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N-Homolupinanoyl and *N*-(ω-lupinylthio)alkanoyl derivatives of some tricyclic systems as ligands for muscarinic M_1 and M_2 receptor subtypes

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Abstract

A set of *N*-homolupinanoyl- and *N*-(ω-lupinylthio)alkanoyl derivatives of tricyclic systems (as phenothiazine, iminodibenzyl and dihydropyridobenzodiazepinone) has been prepared and tested for affinity for rat muscarinic M_1 and M_2 receptor subtypes labeled with [^{3}H]pirenzepine and [^{3}H]AF-DX 384. Good affinity for both M_1 and M_2 subtypes was displayed by most compounds, often with nanomolar K_i values, which for lupinylthiopropionyl- and lupinylthiobutyryl-phenothiazines (**13**–**16**) were comparable to those of pirenzepine and methoctramine, respectively. However, only moderate selectivity for one or the other subtype was seen.

Keywords: *N*-(Homolupinanoyl)phenothiazines; *N*-[(ω-lupinylthio)alkanoyl]phenothiazines; *N*-(*tert*-Aminoalkanoyl)tricyclic systems; Quinolizidine derivatives; Muscarinic receptors ligands

1. Introduction

Specific muscarinic receptor subtypes ligands, acting either as agonists or antagonists, may show interest in several kinds of peripheral and central nervous systems disorders [1–3].

M_1 and/or M_3 receptor antagonists might be useful in the treatment of gastroenteric apparatus dysfunctions (such as gastric acid secretion and irritable bowel syndrome) and chronic obstructive airways diseases, while M_2 antagonists might be used in the treatment of bradycardic disorders. Moreover, central M_4 receptor antagonists could be applied as anti-Parkinson agents [4], while central M_2 receptor antagonists should increase the release of acetylcholine and be useful in the treatment of cholinergic-deficit depending pathologies, such as Alzheimer's disease [5,6]. The same use has been suggested for central M_1 receptor agonists, however, in such a case, unacceptable side effects occurred.

A few years ago [7], we found that *N*-homolupinanoyl phenothiazine **1** (Chart 1), studied as antiarrhythmic agent along with other analogues, exhibited high affinity for M_1 subtype with $IC_{50} = 30$ nM, while the affinity for M_2 and M_3 subtypes was more than two orders of magnitude lower (IC_{50} around 10 μM), resulting in a selectivity for M_1 subtype superior to that of pirenzepine.

In compound **5**, bearing the same homolupinanoyl residue on the pyridobenzodiazepinone tricyclic system, which characterizes pirenzepine, the affinity for M_1 subtype was lower ($IC_{50} = 110$ nM) and the selectivity versus M_2 and M_3 subtypes was also reduced [8].

However, when the homolupinanoyl residue was replaced with the longer lupinylthioacetyl moiety (**6**), the high affinity for M_1 subtype was restored, though the selectivity versus M_2 and M_3 subtypes remained a little lower than that of pirenzepine.

Therefore, both the nature of the tricyclic system and, even more, the length of the chain connecting the quinolizidine ring to the tricyclic system seem to be important for the affinity to M_1 subtype.

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Preliminary functional tests suggested M_1 and M_2 muscarinic antagonism.

On the basis of the above, we have prepared two sets of quinolizidine derivatives which are interesting as muscarinic ligands (Chart 1).

In the first, a lupinylthioacetyl residue is linked to variously substituted iminodibenzyl or phenothiazine rings (**7**–**12**), while in the second set, a lupinylthioalkanoyl moiety of increasing length is linked to phenothiazine or 2-methoxyphenothiazine (**13**–**20**).

The folding of the longer side-chain might allow a more suitable spatial placement of the terminal protonated nitrogen in relation to the tricyclic moiety, which could influence the affinity and selectivity for the muscarinic receptor subtypes, as has already been pointed out by Eberlein et al. [9] for their pyridobenzodiazepinone derivatives.

The quinolizidine moiety of these compounds retains the absolute configuration of natural L-lupinine, from which they derive.

In a first attempt to investigate the effect of a configuration change in the quinolizidine moiety, the epimeric analogue of compound **1** (**1-epi**) was also prepared, starting from D-epi-lupinine.

The novel compounds **1-epi** and **7**–**20**, together with previously described compounds **1**–**6**, were tested for their affinity to M_1 and M_2 receptor subtypes, by displacing specific radioligands from membrane preparations: [^{3}H]pirenzepine from rat brain M_1 receptors and [^{3}H]AF-DX 384 from rat heart M_2 receptors.

