Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 8, pp. 785-790, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00113-1

DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME TETRAAMINES RELATED TO METHOCTRAMINE AND 4–DAMP

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Abstract. Three novel tetraamines (**1–3**) related to methoctramine were designed, and their biological profiles at muscarinic receptor subtypes were assessed by binding assays. It turned out that the constrained analogue spirotramine (**3**, FC 15–94) of 4–DAMP has an affinity profile better than that of pirenzepine owing to a high affinity for muscarinic M_1 receptors and a significantly lower affinity for the M_2, M_3 and M_4 subtypes.

Molecular biology studies have demonstrated the heterogeneity of cholinergic muscarinic receptors.[1-3] Five different subtypes (m_1-m_5), which share about 70% identity of aminoacids, have been identified so far by molecular cloning. Muscarinic receptors that have been characterized pharmacologically and classified as M_1-M_4, appear to correspond to cloned m_1-m_4 receptors. At present, little information is available about the nature and the cellular location of the m_5 subtype.

Despite the impressive results obtained by molecular biology studies, the availability of selective ligands, able to recognize only one among the muscarinic receptor subtypes, is difficult owing to a high percentage of aminoacids which are identical in the active binding pocket of the different muscarinic receptor subtypes. Achievement of subtype selectivity is inherently more difficult for the agonists than for the antagonists probably because the agonists are small molecules that interact almost all in the same way and with the same conserved amino acids in receptor subtype regions that are presumed to bind agonists. It is well known that the agonists bearing structural kinship to acetylcholine do not display receptor subtype selectivity. The antagonists have a larger and longer structure than agonists which may result in an increased number of specific contacts with receptor regions unique to one muscarinic receptor subtype, rather than to another, thus leading to selectivity. Although, several so–called selective muscarinic receptor antagonists are available, it should be emphasized, however, that the ideal selective ligand, as defined above, is not available yet and remains a formidable challenge to medicinal chemists.

The development of polymethylenetetraamines, the prototype of which is methoctramine,[4-7] has contributed to the characterization of muscarinic receptors subtypes. However, methoctramine, despite its high affinity for M_2 receptors and low affinity for M_3 receptors, failed, like all other selective M_2 antagonists, to discriminate between muscarinic M_2 and M_4 muscarinic receptor subtypes. Very recently, we synthesized a series of tetraamines by replacing the terminal 2-methoxybenzyl groups of methoctramine with an 11-acetyl-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]-benzodiazepin-6-one moiety.[8, 9] Tripitramine resulted the most potent and the most selective M_2

muscarinic receptor antagonist so far available, able to discriminate also between M_2 and M_4 muscarinic receptor subtypes.[8-10]

