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Syntheses and biological activities of chiral piperidines-tachykinin NK3 antagonists

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KEY WORDS piperidines; tachykinins; neurokinin K; stereoisomerism; radioligand assay; intestinal absorption; structure-activity relationship

ABSTRACT

AIM: To develop nonpeptide tachykinin NK3 antagonists. **METHODS**: Five tachykinin NK3 antagonists were synthesized. Receptor binding assay and oral absorption study were made. **RESULTS**: The 4,4-disubstituted piperidine compounds (1b, 1c, and 1d) showed stronger activities (IC_{50} = 5.9, 6.2, and 11 nmol·L^{-1}, respectively) than the monosubstituted ring compound 1e (IC_{50} = 17 nmol·L^{-1}). 4-Phenyl (1b) and 4-phenylsulfonylmethyl (1c) compounds were more active than the 4-fluorobenzyl compound (1d). All antagonists were found to be orally absorbable, the $T_{1/2}$ of 1b (6.4 h) was more than three-fold longer than that of 1a (1.9 h). **CONCLUSION**: Compound 1b had the best binding activity (IC_{50} = 5.9 nmol·L^{-1}) and the best AUC (2081 μg·h·L^{-1}).

INTRODUCTION

Over the last decade, major advances have been made in understanding the biology of mammalian tachykinin neuropeptides. It is now well established that substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), all of which share a common C-terminal sequence Phe-X-Gly-Leu-Met-Phe-X-Gly-Leu-Met-NH_2[1], are widely distributed in the peripheral and central nervous system (CNS), where they appear to interact with at least three receptor types referred to as NK1, NK2, and NK3 sites. Substance P has the highest affinity for the NK1, whereas NKA and NKB bind preferentially to NK2 and NK3, respectively[2]. Although several tachykinin receptor antagonists have been described[3], most have been developed through modification and/or deletion of one or more of the amino acids that comprise the endogenous mammalian tachykinins such that the resulting molecules are still peptides that possess poor pharmacokinetic properties and limited *in vivo* activity. Over the past several years, a number of high-affinity nonpeptide tachykinin antagonists have been reported. Snider[4] and Garret[5] described CP 96345 and RP 67580 respectively, as NK1 receptor ligands, while Advenier[6] presented data on SR 48968 showing its high affinity and selectivity for NK2 receptors. Recently, the pharmacology of the first potent and selective nonpeptide NK3 receptor antagonist, SR 142801, was disclosed by X Emonds-Alt and coworkers[7], and several synthetic approaches were later reported[8]. More recently, another potent NK3 antagonist SB 223412 was revealed by Smith-Kline[9]. SR 142801 (1a) is the most potent nonpeptide NK3 antagonist which has ever been reported in the literature. Our laboratory has been interested in developing nonpeptide NK3 antagonists, and independently developed a practical synthetic

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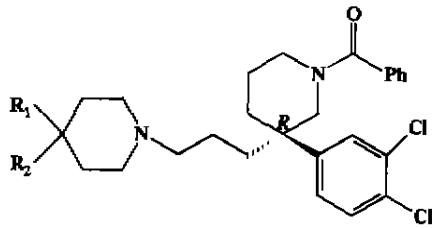
method for the scale-up of SR 142801 as a reference agent[8]. Given the availability of an efficient synthetic route, we thought it reasonable to utilize this route to synthesize a small group of related, but potentially novel analogs, in which the piperidine moiety was replaced. In this paper, we report the synthesis and preliminary biological results of several NK3 antagonists *R*-*d*-1.



	R_1	R_2
1a	Ac(Me)N –	Ph(SR 142801)
1b	Ph	OH
1c	$PhSO_2CH_2$ –	OH
1d	*p* – F – Bn –	OH
1e	Ph	H

Chemistry The retro synthetic analysis of *R*-*d*-1 is shown in Scheme 1. The disconnection of the C-N bond of the side chain of 1 gives iodide 2 and substituted piperidines 3 as precursors (Scheme 1).