10-(*tert*-Aminoacyl)phenothiazines endowed with outstanding spasmolytic properties in vitro have already been described in the past [10–12]. Among these, 10-(4-diethylaminobutyryl)phenothiazine was as potent as atropine against acetylcholine-induced spasms.

However, as far as we know, these compounds were not tested for affinity to muscarinic receptor subtypes.

Several 10-(aminoacyl)phenothiazines display high antinicotinic and antimuscarinic activity and are therefore able to protect animals up to 10 times better than atropine from powerful acetylcholinesterase inhibitors such as Tabun (dimethylamidoethoxyphosphoryl cyanide) [13,14]. Interestingly, 10-(2-diethylaminopropionyl)phenothiazine (Astra 1397) is reported to be a selective inhibitor of human plasma butyrylcholinester-

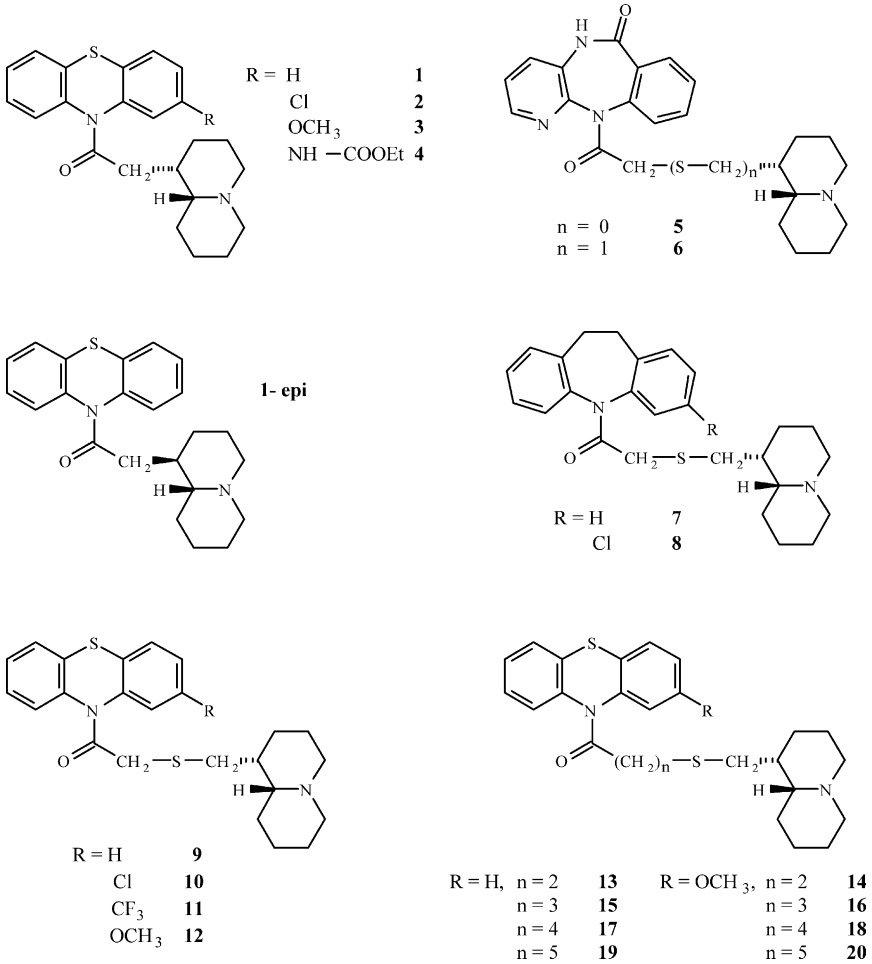


Chart 1.

ase with respect to human red cell acetylcholinesterase [15].

2. Chemistry

Compounds **1**–**6** were prepared as already described [7,8].

Compound **1-epi** was prepared in the same manner as compound **1** [7] starting from epi-homolupinanoylchloride hydrochloride [16].

The new compounds **7**–**20** were prepared by reacting the proper tricycle (substituted iminodibenzyl or phenothiazine) with the suitable ω-haloalkanoyl chloride in refluxing toluene and subsequent reaction of the *N*-(ω-halo)alkanoyl tricycle with thiolupinine [17] in ethanol or acetonitrile (Scheme 1).

It is worth noting that, while *N*-chloroacetyl and *N*-chloropropionyl-2-substituted phenothiazines react smoothly with thiolupinine in ethanol, *N*-(4-chlorobutyryl)phenothiazines do not react, but lose the aliphatic chain to give *N*-unsubstituted phenothiazines, ethylchlorobutyrate and butyrolactone. If the reaction between thiolupinine and chlorobutyrylphenothiazine is run in dry benzene, the latter reactant is recovered unchanged even after 70 h of heating at 100 °C in a sealed tube. Finally, the required *N*-[(4-lupinylthio)butyryl]phenothiazines were obtained (with about 40% yield) by reacting the *N*-(4-bromobutyryl)-2-substituted phenothiazines with thiolupinine in acetonitrile. The same experimental conditions were used to obtain higher homologues.

