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Muscarinic Agonist SAR of Azaspirodioxolanes

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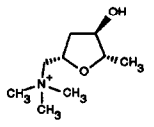
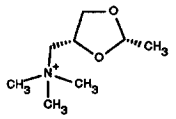
Abstract: The *in vitro* muscarinic activity and a model for binding to the m1 receptor are presented for a series of azaspirodioxolanes.

Central activity or blood brain barrier penetration is a prerequisite for any muscarinic agonist which is to have therapeutic potential for Alzheimer's Disease (AD). The potent agonists muscarine and cis-dioxolane (CD) are quaternary ammonium compounds which do not penetrate into the CNS. Furthermore the corresponding *nor*- tertiary amine analogs of these agonists are only weakly active.[1] As part of a program to test muscarinic agonists in AD, we set out to prepare spirocyclic nonquaternary dioxolane analogs and determine if potent CNS active compounds of this substance class were in fact accessible.



Muscarine



cis-dioxolane

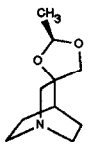
Synthesis

Methods for synthesizing azaspirodioxolanes and their sulfur analogs have been published.[2,3] There are three basic methods starting from a ketone. Method 1 involves preparation of a cyanohydrin then elaboration to a diol and finally acetal formation. Method 2 proceeds via one carbon homologation of the ketone to an oxirane or thiirane which is directly converted to an acetal by treatment with BF_3-Et_2O and a carbonyl component. Finally, with method 3, the oxirane or thiirane may be ring opened with acetate or thioacetate ion. In this case acetate hydrolysis is followed by standard acetalization with a carbonyl component.

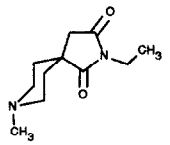
SAR

At the outset of our studies there was already some evidence supporting the possibility of preparing potent nonquaternary analogs of CD. The quinuclidine dioxolane analog AF-30 was a known agonist of moderate potency.[4] Furthermore, the spirocyclic piperidine succinimide RS86[5], which had been clinically investigated, was a centrally active muscarinic agonist only tenfold less potent than muscarine. On this basis the first compound prepared was the N-methylpiperidine spirodioxolane 1. This compound proved to be equiactive to RS86, exhibiting high agonist potency with pD_2 values of 6.0 and 6.7 in the ileum and ganglion models and 100nM cortex affinity in displacing [^{3}H]-CD. A very strict SAR relationship was observed for the series of subsequently prepared spirodioxolane analogs in which the activity of **1** was not surpassed. The *in vitro* pharmacological data for the spirodioxolane analogs **1-27** are summarized in Table 1. Compounds **1-15** are spiropiperidines and compounds **17-20** are spiropyrrolidines. A detailed description of

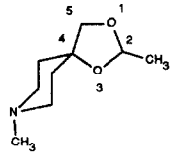
the pharmacological tests employed may be found elsewhere.[6] In brief, the ileum model serves as a functional



AF30



RS86



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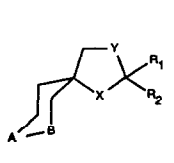
measure of peripheral muscarinic activity while the ganglion model is a functional model of central muscarinic activity. [^{3}H]-CD-binding provides a measure of agonist affinity[7] and [^{3}H]-pirenzepine binding provides a measure of antagonist affinity.

Dioxolane ring variation

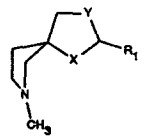
The C2 position of the dioxolane ring plays a key role in activity appearing to mimic the acyl carbon of Ach. A methyl group at C2 is optimal and necessary for the agonist activity of **1**. The unsubstituted compound **2** retains only marginal activity. Bulkier substituents at C2 cause a drop in agonist activity and, from a certain size, a shift towards antagonism. The ethyl substituted analog **3** experiences a tenfold drop in potency relative to **1**.[8] Compound **4** having a methoxy group at C2 is equiactive to **3** while the acetylene analog **5** is slightly more active than **3** particularly regarding ganglion potency and efficacy. Compound **8** having dimethylsubstitution at C2 is inactive. A single phenyl substitution at C2 yields the weak antagonist **7** whereas diphenylsubstitution yields the potent antagonist **9**. While the 2S absolute stereochemistry is optimal,[9] **1**-(R), the (+)-2R enantiomer of **1**, is only 5 fold less potent than **1**-(S), the (-)-2S antipode.[10] Oxathiolane **12** in which the O1 acetal oxygen of **1** is exchanged for sulfur is equiactive to **1** in the ileum model and displays lowered ganglion potency but increased ganglion efficacy. The CD binding affinity of **12** is lowered by a factor of 3. Oxathiolane **13** in which the O3 acetal oxygen is exchanged for sulfur is about 10 fold less active than **1** in all tests. Dithiolane **14** displays activity comparable to **13**. Exchange of the O3 dioxolane oxygen with sulfur for pyrrolidine **17**, AF-30 and CD[11] leads to little or no loss of activity.