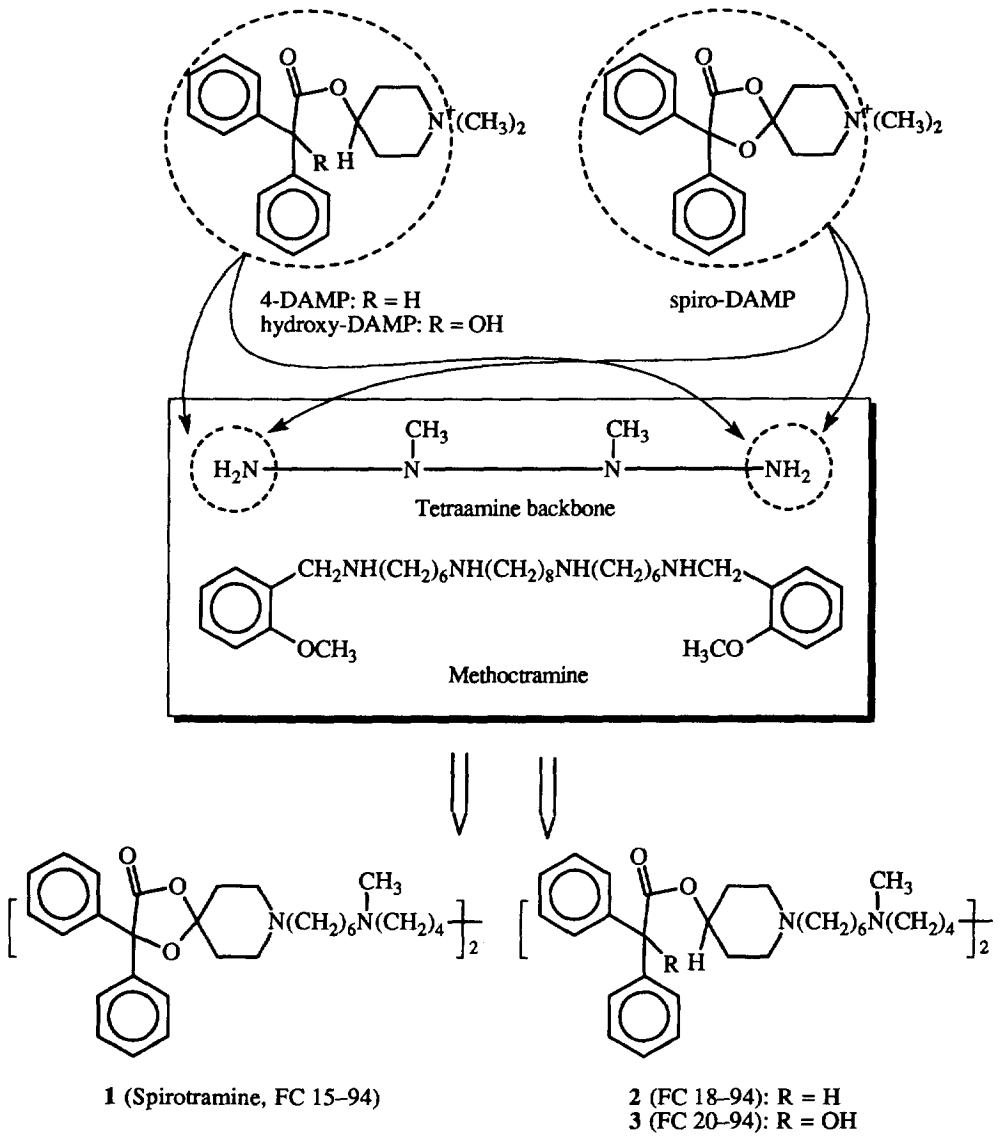
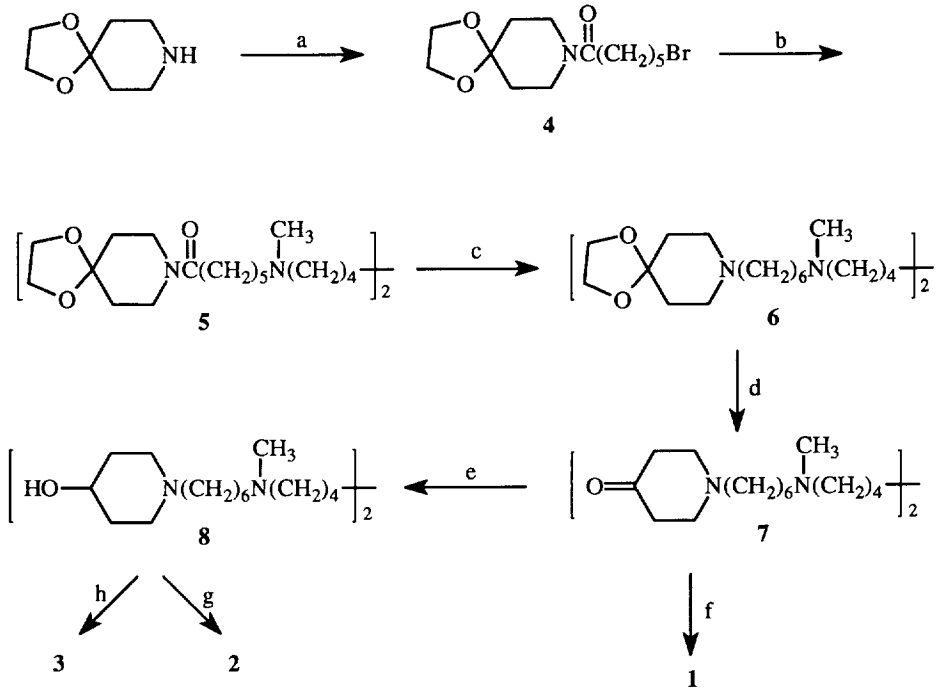


Figure 1. Design strategy for the synthesis of hybrid structures **1-3** by inserting the structural features of 4-DAMP and of related compounds (hydroxy-DAMP and spiro-DAMP)[11] on the terminal nitrogens of the tetraamine backbone of methoctramine.

