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SR 46559 A AND RELATED AMINOPYRIDAZINES ARE POTENT MUSCARINIC AGONISTS WITH NO CHOLINERGIC SYNDROME

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Abstract. The development of an aminopyridazine lead structure (minaprine) yielded compound SR 46559 A 3-[N-(2-diethyl-amino-2-methylpropyl)-6-phenyl-5-propyl] pyridazinamine sesquifumarate, a novel and potent muscarinic agonist with no cholinergic syndrome.

Our interest in muscarinic M1 agonists has its origin in the biochemical and pharmacological study of minaprine (compound **1**, Table 1),[1] a 3-amino-6-aryl-pyridazine with an atypical antidepressant profile, and prepared by us some years ago.[2-4] Surprisingly, in contrast to classical tricyclic antidepressants, minaprine was devoid of anticholinergic effects and even had cholinomimetic properties. In this respect, minaprine and its orthohydroxy metabolite SR 95070B[1] selectively bound to cerebral muscarinic M1 receptors, dose-dependently antagonized pirenzepine-induced turning in mice and showed memory-enhancing properties.[1] Interestingly, at pharmacologically active doses, minaprine did not induce cholinergic symptoms (salivation, lacrymation, tremors and hypothermia), most of this symptomatology being reported to be due to activation of muscarinic M2 receptors.[5] Along with the hypothesis that selective muscarinic M1 agonists would be useful in the symptomatic treatment of senile dementia of Alzheimer type,[6] these results prompted us to design aminopyridazine analogues that are non-toxic and potent agonists at M1 sites.

Structure-activity relationship studies

Our main initial *in vitro* test was displacement experiments using [^{3}H] pirenzepine, a selective antagonist at cerebral muscarinic M1 receptors. The classical structure-activity relationship (SAR) studies carried out after the synthesis and testing of approximately 250 compounds can be summarized as follows.[7-10]

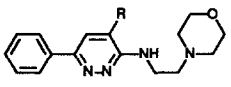
Influence of the substituents at the 4- and 5-position. The replacement of the minaprine 4-methyl group by ethyl or phenyl groups is favorable (affinities 5 to 10 times higher than minaprine) whereas the introduction of electro-attracting groups (CN, CHO, COOH) yields compounds showing weaker affinities (Table 1). However, a size limitation seems to exist, since 4-benzyl and ß-napthyl-methylene derivatives showed lesser affinities.

Moving of the the 4-methyl group of minaprine to the 5-position (minaprine **1** **11**, see Table 2) produced a 30-fold increase in affinity. More generally, any aliphatic or aromatic substitution at position 5 appeared to be beneficial, compared to the 5-unsubstituted analog.

Among these compounds the semi-rigid minaprine analog SR 95639A **12** (Table 2) was selected for a detailed study.[11] It was shown to possess a similar activity profile, both *in vitro* and *in vivo*, to minaprine or its ortho-hydroxy metabolite SR 95070B and to behave as a partial muscarinic agonist.[12] Compound **12** (SR 95639A) was discarded because of a weak activity *in vivo* and a short duration of action. In the search of compounds with duration of action in pharmacological tests longer than **12**, it was decided to focus our studies on 5-alkyl pyridazines, special attention being devoted to modifications of the 3-aminoalkylamino chain.

3-Aminoalkylamino chain modifications. Lipophilic amines showed more affinity than hydrophilic oxygen-bearing amines such as morpholine or 4-hydroxy-piperidine (Tables 3 and 5). A cationic head was essential for binding, as shown by the lack of affinity of the morpholone **17**. The ideal length for the side-chain corresponded to a two carbon chain between the exocyclic amidine nitrogen and the cationic head, lengthening to a propylene chain induced generally a 5-10 times fall in affinity (Table 4). One of the most favorable side-chains, the 2-N-ethylpyrrolidinylmethyl group, the side-chain of sulpiride, which contains a chiral center, was present in compound **26** (Table 5). As both enantiomers **27** and **28** showed only a slight difference in M1 affinity, it was decided to cancel the chiral center by introducing a symmetrical open chain, as found in the structure of **29**. Starting from the latter compound, replacing the 5-methyl group in its structure, by a large alkyl group (c-Pr, n-Pr) or a phenyl ring afforded highly potent ligands (compounds **30, 31** and **32** in Table 6).