Aza ring variation

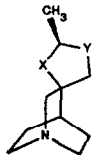
The geometrical disposition for the amino nitrogen of **1**, two methylene units removed from C4 of the dioxolane ring, is well defined with two alternative chair conformations of the piperidine ring being highly favored. Compound **15** where only one methylene unit separates the spatially well defined piperidine ring nitrogen is virtually inactive. When the amine function is incorporated in the conformationally distinct and more flexible pyrrolidine ring of **17**, activity is restored. As with CD[12] and AF-30[4] the *cis* configuration of the methyl group and aminomethylene element in **17** is preferred and *trans* compound **18** is less active. Compound **17** is roughly equiactive to AF-30 but tenfold less potent than **1** in functional tests. The azabicycloheptane-spirodioxolane **21** was prepared[13] as a 70:30 mixture of C2 methyl epimers where the O3 of the dioxolane ring system is *exo* to the azanorbornane ring. In functional tests this mixture is only slightly less potent than **1**. Compound **22**[14] where O3 is *endo* and the methyl group and aminomethylene unit are *cis* is less active than **21** and *endo-trans* isomer **23** is only weakly active. Tropane analogs **26,27**[15] and *cis*-2,6-dimethylpiperidine analogs **24,25**[16] which incorporate the structure of **1** but do not allow piperidine ring inversion were all inactive. Quaternization of **1** to the dimethylammonium analog **10** results in a ca. tenfold drop in potency but an increase in ganglion efficacy. The secondary amine analog **11** lacking the N-methyl



Piperidines



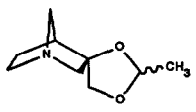
Pyrrolidines



AF30 analogs

TABLE 1

Compound#	X	Y	R1	R2	A	B	pD2 ileum	eff.%	pD2 ganglion	eff.%	CD nM	Pir nM
1-racemic	O	O	Me	H	NMe	CH2	6.0	100	6.7	80	100	5825
(+)-1-(R)	O	O	Me(R)	H	NMe	CH2	5.5	100	6 4	80	240	7690
(-)-1-(S)	O	O	Me(S)	H	NMe	CH2	6.3	100	7.0	80	85	5325
2	O	O	H	H	NMe	CH2	4.6	70	4 6	90	1200	>10,000
3	O	O	Et	H	NMe	CH2	5.0	70	5.5	85	745	1325
4	O	O	OMe	H	NMe	CH2	4.7	80	5.5	100	870	>10,000
5	O	O	C≡CH	H	NMe	CH2	5 2	100	5.9	120	295	3000
6	O	O	CCl3	H	NMe	CH2	4.1	60	<4 0		960	200
7	O	O	Ph	H	NMe	CH2	pA2 5.3		n.d.		2800	620
8	O	O	Me	Me	NMe	CH2	<4.0		<4.0		>10,000	>10,000
9	O	O	Ph	Ph	NMe	CH2	pA2 8.0		pA2 8.2		8.1	0.60
10	O	O	Me	H	NMe2	CH2	5.2	105	6.1	110	210	5000
11	O	O	Me	H	NH	CH2	5.1	100	5.9		145	>10,000
12	O	S	Me	H	NMe	CH2	6 0	100	6 1	100	185	2650
13	S	O	Me	H	NMe	CH2	5.1	100	5.6	70	720	3160
14	S	S	Me	H	NMe	CH2	4.9	100	5.0	100	530	2100
15	O	O	Me	H	CH2	NMe	<4.0		<4.0			
RS 86	CO	CO	Et	H	NMe	CH2	6.1	100	6 7	80	87	765
16	CO	CO	Me	H	NMe	CH2	5.5	100	6.2		175	4050
pyrrolidines												
17	O	O	Me cis				5.2	100	5 6	90	550	>10,000
18	O	O	Me trans				4.5	100	n d.		1750	>10,000
19	O	S	Me cis				5.2	100	5.7	100	585	6700
20	S	O	Me cis								9150	>10,000
AF-30	O	O	Me cis				4 8	100	6.0	60	90	1575
AF-102b	O	S	Me cis				4.9	50	6.1	80	105	430
AF-102a	O	S	Me trans				5.4	30	5.7	70	515	1250
21							5.9	100	6.5	75	30	1950
22							5 5	100	5.7	85	180	6400
23							4.1	100	4 4		1850	>10,000
muscarine							6 8	100	7.4	100	12.0	5680
CD							7.5	100	7.8	100	6.4	2100