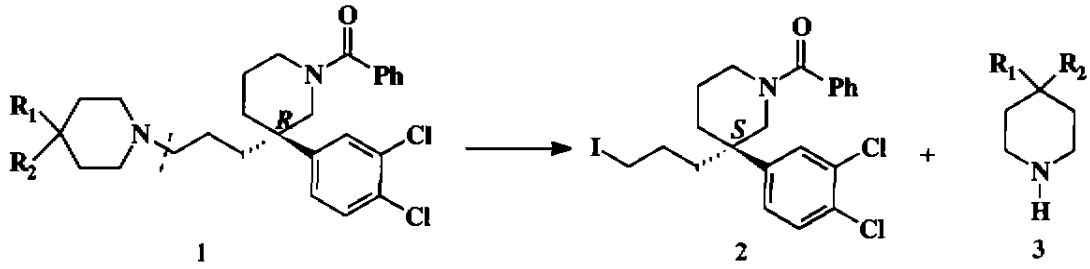
The synthesis and determination of absolute configuration of precursor 2 were published in our earlier paper[8]. The synthesis of precursor 3c is shown in Scheme 2. The reaction of 4-piperidone hydrochloride hydrate with Boc anhydride and DIPEA in methanol gave *N*-Boc protected piperidone 4 in 93 % yield. Deprotonation of methylphenylsulfone by *n*-BuLi at – 40 ℃ in THF, and reaction with *N*-Boc-piperidone 4 gave 92 % yield of the corresponding sulfone alcohol 5. Standard deprotection of the t-butoxycarbonyl group with 50 % TFA in dichloromethane yielded the 4, 4-disubstituted piperidine 3c (92 %) (Scheme 2).

The syntheses of 3b, 3d and 3e are illustrated in Scheme 3. Reaction of *p*-fluorobenzylbromide with Mg chips in THF at 0 ℃ gave the Grignard reagent 6, which reacted with *N*-benzyl-4-piperidone at – 40 ℃ to ambient temperature to yield the hydroxypiperidine 7 (82 % yield). Debenzylation of 7 was accomplished by standard catalytic hydrogenolysis in ethanol to obtain an almost quantitative yield of precursor 3d. The reaction of commercially available phenyl lithium (1 $mol \cdot L^{-1}$ THF solution) with *N*-benzyl-4-piperidone at – 78 ℃ gave tertiary alcohol 8 (75 % yield). Intermediate 3b was obtained by removing the *N*-benzyl group of 8 by catalytic hydrogenolysis (95 % yield). Further removal of the hydroxy group of 3b by hydrogenolysis using 20 % Pd/C in the presence of concentrated HCl yielded intermediate 3e (82 % yield). The synthesis of precursor 3f was described in the previous paper[8] (Scheme 3).

The coupling of iodide 2 with 4,4-disubstituted piperidine 3 using $KHCO_3$ as base in MeCN, at 60-65 ℃ for 18 h, followed by the treatment of ether-HCl, gave high yields of *R*-*d*-1 as HCl salt. The reaction of 3f with 2 in the same manner gave compound 1f in 80 % yield (Scheme 4).

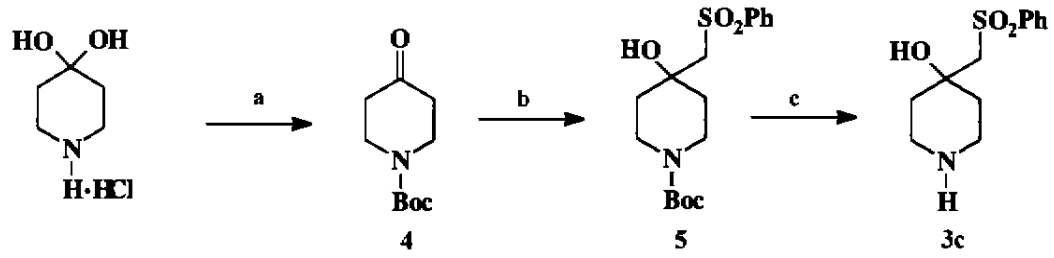
Receptor Binding Assay Agonists and/or antagonists were incubated with 5 g of membrane protein in binding buffer containing 100 – 300 $pmol \cdot L^{-1}$ [^{125}I]-[MePhe7]-neurokinin B, in 96-well filter plates (Millipore). Phosphoramidon (25 $\mu mol \cdot L^{-1}$) was included in the radioligand buffer to prevent peptide degradation. The latter components were incubated for 1 h at room temperature, and the binding was terminated by filtration on a vacuum manifold followed by two washes with ice-cold binding buffer. Filter plates were blotted on Whatmann filter paper to remove excess liquid and air dried. The dried