The finding that the affinity profile of methoctramine–related tetraamines depends on the type of substituents on the terminal nitrogens of a tetraamine backbone, prompted us to further modify methoctramine structure, in order to improve the affinity and selectivity for different muscarinic receptors subtypes. Thus, we describe here the synthesis and the pharmacological profile of the novel tetraamines **1-3**, designed by substituting the 2-methoxybenzyl groups of methoctramine with the structural features of 4-DAMP, a relatively selective muscarinic M_1/M_3 receptor antagonist, and of its analogues spiro-DAMP and hydroxy-DAMP.[11] To prevent *N*-alkylation of the two inner nitrogens, we choose as a common backbone a tetraamine bearing on these nitrogens a methyl group. The design strategy for our compounds is shown in Figure 1.

All the compounds were synthesized by standard procedures (Scheme 1) and were characterized by ^1H NMR, ^{13}C NMR and elemental analysis.[12]

Scheme 1



(a) $ClCO(CH_2)_5Br$, NEt_3, CH_2Cl_2, r.t., 6 h, 88%; (b) $MeNH(CH_2)_8NHMe$, K_2CO_3, 2-PrOH, reflux, 24 h, silica gel (MeOH-EtOAc-CH_2Cl_2-cyclohexane-30% ammonia; 1:0.5:8:0.5:0.1), 33%; (c) $LiALH_4$, THF, reflux, 18 h, 95%; (d) concd HCl, reflux, 18 h, silica gel (MeOH-$CHCl_3$-30% ammonia; 9:1:0.3), 80%; (e) $NaBH_4$, EtOH, r.t., 18 h, 85%; (f) benzilic acid, toluene-4-sulfonic acid, benzene, reflux, 36 h, silica gel (MeOH-30% ammonia; 10:0.1 then MeOH-30% ammonia; 10:0.2), 43%; (g) diphenylacetic acid, $SOCl_2$, then NEt_3, benzene, reflux, 18 h, silica gel (MeOH-$CHCl_3$-30% ammonia; 8.5:1.5:0.2), 35%; (h) 1,1'-carbonyldiimidazole, benzilic acid, CH_2Cl_2, r.t., 1 h, then benzene, reflux, 36 h, silica gel (MeOH-$CHCl_3$-30% ammonia; 9:1:0.3), 20%.

Amidation of 6-bromohexanoyl chloride with 1,4-dioxa-8-azaspiro[4,5]decane gave intermediate **4**, which afforded upon alkylation with *N,N'*-dimethyl-1,8-octanediamine compound **5**. Reduction of **5** gave tetraamine **6**,

that was deprotected to yield keto-tetraamine **7**. Reduction of **7** afforded the corresponding alcohol **8**. Compound **1** was synthesized through condensation of **7** with benzilic acid and was characterized as tetracitrate salt.[13] Compounds **2** and **3** were obtained by esterification of **8** with activated diphenylacetic acid and benzilic acid, respectively, and both were characterized as tetraoxalate salts.[14, 15]

The muscarinic receptor subtype selectivity was assessed by employing receptor binding assays as reported previously.[8, 9] [^{3}H]N-Methylscopolamine ([^{3}H]NMS; specific activity 79.5 Ci/mmol) was used to label M_2, M_3 and M_4 muscarinic receptors binding sites of rat heart (K_d 0.32±0.042 nM; B_{max} 77.8±15.3 fmol/mg of protein) and submaxillary gland (K_d 0.485±0.035 nM; B_{max} 1102±85 fmol/mg of protein), and NG 108-15 cell homogenates (K_d 0.544±0.032 nM; B_{max} 19±4 fmol/mg of protein), respectively. [^{3}H]Pirenzepine (specific activity 86.2 Ci/mmol) was the tracer to label M_1 muscarinic receptors binding sites of the rat cerebral cortex (K_d 2.15±0.19 nM; B_{max} 49±13 pmol/mg of protein). Binding affinities were expressed as pK_i values. Methoctramine, tripitramine, 4-DAMP and pirenzepine were used as the standard compounds.

