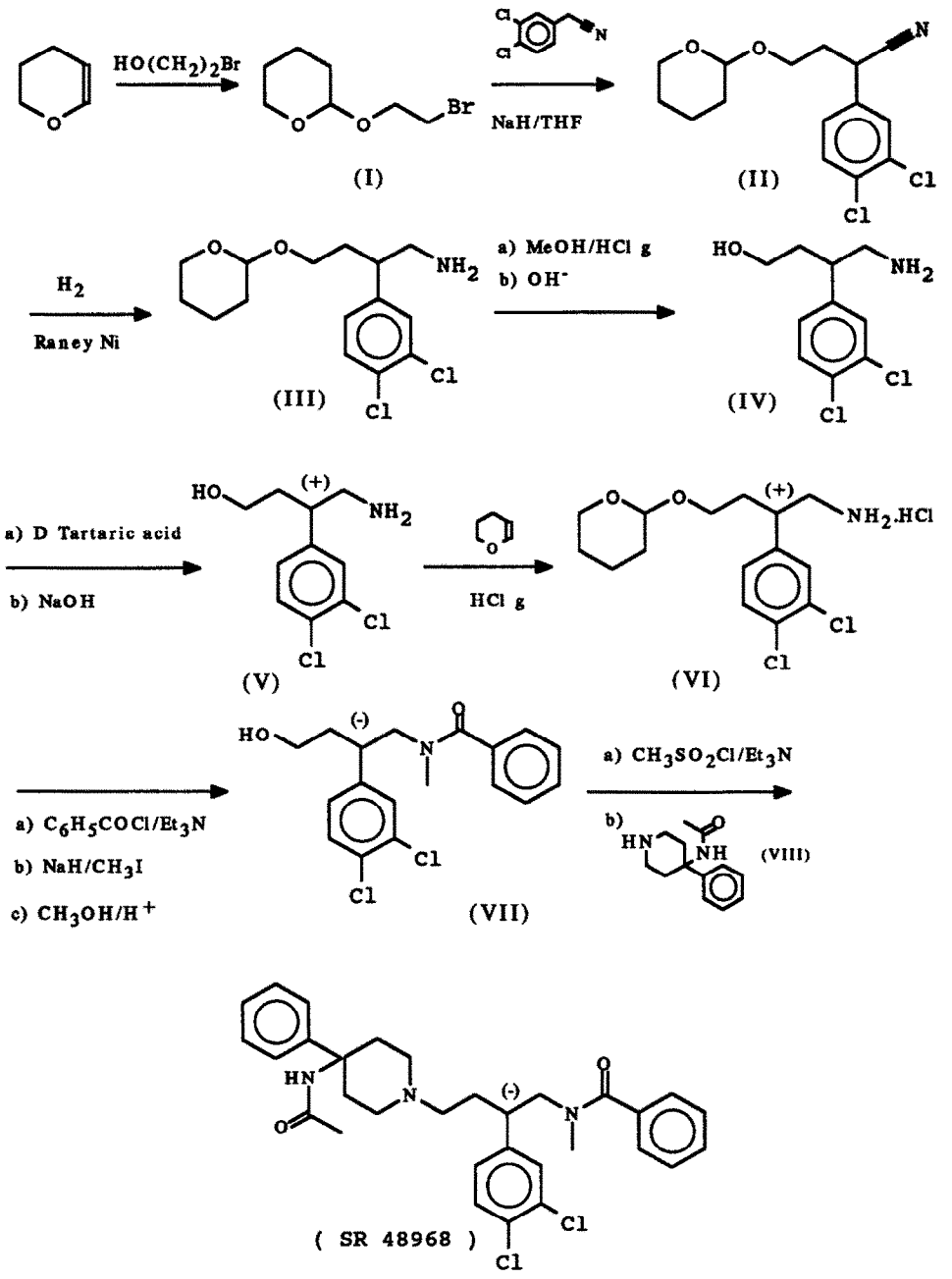
Bronchonchoconstriction was evaluated as previously described[9]. SR 48968 was administered per os at the dose of 1 mg/kg. [Nle^{10}]-NKA(4-10) was administered at the dose of 5 μg/kg i.v. on various times after administration of SR 48968. Results are means ± SEM (n=6).

Spasmogen	Control	Time after SR 48968 administration : 15 min.	Time after SR 48968 administration : 60 min.
Histamine	3.7 ± 0.4	3.2 ± 0.4	3.9 ± 0.4
Serotonine	5.1 ±0.5	4.5 ± 0.6	4.7 ± 0.5
Acetylcholine	3.8 ± 0.3	2.8 ± 0.1	3.2 ± 0.2
[Sar^9,Met(O$_2$)^{11}]-SP	4.7 ± 0.4	4.4 ± 0.4	4.2 ± 0.3

Table 1

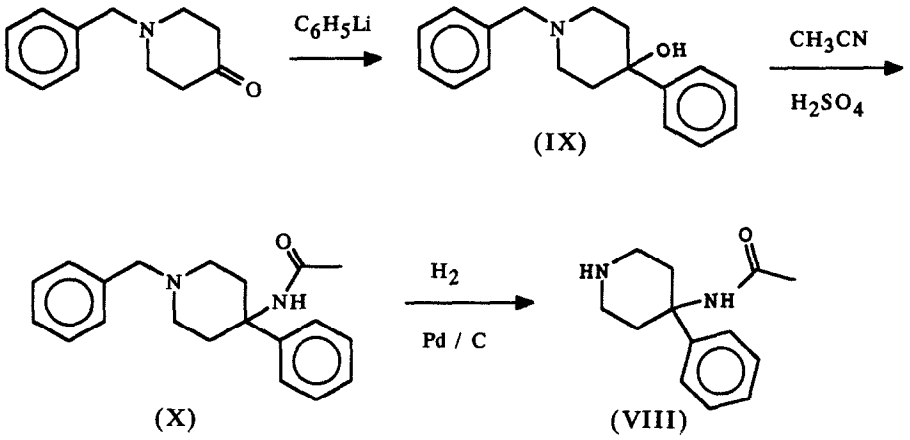
Effect of SR 48968 on bronchocontriction induced by various spasmogens in the guinea pig.