Scheme 1



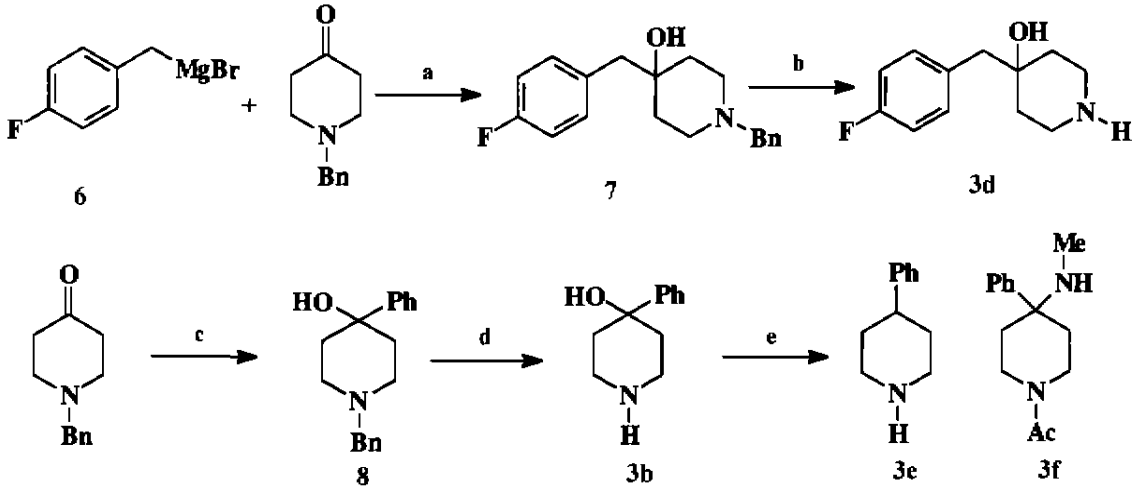
1b, 3b R_1 = Ph, R_2 = OH; 1d, 3d R_1 = *p*-F-Bn-, R_2 = OH; 1c, 3c R_1 = $PhSO_2CH_2$-, R_2 = OH; 1e, 3e R_1 = Ph, R_2 = H

Scheme 2



(a) Boc_2O, *i*-Pr_2NEt, MeOH, 93 %; (B) $PhSO_2CH_3$, nBuLi, −40 ℃, 92 %; (c) 50 % TFA in CH_2Cl_2, 92 %

Scheme 3



(a) THF, −40 ℃ to rt, 82 %; (b) H_2, Pd/C, EtOH, 98 %; (c) PhLi, THF, −78 ℃, 75 %; (d) H_2, Pd/C, 95 %; (e) H_2, Pd/C, concentrated HCl, 82 %.

filter plates were coated with scintillant by submerging the bottoms of filter plates briefly in a melted meltilex scintillator sheet (Wallac), and the scintillant-coated filter plates were placed in a Wallac 1450 microbeta scintillation counter for counting radioactivity. K_d and B_{max} values of