Table I. Affinity Values for Inhibiting [^{3}H]Pirenzepine Binding in Rat Cortex Homogenates (M_1), and [^{3}H]N-Methyl-scopolamine Binding in Rat Heart (M_2), Rat Submaxillary Gland (M_3) and NG 108–15 Cell (M_4) Homogenates

Compound	pK_i[a]				Affinity Profile[b]
	M_1	M_2	M_3	M_4	
Methoctramine[c]	7.43±0.11	7.84±0.09	5.96±0.18	7.58±0.13	$M_2 = M_4 = M_1 >> M_3$
4-DAMP	9.23±0.09	8.40±0.10	8.94±0.07	9.29±0.11	$M_4 = M_1 = M_3 \geq M_2$
Pirenzepine[c]	8.19±0.08	6.10±0.09	6.76±0.10	7.46±0.17	$M_1 > M_4 \geq M_3 \geq M_2$
Tripitramine[c]	7.63±0.09	9.54±0.08	6.19±0.14	7.93±0.11	$M_2 >> M_4 = M_1 >> M_3$
Spirotramine (**1**)	7.88±0.10	6.20±0.17	6.01±0.18	6.27±0.09	$M_1 >> M_4 = M_2 = M_3$
2	7.12±0.12	6.74±0.13	5.67±0.17	7.40±0.09	$M_4 = M_1 = M_2 > M_3$
3	8.30±0.13	7.85±0.06	6.52±0.13	7.50±0.12	$M_1 = M_2 = M_4 > M_3$

[a] Values are the mean ± SE of at least three separate experiments performed in triplicate. All Hill number (nH) were not significantly different from unity ($p > 0.05$). Equilibrium dissociation constants (K_i) were derived using the Cheng-Prusoff equation,[16] $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of [^{3}H]NMS or [^{3}H]pirenzepine, respectively. Scatchard plots were linear or almost linear in all preparation tested. In competition studies, fixed concentrations of 0.7 - 0.8 nM [^{3}H]NMS were used in rat heart and submaxillary gland, and NG 108-15 binding assays, whereas 5 nM was the concentration of [^{3}H]pirenzepine in rat cortex homogenates. Non specific binding was assessed in the presence of 1 μM atropine. [b] Differences in antagonistic affinities for muscarinic receptor subtypes by a factor of ≤3, >3÷5, >5÷20, and >20 are indicated by =, ≥, >, and >>, respectively. [c] Data from ref 9. However, the standard compounds methoctramine, pirenzepine and tripitramine were also tested by performing few experiments while assaying **1–3**. It was verified that K_i values of standard compounds were not significantly different ($p > 0.01$) from those reported in ref 9.

The biological results of tetraamines **1–3**[17] are shown in Table I together with those of standard compounds methoctramine, tripitramine, 4–DAMP and pirenzepine, which were included in this study for a comparison. It

can be seen that replacing 2-methoxybenzyl groups of methoctramine by other moieties affording tripitramine[8,9] or **1–3** altered markedly both affinity and selectivity toward muscarinic receptor subtypes. It is evident that an appropriate substitution of the terminal nitrogens of the tetraamine backbone of methoctramine affords potent antimuscarinics that display different selectivity profiles. The insertion of 11-acetyl-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]-benzodiazepin-6-one groups on a tetraamine backbone afforded tripitramine which possesses outstanding properties toward M_2 muscarinic receptors in comparison to the parent compound methoctramine.[8–10] However, the inclusion of 4–DAMP related moieties (Figure 1) on the terminal nitrogens of an identical tetraamine backbone afforded spirotramine (**1**) which displays an inverse selectivity profile in comparison to both methoctramine and tripitramine owing to a higher affinity for M_1 muscarinic receptors and a significantly lower affinity for all other muscarinic receptor subtypes so far investigated.