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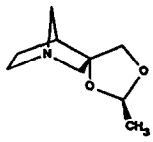

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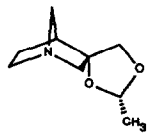

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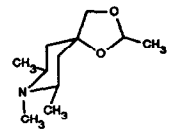

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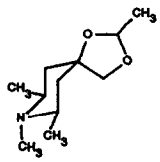

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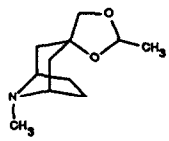

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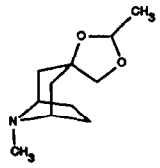

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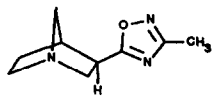


28

group suffers a roughly tenfold drop in potency with a somewhat less marked loss of CD-affinity.

Receptor Binding Model

Recently, we have proposed a hypothetical model for the binding of muscarinic agonists to the m1 receptor.[6] In this dynamic model the triad of residues Asp-105, Ser-109 and Ser-112 aligned on one side of the third putative transmembrane idealized alpha helix binds agonists via a salt bridge and one or two hydrogen bonds. For different agonists distinct receptor conformations were modeled demonstrating the dynamic nature of the receptor and the limitations intrinsic to a precise pharmacophore where receptor mobility is neglected. The extraordinary potency of the azabicycloheptane oxadiazole **28**[17] shown in Figure 1 (crossed stereoscopic view) was ascribed to an ideal receptor fit with a strong salt bridge and two equally strong hydrogen bonding interactions.[6] The most active (2'S,3R) isomer of the quinuclidine spirodioxolane AF-30 can be accommodated in the same dynamic model (Fig. 2). The protonated quinuclidine nitrogen forms an ideal linear salt bridge (2.7Å) to Asp-105, Ser-109 forms a good hydrogen bond to the O3 dioxolane (1.9Å) and Ser-112 forms a weaker hydrogen bond to O1 (2.3Å). The azabicycloheptane analogs **21** and **22** fit in an analogous manner. In the case of the *exo*-isomer **21**, the methylene bridge occupies the same region as the ethylene unit of AF-30 which is seen extending out of the plane (Fig. 2). One may postulate this orientation of the azabicyloheptane ring, which was also found for **28**, to be preferrable. Both chair conformations of spiropiperidine **1**-(S) gave equally good fits. To obtain a good fit for the conformation where O3 is equatorial, the N-methyl group must also be equatorial, and the torsion angle for Asp105 differs slightly (Fig. 3). An optimal salt bridge is formed and there is a good hydrogen bond (1.9Å) between Ser109 and O3 of the dioxolane ring. In the conformation where O3 is axial the N-methyl group must also be axial (fit not shown). In this fit there is also only a single hydrogen bonding interaction (Ser109 to O3). A binding mode for **1**-(S) in which there are two hydrogen bonds as for AF-30 requires a large deviation of the salt bridge from optimal linearity. Pyrrolidine dioxolane **17** could be fit analogously to AF-30, or more exactly **21**, with the N-methyl group occupying a pseudoaxial position (not shown). Alternatively a fit similar to that shown for **1**-(S) was possible (Fig. 4). The fits shown for AF-30, **1**-(S) and **17** are consistent with the effects of sulfur oxygen exchange on activity. Substitution of O1 of AF-30 with sulfur yields AF-102b which does not lose potency but does lose efficacy and is therefore a weaker agonist. This is consistent with the moderate hydrogen bonding role for O1 in AF-30. Substitution of sulfur for O1 in **1** and **17** results in slight and no loss of agonist activity, respectively. This is consistent with the depicted binding modes for these compounds where O1 is not involved in hydrogen bonding. It should be noted that the important hydrophobic binding site occupied by the dioxolane methyl group is not identified in this model. For **1**, the **1**-(R) enantiomer must have an active conformation and/or a binding mode distinct from the **1**-(S) since the dimethylsubstituted **8** is completely inactive. When the dioxolane ring of **1** adopts an envelope conformation with C2 at the flap position, a good superposition of the C2-methyl groups of the **1**-(S) and **1**-(R) enantiomers may be achieved by simple inversion of the flap position. Interestingly, the same conformational relationship exists between the *cis* and *trans* isomers of the pyrrolidine, quinuclidine and azabicycloheptane analogs for which, pairwise, a similar 5-10fold difference in agonist activity is observed.[18] The SAR clearly shows that the agonist binding site is a very tight pocket with little tolerance for excess volume. Greater steric bulk generally leads to loss of agonist potency and/or efficacy (shift toward antagonism). Finally, this study emphasizes the potential importance of multiple binding modes for a given agonist.