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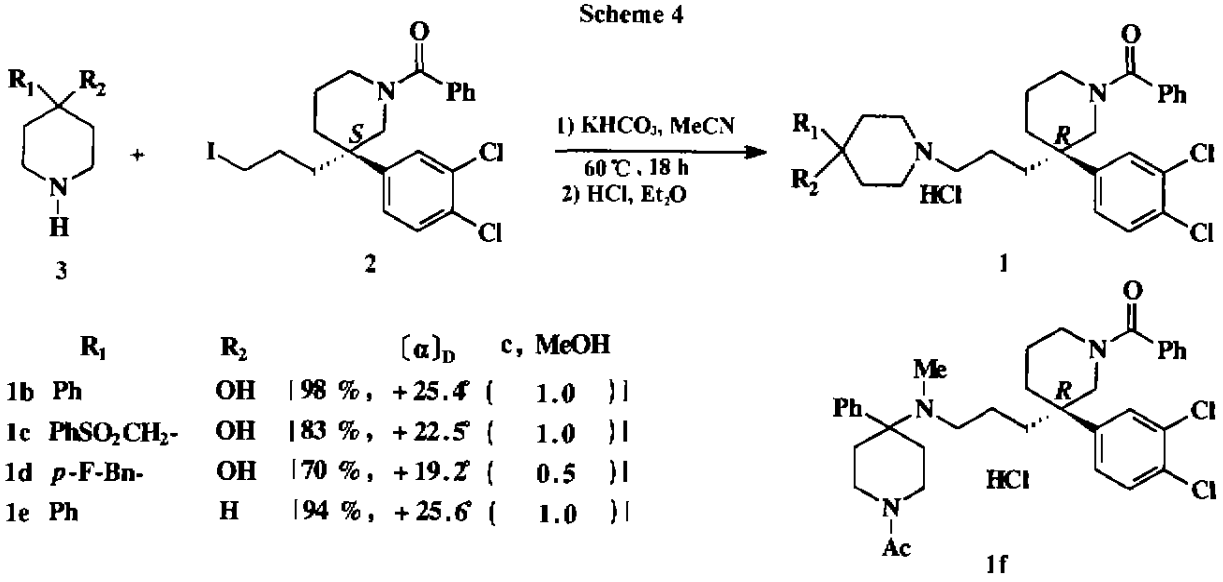
Scheme 4



	R_1	R_2	$[\alpha]_D$	c, MeOH
1b	Ph	OH	98 %, +25.4°	1.0
1c	$PhSO_2CH_2$-	OH	83 %, +22.5°	1.0
1d	*p*-F-Bn-	OH	70 %, +19.2°	0.5
1e	Ph	H	94 %, +25.6°	1.0

[MePhe7]-neurokinin B in different NK3 receptors were estimated from competition binding experiments using the equations, $K_d = IC_{50} - L$ and $B_{max} = B_0 \times IC_{50}/L$ (L = concentration of free radioligand, B_0 = specific bound radioligand)[10]. K_i values were determined using the equation $K_i = IC_{50}/(1 + L/K_d)$[11].

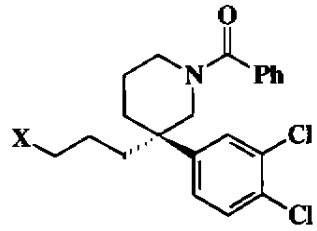
Oral absorption study Oral absorption of the antagonists were determined in fasted male Wistar rats. Compounds were administered ig at a dose of 20 mg·kg^{-1} using GelucireR (44/14) as dosing vehicle. Several blood samples were collected over 24 h via cannulated jugular vein. Plasma concentrations of these compounds were simultaneously determined by HPLC using a sample pooling technique. The pooled plasma samples at each time point were extracted with *n*-butyl chloride/chloroform (1/1, vol/vol). The reconstituted samples were chromatographed, using a gradient elution of 100 % mobile phase A to 100 % B over 40 min. Oral plasma concentration-time curves were calculated by the trapizoidal rule, and the elimination rate constants for each compound were determined by nonlinear regression.

