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SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF ALKYL SUBSTITUTED ANALOGUES OF THE FUNCTIONAL M_1 SELECTIVE MUSCARINIC RECEPTOR AGONIST XANOMELINE

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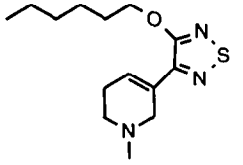
Abstract: A series of 3-(4-substituted-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydrodimethylpyridines have been synthesized and tested in vitro for muscarinic receptor affinity. Methyl substitution at the 5 or 6 position of the tetrahydropyridine resulted in receptor affinities comparable to xanomeline. The use of a sodium borohydride/ cerium trichloride reduction was essential for the synthesis of the 3,5-disubstituted tetrahydropyridines.

Alzheimer's Disease is a neurodegenerative disease that has been associated with central cholinergic deficiencies in the brain's of patients afflicted with the disease[1]. According to the cholinergic hypothesis of Alzheimer's Disease, it may be possible to treat symptoms of the disease with cholinomimetics. The first step in identifying muscarinic agonists is to study the affinity and efficacy of newly synthesized compounds at muscarinic receptors.

3-(4-substituted-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridines, specifically xanomeline, have been reported previously as a novel class of potent, functionally selective M_1 muscarinic receptor agonists. The preclinical pharmacology[2] and neurochemical effects[3] of xanomeline itself has been evaluated in previous reports. Affinity and efficacy at the M_1 receptor was studied using the structure activity relationship of alkoxy- and alkylthio- substituents on 3-(4-substituted-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine[4]. The structure-activity relationship between the 1,2,5-thiadiazole and M_1 receptor affinity and efficacy also has been previously studied by substitution of various five membered aromatic heterocyclic rings at the 3 position of the tetrahydropyridine[5].



Xanomeline

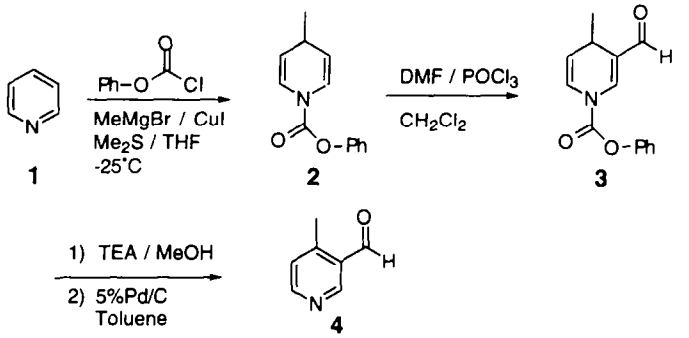
Previous studies[2,3,4,5] indicate that unbranched C_{5-6} alkoxy/alkylthio-1,2,5-thiadiazoles on the 1,2,5,6-tetrahydropyridine provide optimum affinity and efficacy for the M_1 receptor. We now report the structure-activity relationship of alkyl substitutions on the 1,2,5,6-tetrahydropyridine.

Methyl substitutions were made at the 2, 4, 5, and 6 positions on the tetrahydropyridine ring of 3-(4-alkoxy-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine. The structure-activity relationship of the methyl

substitutions were made by evaluating affinity and efficacy of the compounds for muscarinic receptors.

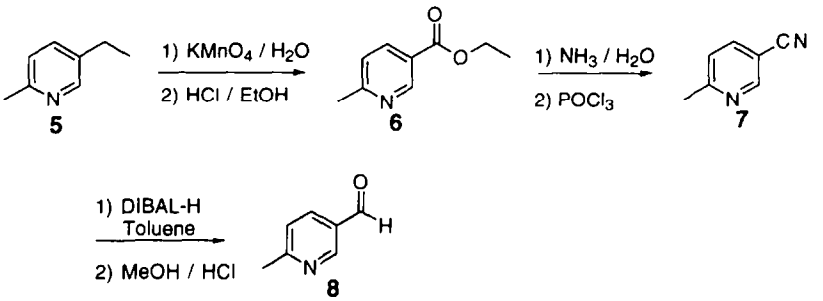
The synthesis of 4-methyl-3-pyridinecarboxaldehyde(**4**) is described in **Scheme 1.** Pyridine (**1**) was treated with phenylchloroformate and methyl grignard to give the dihydro-4-methylpyridine (**2**)[6]. The dihydropyridine was formylated with N,N-dimethylformamide and phosphorus oxychloride to give the aldehyde (**3**)[7]. **3** was rearomatized with triethylamine in methanol followed by palladium on carbon in refluxing toluene[8] to yield 4-methyl-3-pyridinecarboxaldehyde(**4**).