Table 1 : Influence of the substituent at the 4-position



COMPOUND	R	IC_{50} (μM) M_1 [^{3}H]-PZ
1 (Minaprine)	CH_3	17
2	H	12
3	C_2H_5	1.6
4	PHENYL	0.6
5	BENZYL	4.6
6	CH_2-β-NAPHTYL	16
7	CHO	>100
8	CO_2H	>100
9	CN	60

a : PZ : pirenzepine

Table 2 : Influence of the methyl group in the positions 4 and 5

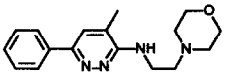
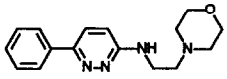
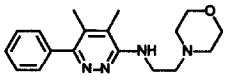
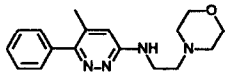
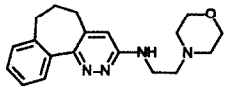
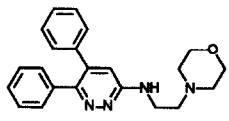
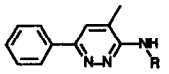
COMPOUND		IC_{50} (μM) M_1 [^{3}H]-PZ
1		17
2		12
10		0.70
11		0.55
12		0.37
13		0.17

Table 3 : Side chain modifications



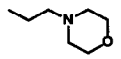
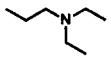
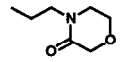
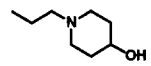
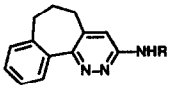
COMPOUND	R	IC_{50}(μM) M_1[^{3}H]-PZ
1 (Minaprine)		17
14		0.23
15		0.24
16		0.80
17		>100
18		>100

Table 4 : Influence of the side chain length



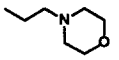
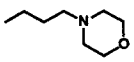
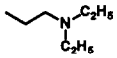
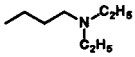
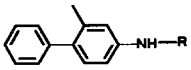
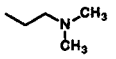
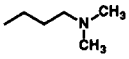
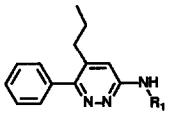
COMPOUND	R	IC_{50}(μM) M_1[^{3}H]-PZ
		0.37
19		2.0
20		0.05
21		0.24
		
22		0.14
23		0.50

Table 5 : Influence of the aminoalkylamino chain in position 3



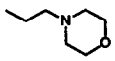
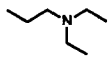
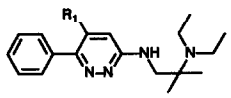
COMPOUND	R_1	IC_{50}(μM) M_1[^{3}H]-PZ
24		1.6
25		0.4
26		0.03
27		0.06
28		0.01

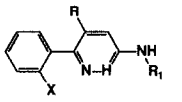
Table 6 : Influence of the substituent in position 5



COMPOUND	R_1	IC_{50}(μM) M_1[^{3}H]-PZ
29	CH_3	2.3
30		0.4
31	$n\text{-}C_3H_7$	0.11
32		0.04

Further modifications at the 6- and 5-positions. Introducing an hydroxyl group in the ortho position of the 6-phenyl ring generally enhanced the affinity for muscarinic M1 receptors (Table 7). The 6-phenyl ring of minaprine can be replaced by other aromatic rings or even by aliphatic rings without significant changes in affinity. Thus the IC_{50} values for [^{3}H]-PZ binding are 4, 4, 7.5 and 6 μM for the α-thienyl, ß-thienyl, α-naphtyl and cyclohexyl analogues respectively. These observations combined with the good potency of the 5,6-diphenyl derivatives **13** and **32** prompted us to synthesize the 5-phenyl-6-alkyl analogues listed in Table 8. All these compounds showed about the same affinity than SR 46559 A, with IC_{50} values in the range of 0.02-0.1 μM.