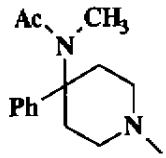
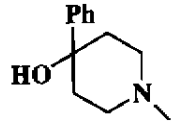
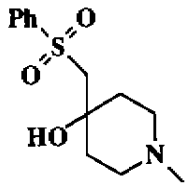
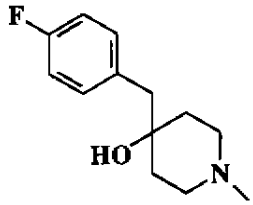
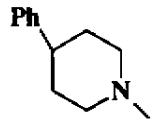
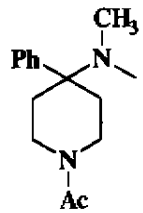
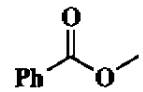
RESULTS AND DISCUSSION

There are numerous sites in the structure of 1a (SR 142801) that can potentially be modified. Our first modification was on the achiral piperidine moiety of compound 1a. The results are listed in Tab 1. Replacement of the 4-substituted piperidine ring in 1a with a benzoyloxy group (1g) or disubstituted nitrogen (1f) caused complete loss of activity (compounds 1f, 1g, $IC_{50} > 1000$, *vs* 1b, 1c, 1d, and 1e, $IC_{50} = 5.9 \pm 0.7$, 6.2 ± 0.6, 11 ± 2, 17 ± 3 nmol·L^{-1} respectively). The 4,4-disubstituted piperidine compounds (1b, 1c, 1d) showed better activity than the monosubstituted ring (1e) (1b, 1c, 1d, $IC_{50} = 5.9 \pm 0.7$, 6.2 ± 0.6, 11 ± 2 *vs* 1e, $IC_{50} = 17 \pm 3$ nmol·L^{-1}). 4-Phenyl (1b) and 4-phenylsulfonylmethyl (1c) compounds are around two fold more active than the 4-fluorobenzyl (1d) compounds (1b, 1c, $IC_{50} = 5.9 \pm 0.7$, 6.2 ± 0.6 *vs* 1d, $IC_{50} = 11 \pm 2$ nmol·L^{-1}). By comparing compounds 1b and 1e, it is evident that the hydroxy group enhances the activity nearly 3-fold (1b, $IC_{50} = 5.9 \pm 0.7$ *vs* 1e, $IC_{50} = 17 \pm 3$ nmol·L^{-1}) (Tab 1).

Following oral dosing of rats, all antagonists were found to be orally absorbable, with plasma

Tab 1. Activities and pharmacokinetics of 7 compounds.



Compounds	X	IC_{50} /nmol·L^{-1}	C_{max} /μg·L^{-1}	T_{max} /h	$T_{1/2}$ / h	AUC / μg·h·L^{-1}
1a*		0.8	183	2.0	1.9	1080
1b		5.9	254	2.0	6.4	2081
1c		6.2	167	2.0	3.5	1440
1d		11	N/A	N/A	N/A	N/A
1e		17	179	2.0	5.2	1770
1f		>1000	N/A	N/A	N/A	N/A
1g		>1000	N/A	N/A	N/A	N/A

*1a: Sanofi SR 142801

elimination half lives ($T_{1/2}$) of 6.4, 5.2, 3.5, and 1.9 h for 1b, 1e, 1c, and 1a, respectively. The $T_{1/2}$ values were consistent with the area under the plasma concentration-time curves (AUC). The $T_{1/2}$ of 1b (6.4 h) is more than three-fold longer than that of 1a. These data

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suggest that the clearance of 1b may be the slowest, thus possibly the best bioavailable compound among all the antagonists tested (Tab 1).

CONCLUSION

The chemical modification of 1a (Sanofi SR 142801) may have resulted in a possibly more bioavailable compound 1b as shown in the preliminary rat pharmacokinetic data, although 1b has a higher IC_{50} value than 1a. The $T_{1/2}$ of 1c, and 1e are also longer than that of 1a (3.5, 5.2, and 1.9). Based on these biological results, further pharmacological studies are in progress on compound 1b in our labs.

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速激肽 NK3 拮抗剂-手性哌啶类化合物的合成与生物活性 R9145

陈懔谷, 鲍富荣, Bruce D ROTH, 郭必勝, James ATHERTON, 余承慧 R962

关键词 哌啶类; 速激肽; 神经激肽 K; 立体异构; 放射配位体测定; 肠吸收; 结构-活性关系

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