Scheme 1



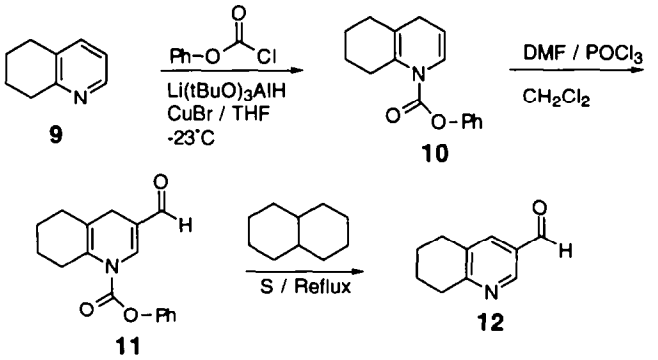
The synthesis of 6-methyl-3-pyridinecarboxaldehyde[9] (**8**) is described in **Scheme 2.** 3-Ethyl-6-methylpyridine (**5**) was oxidized to the corresponding acid using potassium permanganate then converted to the ethyl ester (**6**) using hydrochloric acid and ethanol. The ethyl ester was converted to the amide with ammonia then dehydrated with phosphorous oxychloride to give 6-methyl-3-pyridinecarbonitrile(**7**). The nitrile was reduced with DIBAL-H then converted to the 6-methyl-3-pyridinecarboxaldehyde (**8**) with hydrochloric acid and methanol.

Scheme 2



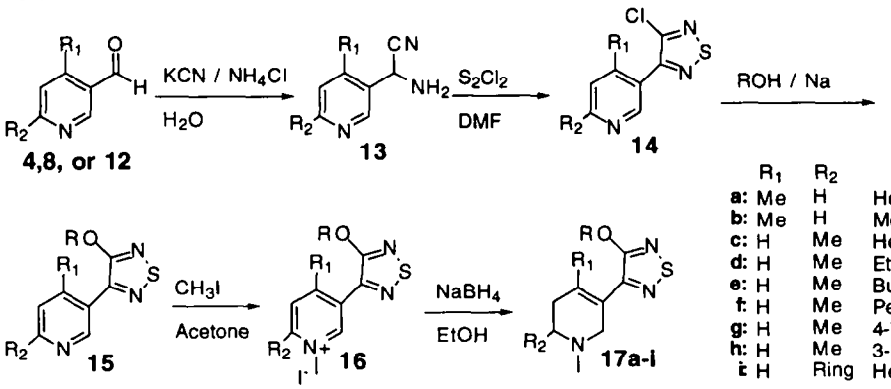
5,6-Cyclohexeno-3-pyridinecarboxaldehyde (**12**) was synthesized as described in **Scheme 3.** 2,3-Cyclohexenopyridine (**9**) was treated with phenyl chloroformate and Comins' copper hydride in THF to yield the 1,4-dihydropyridine (**10**)[10]. **10** was formylated with N,N-dimethylformamide and phosphorous oxychloride to give **11.** The dihydropyridine (**11**) was rearomatized using sulfur in refluxing decahydronapthalene to give 5,6-Cyclohexeno-3-pyridinecarboxaldehyde (**12**)[6].

Scheme 3



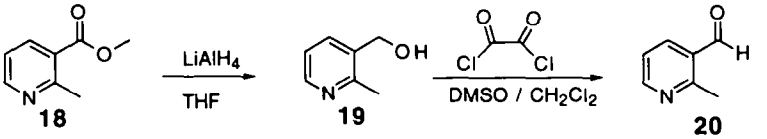
The 4-methyl, 6-methyl-, and 5,6-cyclohexano- substituted tetrahydropyridines were synthesized as described in **Scheme 4**[1]. Aldehydes **4**, **8**, or **12** were treated with potassium cyanide and ammonium chloride in water to give the corresponding aminonitrile (**13**). The aminonitrile was cyclized with sulfur monochloride to give substituted-3-(4-chloro-1,2,5-thiadiazol-3-yl)-pyridine (**14**). **14** was treated with the appropriate alcohol and sodium hydride to give the alkoxy compound (**15**) which was quaternized with iodomethane to yield **16**. The quaternized pyridine was reduced with sodium borohydride in ethanol to yield the desired tetrahydro-alkyl-substituted-pyridine (**17**).

Scheme 4



The synthesis of 2-methyl-3-pyridinecarboxaldehyde(**20**) is described in **Scheme 5**. Methyl-2-methylnicotinate (**18**) was reduced with lithium aluminum hydride to the carbinol (**19**) which was oxidized to 2-methyl-3-pyridinecarboxaldehyde (**20**) using standard Swern oxidation conditions.