Bronchoconstriction was evaluated as previously described[9]. SR 48968 was administered by i.v. route at the dose of 100 μg/kg before the different spasmogens. Histamine, serotonine, acetylcholine and [Sar^9,Met(O$_2$)^{11}]-SP were administered by i.v. route at 10, 10, 60, 5 μg/kg, respectively. Results are expressed as volume of air in excess (ml) and are means ± SEM (n=5).



Scheme 1.

Synthesis of SR 48968



Scheme 2.

Synthesis of 4-acetylamino-4-phenyl-piperidine

SR 48968 and its analogues were prepared by the route described in scheme 1[14]. The key intermediate IV was obtained via alkylation of 3,4-dichlorophenylacetonitrile and catalytic hydrogenation over Raney Ni. Resolution of the compound IV was carried out by 2 crystallizations in methanol of its D-tartaric salt [e,e was determined by chiral HPLC using Crownpak CR(+) and was > 99.5 %; $[\alpha]_D$ = + 9.2 (c=1,methanol, 20° C)]. Compound VII {$[\alpha]_D$ = - 19.9 (c=1, methanol, 20° C)} was isolated by crystallization after protection of the alcohol function of compound V, acylation of the amino group of compound VI with benzoyl chloride, N-methylation of the amide function and then, deprotection in acidic medium. SR 48968 {$[\alpha]_D$ = -30 (c=1, methanol, 20° C)} was then obtained by substitution of the mesylate of compound VII with the substituted piperidine VIII. The substituted piperidine VIII was prepared by the route described in Scheme 2. Compound X [m.p. = 181° - 182° C] was obtained from compound IX via the Ritter reaction and crystallization. Catalytic hydrogenation over Pd/C led to compound VIII [m.p. of its hydrochloride = 286.5° -288° C].

Structure activity relationship is shown in table 2. Data comparison of compounds 1 (SR 48968), 3 to 8 clearly shows that X substituent greatly influenced compound affinity (Ki) for NK_2 receptor. At high affinity, X substituent also influenced in vivo activity (comparison of compounds 1, 3, 5 and 6). Moreover, R substituent had a dramatic effect on compound affinity for NK_2 receptor (comparison of compounds 1 [R = methyl] and 9 [R = H]). Compared to Ar = phenyl, other Ar substituents did not greatly modified (compounds 10, 11) or reduced (compounds 12, 13) in vivo activity.

In conclusion, all the biochemical and pharmacological results have clearly shown the potency and selectivity of SR 48968 as NK_2 receptor antagonist. SR 48968 can be used as a powerful tool to study the physiological and physiopathological role of NKA.

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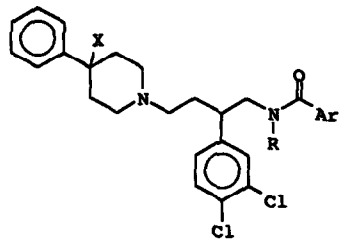

Compound	Substituents	K_i (nM)	pA_2	% inhibition of bronchoconstriction 0.2 mg/kg i.v.	5 mg/kg i.d.
1 (S-configuration) (SR 48968)	X = NHAc R = methyl Ar = phenyl	0.5	10.48	100	100
2 (R-configuration)	X= NHAc R = methyl Ar = phenyl	945	ND	ND	ND
3 (Racemate)	X = OH R = methyl Ar = phenyl	1.0	9.74	93	ND
4 (Racemate)	X = CH_2OH R = methyl Ar = phenyl	50	ND	ND	ND
5 (Racemate)	X = OEt R = methyl Ar = phenyl	0.9	9.77	40	ND
6 (Racemate)	X = OAc R = methyl Ar = phenyl	0.3	ND	98	36
7 (Racemate)	X = CH_2NHAc R = methyl Ar = phenyl	23	ND	ND	ND
8 (Racemate)	X= H R = methyl Ar = phenyl	>100	ND	ND	ND
9 (Racemate)	X = NHAc R = H Ar = phenyl	>100	ND	ND	ND
10 (Racemate)	X = OH R = methyl Ar = 2-thienyl	1.4	10.17	93	73

11 (Racemate)	X = OAc R = ethyl Ar = 2-thienyl	0.3	9.35	97	34
12 (Racemate)	X = OH R = methyl Ar = 3-thienyl	1.0	9.64	77	ND
13 (Racemate)	X = OH R = methyl Ar = α-naphtyl	1.5	8.63	0	ND

Table 2

Structure activity relationship of SR 48968 and its analogues

All the experimental procedures are previously described in reference 9. K_i is the inhibition constant of [^{125}I]-NKA binding to its receptor from rat duodenum membranes. pA_2 are related to inhibition of [Nle^{10}]-NKA(4-10)-induced contraction of the rabbit pulmonary artery. Bronchoconstriction in the guinea pig was induced by 5 μg/kg i.v. [Nle^{10}]-NKA(4-10), 30 min. after drug administration by intravenous (i.v.) or intraduodenal (i.d.) route. ND : not determined.

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