The structures of all the prepared compounds were supported by elemental analyses and spectral (IR and NMR) data. NMR spectra were consistent to the assigned structures and did not exhibit any peculiar features, thus only a few are described in Section 3 as examples.

3. Experimental

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected.

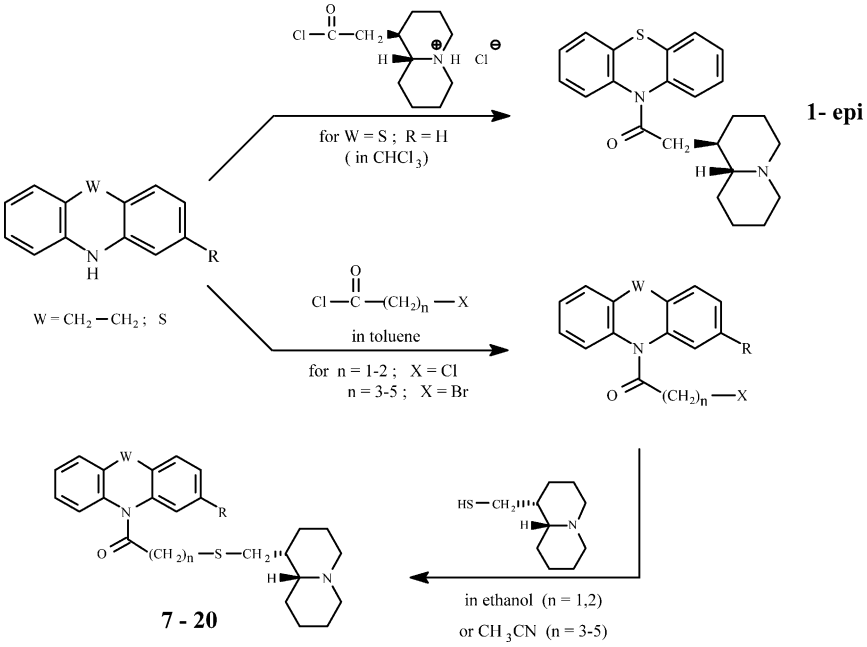
Elemental analyses were performed with CE EA 1110 CHNS-O instruments and the results obtained for C, H and N were within ±0.3% of the calculated values; for several compounds, the sulfur content resulted lower than the calculated value for more than 0.3%.

IR spectra were recorded on Perkin–Elmer Paragon 1000PC spectrophotometer; ^{1}H NMR were taken on a Varian Gemini 200 spectrometer, using $CDCl_3$ or d_6-DMSO as solvent, with TMS as internal standard.

3.1. *N*-(*epi*-Homolupinanoyl)phenothiazine (**1-epi**)

The epi-homolupinanoic acid hydrochloride (1 g, 4.3 mmol) was converted to the corresponding chloride by means of oxalyl chloride in chloroform, as already described [16].

A slurry of phenothiazine (0.86 g, 4.3 mmol) in 10 ml of chloroform was added to the solution of the above chloride in 10 ml of the same solvent and the mixture was heated under reflux for 3 h. After removing the solvent under reduced pressure, the residue was treated



Scheme 1.

with acidic water and the unreacted phenothiazine was extracted with ether.

The acid solution was alkalized with 2 N NaOH solution and extracted with ether. The solvent was evaporated and the residue was crystallized from dry ether.

^{1}H NMR ($CDCl_3$): δ 0.74–1.30 (m, 3H of quinolizidine ring = Q), 1.30–2.06 (m, 11H, Q), 2.15 (dd, J = 14.65 and 9.28 Hz; 1H of C(O)–CH_2–Q), 2.65–2.90 (m, 3H; 1H of C(O)–CH_2–Q and 2H of Q), 7.20–7.40 (m, 4H arom.), 7.40–7.58 (m, 4H arom.).

3.2. *N-(ω-Haloalkanoyl)-2-substituted phenothiazines and other N-(ω-haloalkanoyl)tricycles*

A solution of the suitable ω-haloalkanoyl chloride (10% excess) in 5 ml of dry toluene was added dropwise to a solution of the proper tricyclic compound (2.6–5 mmol) in dry toluene (30 ml). The solution was then heated at reflux for 3–7 h; the completion of the reaction was monitored by TLC on alumina. After cooling, the toluene solution was either washed with water, dried and evaporated under reduced pressure (chloroacetyl and propionyl derivatives) or evaporated directly under reduced pressure, rinsing the residues with pentane (4-bromobutyrylphenothiazines) or dry ether (ω-bromohexanoylphenothiazines). The oily residues were chromatographed on alumina, eluting with dichloromethane.