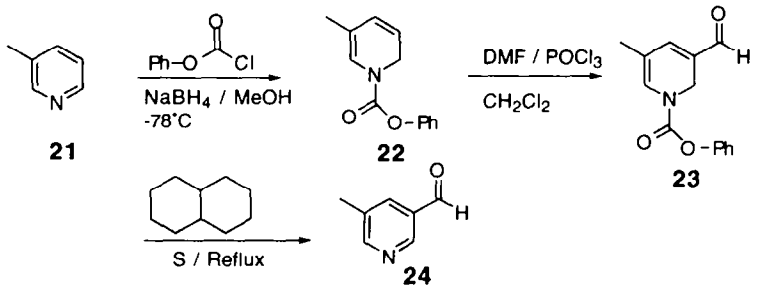
Scheme 5



The synthesis of 5-methyl-3-pyridinecarboxaldehyde(**24**) is described in **Scheme 6**. 3-Methylpyridine (**21**) was

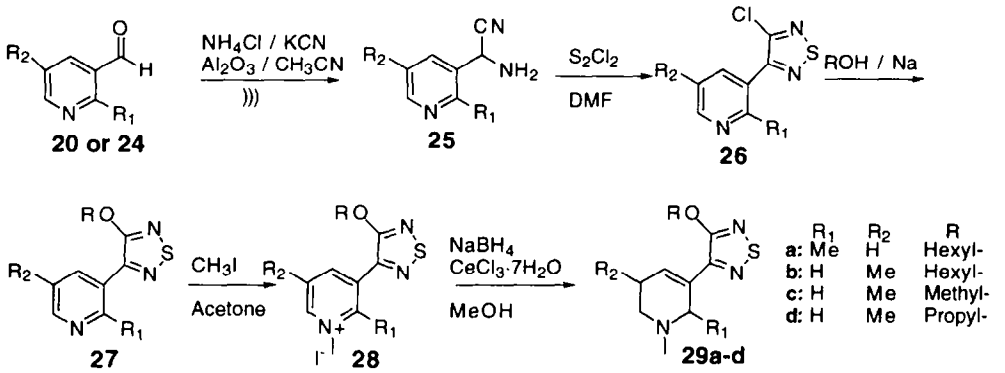
treated with phenylchloroformate and sodium borohydride in methanol to yield the 1,2-dihydropyridine (**22**) The dihydropyridine was formylated as in scheme 1 to give **23.** The dihydropyridine was rearomatized using sulfur in refluxing decahydronapthalene to give 5-methyl-3-pyridinecarboxaldehyde (**24**)[10].

Scheme 6



The 2-methyl and 5-methyl- substituted tetrahydropyridines were synthesized as described in **Scheme 7.** Sonication[11] of aldehydes **20** or **24** with ammonium chloride and potassium cyanide in alumina and acetonitrile gave the aminonitrile (**25**)[12]. The aminonitrile was cyclized with sulfur monochloride to give substituted-3-(4-chloro-1,2,5-thiadiazol-3-yl)-pyridine (**26**). Through the use of ultrasound, the yield of the 3-(4-chloro-1,2,5-thiadiazol-3-yl)-pyridine (**26**) improved from 30% to 80% from the aldehyde. **26** was treated with the appropriate alcohol and sodium hydride to give the alkoxy compound (**27**) which was quaternized with iodomethane to yield **28**. The quaternized pyridine was reduced with sodium borohydride in methanol in the presence of cerium trichloride to give the desired 1,2,5,6-tetrahydrodimethylpyridine (**29**) in a yield of 6% for **29a** and 18% for **29b-d**. Reduction of the pyridinium salt with sodium borohydride in the presence of cerium trichloride gave a mixture of regioisomers, the 1,2,5,6-tetrahydropyridine (minor product) and the 1,2,3,6-tetrahydropyridine (major product). The combined yield of the tetrahydropyridine regioisomers from the reduction of the 2,3- and the 3,5-disubstituted N-methyl pyridinium salts[13] was approximately 40%. Reduction of the pyridinium salt with other hydride reducing agents, such as sodium borohydride, sodium cyanoborohyride, lithium aluminum hydride, copper hydrides, proved to be unsuccessful.