Figure 1

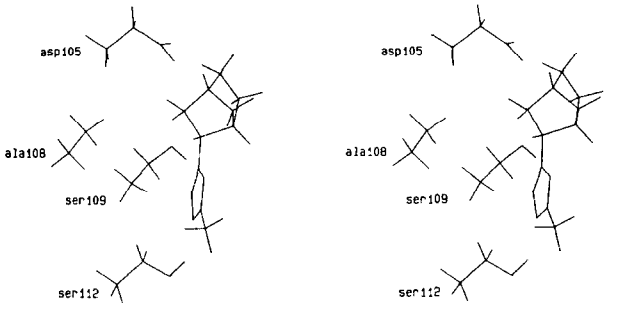


Figure 2

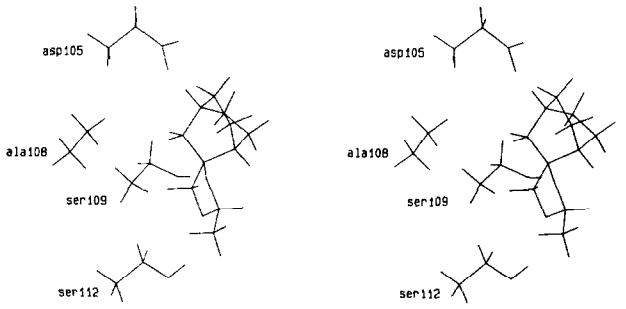


Figure 3

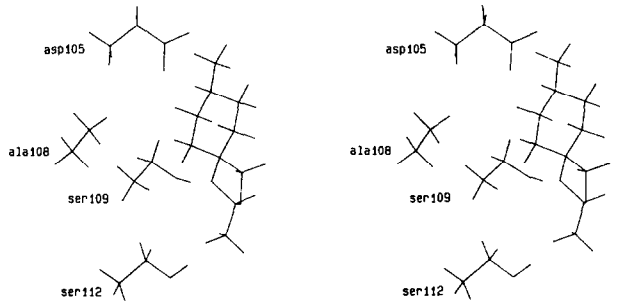
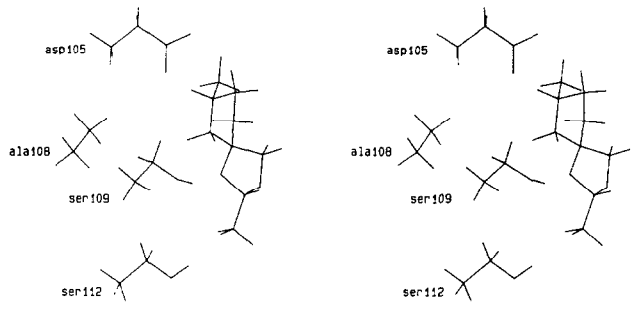


Figure 4



Conclusion

The (-)-S isomer of **1** was developed under the classification of SDZ 210-086 for the indication Alzheimer's Disease. Clinical studies and further characterization with regard to the *in vivo* pharmacological actions of 210-086 have been performed.[19]