This finding clearly indicates that the tetraamine backbone is able to interact with different muscarinic receptor subtypes. Thus, by applying the message–address concept,[18] it can be assumed that the tetraamine backbone carries a "message" responsible for the recognition of muscarinic receptors, while the different *N*-terminal groups play an "address" role in conferring preference for different muscarinic receptor subtypes. If this view is correct it may assume great relevance in the design of novel substituted tetraamines displaying different selectivity profiles thus allowing a better characterization and classification of muscarinic receptor subtypes.

In conclusion, we have demonstrated that appropriate substituents on the terminal nitrogens of a tetraamine template control both affinity and selectivity for muscarinic receptors. This approach may lead to antagonists with biological properties resembling those of an ideal antagonist, i.e. compounds with high affinity for one subtype and modest, if any, affinity for the other subtypes of a given receptor system. The insertion of a 4–DAMP–like moiety into the template afforded the novel tetraamine spirotramine (**1**), which was able to discriminate significantly between M_1 muscarinic receptors and the other subtypes. It is evident that spirotramine has a significantly better affinity profile than pirenzepine, which is classified as a highly M_1–selective antagonist. The results presented in this paper clearly show that the use of tripitramine, a highly M_2–selective tetraamine that fails, however, to discriminate between M_1 and M_4 muscarinic receptors, combined with that of spirotramine, which possesses a complementary affinity profile owing to its ability to discriminate between M_1 and M_4 subtypes (selectivity ratio = 41), may represent a valuable tool for the pharmacological identification of muscarinic receptor subtypes.

Acknowledgment. Supported by a Grant from MURST (Italy).

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12. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXR 300 instrument. The elemental compositions of compounds **1–3** agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography.
13. **1**: mp = 78-80 °C (from EtOH/Et_2O); ^{1}H NMR (free base, $CDCl_3$) δ 1.19-1.36 (m, 16 H), 1.36-1.55 (m, 12 H), 1.81-2.01 (m, 8 H), 2.16 (s, 6 H), 2.27 (t, 8H), 2.35 (t, 4 H), 2.42-2.58 (m, 4 H), 2.60-2.74 (m, 4 H), 7.28-7.43 (m, 12 H), 7.52 (dd, 8H). ^{13}C NMR (free base, $CDCl_3$) δ 171.38, 140.27, 128.49, 128.37, 126.17, 109.65, 83.67, 58.06, 58.01, 57.91, 50.27, 42.38, 36.74, 29.63, 27.63, 27.59, 27.35, 27.26.
14. **2**: mp = 127-131 °C (from MeOH/EtOH); ^{1}H NMR (free base, $CDCl_3$) δ 1.19-1.35 (m, 16 H), 1.35-1.53 (m, 12 H), 1.60-1.73 (m , 4 H), 1.81-1.94 (m, 4 H), 2.18 (s, 6 H), 2.12-2.35 (m, 16 H), 2.46-2.62 (m, 4 H), 4.8-4.91 (m, 2 H), 4.99 (s, 2 H), 7.19-7.35 (m, 20 H). ^{13}C NMR (free base, $CDCl_3$) δ 171.70, 138.65, 128.49, 128.39, 127.04, 70.96, 58.50, 57.80, 57.72, 57.22, 50.51, 42.19, 30.51, 29.47, 27.50, 27.46, 27.15, 27.00.
15. **3**: mp = 92-94 °C (from EtOH/2-PrOH); ^{1}H NMR (free base, $CDCl_3$) δ 1.18-1.35 (m, 16 H), 1.35-1.56 (m, 12 H), 1.60-1.77 (m, 4 H), 1.81-1.95 (m, 4 H), 2.22 (s, 6 H), 2.11-2.28 (m, 8 H), 2.33 (t, 12 H), 4.31 (br s, 2 H), 4.91-5.20 (m, 2 H), 7.25-7.38 (m, 12 H), 7.38-7.50 (m, 8 H). ^{13}C NMR free base ($CDCl_3$) δ 173.85, 142.07, 127.98, 127.91, 127.40, 80.84, 72.94, 58.51, 57.74, 57.65, 50.05, 42.10, 30.30, 29.68, 29.51, 27.49, 27.08, 26.95.
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(*Received in Belgium* 21 *November* 1994; *accepted* 8 *February* 1995)