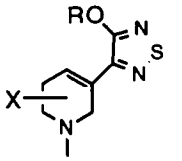
Scheme 7



The affinity of these compounds for muscarinic receptors was determined by in-vitro receptor binding to rat brain membranes[1]. Displacement of [^{3}H]pirenzepine ([^{3}H]Pz), a selective antagonist for the M_1 muscarinic

receptor, was used to determine the affinity for the M_1 receptor in rat cerebral cortex membranes. The ability of the compounds to displace [^{3}H]oxotremorine-M ([^{3}H]Oxo-M), a nonselective muscarinic agonist, was interpreted as the affinity for the agonist conformational state of the muscarinic receptor sites. Efficacy for these compounds was evaluated as their ability to stimulate phospholipid hydrolysis in BHK cells transfected with cloned human m_1 muscarinic receptors as compared to the maximal stimulation of the full agonist carbachol[4].

Table 1



Compound	R	Position of Methyl Substitution, X	Receptor Binding to Rat Brain Membranes [^{3}H]Pz IC50 , nM	[^{3}H]Oxo-M IC50 , nM	% Stimulation[a] PI Hydrolysis BHK m_1 Cells Dose = 100μM
Xanomeline	Hexyl-	unsubstituted	7	10	70
30	Propyl-	unsubstituted	18	2	25
31	Methyl-	unsubstituted	148	22	7
17a	Hexyl-	4	112	100	nt
17b	Methyl-	4	1000	1000	nt
17c	Hexyl-	6	5	6	27
17d	Ethyl-	6	24	11	10
17e	Butyl-	6	8	9	9
17f	Pentyl-	6	5	9	18
17g	4-Pentenyl-	6	8	10	12
17h	3-hexynyl-	6	5	6	12
17i	Hexyl-	5,6[b]	1230	1000	nt
29a	Hexyl-	2	48	83	nt
29b	Hexyl-	5	7	14	12
29c	Methyl-	5	580	600	nt
29d	Propyl-	5	15	17	6

[a] The % stimulation is reported as a percentage of the maximal stimulation produced by100μM carbachol.
[b] A cyclohexane ring was fused to the tetrahydropyridine between the 5 and 6 positions.
nt = not tested

Affinities for muscarinic receptors of the present alkyl substituted tetrahydropyridines were compared to the affinity of the unsubstituted tetrahydropyridine, xanomeline. Methyl substitution at the 4 position of the tetrahydropyridine ring (**17a and b**) resulted in a ten fold decrease in affinity for the receptors labeled with [^{3}H]Pz and [^{3}H]Oxo-M. Fusion of a cyclohexane ring between the 5 and 6 position of the tetrahydropyridine ring(**17i**) resulted in a substantial decrease in the affinity of the compound. Substitution at the 2 position of the tetrahydropyridine ring where R is hexyl (**29a**) resulted in an eight fold decrease in affinity. Compounds that have been substituted with methyl groups at the 5 or 6 position of the tetrahydropyridine ring where R is hexyl (**17c and 29b**) resulted in affinities that are comparable to the affinity of xanomeline at the M_1 muscarinic

receptor. Variation of the alkoxy groups, except for the methoxy substitution, on the 1,2,5-thiadiazole when the tetrahydropyridine ring is methyl substituted at the 5 and 6 positions (**17d-h** and **29b,d**) appeared to have negligible effects on affinity when compared to the corresponding unsubstituted tetrahydropyridines. While methyl substitution around the tetrahydropyridine ring maintained good affinity for the receptors labeled with [^{3}H]Pz and [^{3}H]Oxo-M, alkyl substitutions on the tetrahydropyridine ring greatly reduced the efficacy of these compounds at cloned m_1 receptors in BHK cells. Xanomeline increased phospholipid hydrolysis by 70% while methyl substitutions at the 5 and 6 positions (**17c** and **29b**) increased phospholipid hydrolysis by 12% and 27% respectively.

Substitution of a methyl group at the 2 or 4 position or fusion of a cyclohexane ring between the 5 and 6 position on the tetrahydropyridine ring of 3-(4-alkoxy-1,2,5-thiadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine had a deleterious effect on the affinity and efficacy of these compounds for the M_1 muscarinic receptor. Methyl substitutions at the 5 or 6 position of the tetrahydropyridine ring (**17c and 29b**) resulted in compounds with good affinity at the M_1 muscarinic receptor. While **17c** and **29b** maintain muscarinic affinity comparable to the muscarinic affinity of xanomeline, the substitution of a methyl group produced a significant drop in M_1 efficacy. This decrease in muscarinic efficacy may be due to steric interactions with the muscarinic receptor. Additional longer chain alkyl substitutions at the 5 and 6 position of the tetrahydropyridine ring may provide further insight to the interaction of xanomeline-like compounds at the M_1 muscarinic receptor.

References and Notes

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13) Full experimental details for the sodium borohydride/cerium trichloride reduction of the alkyl substituted pyridinium salt will be published at a later date.

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