REFERENCES

1. Van Rossum, J. M. *Arch. Int. Pharmacodyn.* **1962**, *140*, 592-605.
2. a.Bolliger, G. EP 189370 b.Fischer, A. EP 205247
3. Saunders, J.; Showell, G. A.; Snow, R. J.; Baker R.; Harley, E. A. and Freedman, S. B. *J. Med. Chem.* **1988**, *31*, 486-491.
4. Saunders, J.; Showell, G. A.; Baker R.; Freedman, S. B.; Hill, D.; McKnight, A.; Newberry, N.; Salamone, J. D.; Hirshfield, J. and Springer, J. P. *J. Med. Chem.*, **1987**, *30*, 969-975.
5. a.For a summary of the clinical results with RS 86 see Gray, J.; Enz, A. and Spiegel, R. *Trends Pharmacol. Sci. Suppl.* (Subtypes of Muscarinic Receptors IV) **1989**, 85-88. b.Palacios, J. M.; Bolliger, G.; Closse, A.; Enz, A.; Gmelin, G. and Malanowski, J. *Eur. J. Pharmacol.*, **1986**, *125*, 45.
6. Shapiro, G.; Floersheim, P.; Boelsterli, J.; Amstutz, R.; Bolliger, G.; Gammenthaler, H.; Gmelin, G.;Supavilai, P. and Walkinshaw, M. *J. Med. Chem.* **1992**, *35*, 15.
7. Antagonists also displace CD, whereas full agonists of high efficacy display little or no antagonist binding. The SEM for all pD_2 values is 0.2 and 5% for binding values.
8. Interestingly, in the case of RS86 analogs the ethyl group on the succinimide nitrogen is optimal with the corresponding methyl RS 86 analog **16** being slightly less active. Bolliger, G.; Palacios, J. M.; Closse, A.; Gmelin, G.; and Malanowski, J. "Structure Activity Relationships of RS 86 Analogues" *Alzheimer's and Parkinson's Diseases*, Fisher A. and Hanin, I and Lachman C., Ed.; Plenum Publishing, 1986, pp.585-592.
9. This absolute stereochemistry is also optimal for AF-30[4] and for CD: Belleau, B. and Puranen, J. *J. Med. Chem.* **1963**, *6*, 325.
10. The enantiomers of **1** were resolved via the ditoluoylhydrogentartrate salts. The (-)-S enantiomer of **1** crystallized preferrentially with L-(-)-(R,R)-di-O,O'-p-toluoyltartaric acid. A single crystal X-ray analysis was performed on this salt. The O3 dioxolane oxygen was axial and the N-methyl group equatorial on an ideal chair conformation of the piperidine ring.
11. Teodori, E., Gualtieri, F.; Angeli, P.;Brasili, L.; Giannella, M.and Pigini M. *J. Med. Chem.* **1986**, *29*, 1610-1615.
12. Triggle, D. and Belleau, B. *Can. J. Chem.* **1962**, *40*, 1201.
13. This material was prepared via the cyanohydrin. The *exo*-hydroxy cyanohydrin formed as a kinetic product which precipitated from EtOH but when left to stand went into solution allowing equilibration to the more stable *endo*-hydroxy isomer. The stereochemistry of the diastereomers was not assigned.
14. This material was prepared via method 2 on the *endo*-epoxide followed by chromatographic separation of the two diastereomers **22,23** formed.
15. From a homogeneous tropane (N-benzyl) endo-epoxide, **26** was prepared via method 3 and **27** was prepared by method 2 (stereochemistry assigned by NOE-NMR).
16. From a pure epoxide, a pure diol (N-carboethoxy) precursor was obtained by method 3. Acetalization of this diol could not be performed without facile equilibration at the spirocenter (60:40). Purification of the individual isomers to ca. 85% was achieved by simple chromatography and recrystallization. After carbamate reduction and fumarate salt formation **24** was isolated ca. 95% pure while **25** contained 30% **24** even after recrystallization (stereochemical assignment by NOE-NMR).
17. Showell, G. A.; Baker, R.; Davis, J.; Hargreaves, R.; Freedman, S. B.; Hoogsteen, K.; Patel, S. and Snow, R. J. *J. Med. Chem.*, **1992**, *35*, 911-916.
18. The (2'R,3R) *trans* isomer of AF-30 is ca. 10fold less active than the (2'S,3R) *cis*. Both *cis* and *trans* isomers with 3S absolute stereochemistry are virtually inactive.[4]
19. The *in vivo* effects of the drug on PI-turnover and acetylcholine levels in the rat brain are consistent with the *in vitro* data indicating it to be a potent, centrally active muscarinic agonist with good bioavailability. In a sleep study in man SDZ 210-086 was found to exert central effects at doses found in tolerability studies to be devoid of cholinergic side effects. Unfortunately, elevation of liver transaminases was observed at the expected therapeutic dose level precluding further studies of this muscarinic agonist in Alzheimer subjects. Enz, A.; Bolliger, G.; Gmelin, G.; Palacios, J.M.; Sauter, A.; Laplanche, R.; and Spiegel, R. *Proc. of the 5th meeting of the Int. Study group on the Pharmacology of Memory Disorders associated with Aging*. Wurtman, Corkin, Growdon and Ritter-Walker, Ed.; Center for Brain Sciences and Metabolism Charitable Trust, Cambridge MA. 1989,pp 641-647.