Table 7 : The ortho-hydroxy effect



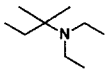
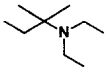
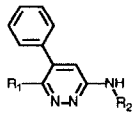
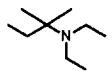
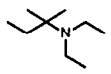
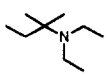
R1	X = H COMPOUND	R	M1	X = OH COMPOUND	R	M1
	33	CH_3	0.26	34	CH_3	0.008
	29	CH_3	2.3	35	CH_3	0.24
	31	$n\text{-}C_3H_7$	0.11	36	$n\text{-}C_3H_7$	0.13

Table 8 : 6-alkyl-5-phenyl pyridazine series



COMPOUND	R_1	R_2	IC_{50}(μM) M_1 [^{3}H]-PZ
37	CH_3		0.02
38	CH_3		0.18
39	$n\text{-}C_3H_7$		0.05
40	$i\text{-}C_4H_9$		0.02

Pharmacological studies

Table 9 summarizes data obtained with four selected compounds having IC_{50} values, equal to or lower than 0.1 μM, for rat cerebral muscarinic receptors labeled with [^{3}H] pirenzepine. In mice, none of these compounds induced, up to sub-lethal doses, cholinergic symptoms indicating no efficacy for muscarinic M2 receptors (affinities not shown). Using antagonism of pirenzepine-induced turning in mice as a screening test,[13] **37** appeared inactive in our experimental conditions, i.e. when injected 4 h before testing. Although **32** and **40** strongly reduced at that time the number of turns induced in mice by intrastriatal injection of pirenzepine, they were rejected because oral toxicity (LD_{50} around 250 mg/kg) and high inter-species variability using tests for *in vitro* hepatic metabolism. Compound **31** (SR 46559 A) which exhibited no toxicity up to 300 mg/kg (p.o.) was finally retained.[14] At this dose, compound **31** did not produce any of the classical cholinomimetic effects, i.e. salivation, diarrhea, lacrymation and tremors. This compound was a competitive ligand at muscarinic M1 sites (Ki = 112 nM), its affinity for muscarinic M2 (cardiac) receptors being 6 times lower. Compound **31** did not interact with brain nicotinic receptors, high affinity choline uptake sites and acetylcholinesterase activity. Up to 1 mM, **31** slightly (15-20 %) and inconsistently stimulated inositol phospholipids breakdown and dit not inhibit the forskolin-induced activation of adenylyl cyclase activity. However, compound **31** potently stimulated diacylglycerol formation (IC_{50} # 50 μM), an effect antagonized by atropine. Furthermore, like muscarinic agonists, **31** inhibited (IC_{50} # 10 μM) the K^+-evoked release of [^{3}H]GABA from rat striatal slices and reduced at 0.5 and 1 μM, the population spike amplitude of the CA1 pyramidal cells induced by stimulation of the Schaffer's collateral commissural pathway in rat hippocampal slices, both effects being antagonized. Like muscarinic agonists, **31** (0.1 mg/kg p.o.) potentiated haloperidol-induced catalepsy in rats and antagonized (ED_{50} = 0.12 mg/kg p.o.) rotations induced in mice by intrastriatal injection of pirenzepine. Compound **31** antagonized the scopolamine- or pirenzepine-induced deficit in passive avoidance learning (ED_{50}'s= 0.25 and 0.027 mg/kg p.o., respectively. Moreover, using the social memory test, **31** (0.1 - 3 mg/kg p.o.) enhanced short-term retention in adult rats and improved memory deficits observed in aged mice and in rats subjected to cerebral ischemic insult. Compound **31** (1 - 3 mg/kg p.o.) also reversed the ischemia-induced alterations of rat's exploratory behaviour.

Table 9: ***In vivo*** activity

Compounds	Affinity for M1 receptors (IC50 μM)	Antagonism of pirenzepine-induced turning in mice (a) (mg/kg)	
		0.3	3
31	0.11	51 %**	78 %**
32	0.04	87 %**	113 %
37	0.02	Inactive	
38	0.02	65 %	85 %**

(a) Data are expressed as percentage inhibition of the number of pirenzepine-induced rotations, compounds being orally administered (0.3 and 3 mg/kg) 4 h before test.
**p < 0.01, Student'st-test.

Together, these results suggest that SR 46559 A behaves as a partial M1 muscarinic agonist with marked ability to improve experimentally induced cognitive deficits in rodents without producing cholinergic symptomatology. Thus **31** could be a potential useful drug for the symptomatic treatment of senile dementia of Alzheimer's type.

References and Notes

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