Yields were in the 84–98% range.

Most compounds have already been described [11,18–24] and measured melting points were in agreement with the published data.

The new *N*-(4-bromobutyryl)-2-methoxyphenothiazine melted at 132–134 °C, while the other new compounds [*N*-(chloroacetyl)iminodibenzyl, *N*-(5-bromopentanoyl)phenothiazine, *N*-(5-bromopentanoyl)-2-methoxyphenothiazine and *N*-(6-bromohexanoyl)-2-methoxyphenothiazine] were oils and after the chromatography were used directly in the next step.

All the *N*-(ω-haloalkanoyl)tricycles exhibited a sharp carbonyl band in the IR spectrum (KBr or film): 1661–1666 cm^{-1} for iminodibenzyl derivatives; 1664–1681 cm^{-1} for phenothiazine derivatives.

3.3. *N-[(2-lupinylthio)acetyl]3-substituted iminodibenzyls (**7** and **8**) and N-[(2/3-lupinylthio)acetyl/propionyl]-2-substituted phenothiazines (**9**–**14**)*

A solution of 2–5 mmol of thiolupinine [17] in 5 ml of absolute ethanol was rapidly added to a solution of *N*-(2/3-chloroacetyl/propionyl)tricycle (2–5 mmol) in absolute ethanol (30 ml).

The solution was refluxed under nitrogen for 5–7 h and then evaporated under reduced pressure. The residue was dissolved in acidic water and extracted three times with ether to remove the unreacted haloalkanoyltricycle. The acid solution was basified with 2 N NaOH solution and the free base was worked as needed.

The solid compounds were collected and crystallized from ethanol/water (**9** and **10**). Oily compounds were extracted with ether; the ether solution was washed with water, dried and evaporated to dryness. The residue was rinsed with little dry ether and crystallized from the same solvent (**7**, **11**, **14**). Compounds **8**, **12** and **13** were chromatographed on neutral alumina, eluting with ether. After chromatography, compound **12** contained a small amount of lupinyldisulfide. Therefore, it was dissolved in ethanol and treated with 1 N HCl and zinc dust; the solution was basified and extracted with ether. The ether solution was chromatographed on basic alumina, eluting with ether.

Characteristics of compounds **7**–**14** are collected in Table 1.

^{1}H NMR ($CDCl_3$) of compound **7**: δ 1.07–2.11 (m, 14H, Q), 2.61–3.06 (m, 6H, 4H of CH_2 of dibenzyl, 1H of S–CH_2–Q and 1H of Q), 3.06–3.68 (m, 4H, 2H of C(O)–CH_2–S, 1H of S–CH_2–Q and 1H of Q), 7.05–7.48 (m, 8H arom.) ppm.

^{1}H NMR ($CDCl_3$) of compound **9**: δ 1.08–2.13 (m, 14H, Q), 2.68–2.89 (m, 3H, 1H of S–CH_2–Q and 2H of Q), 2.99 (dd, J = 4.8 and 12.9 Hz, 1H of S–CH_2–Q), 3.37 (s, 2H, C(O)–CH_2–S), 7.16–7.40 (m, 4H arom.), 7.46 (dd, J = 1.6 and 7.4 Hz, 2H arom.), 7.60 (d, J = 7.8 Hz, 2H arom.) ppm.

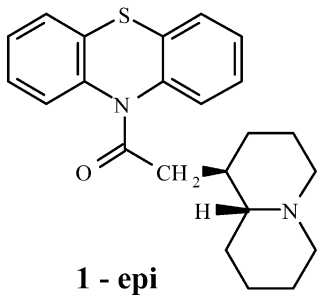
^{1}H NMR ($CDCl_3$) of compound **14**: δ 1.08–2.09 (m, 14H, Q), 2.50–2.70 (m, 2H, 1H of S–CH_2–Q and 1H of Q), 2.70–2.94 (m, 6H, 4H of C(O)–CH_2–CH_2–S, 1H of S–CH_2–Q and 1H of Q), 3.82 (s, 3H of OCH_3), 6.83 (dd, J = 2.7 and 8.7 Hz, 1H arom.), 7.12 (d, J = 2.6 Hz, 1H arom.), 7.16–7.40 (m, 3H arom.), 7.40–7.55 (m, 2H arom.) ppm.

3.4. *N-[(ω-Lupinylthio)alkanoyl]-2-substituted phenothiazines (**15**–**20**)*

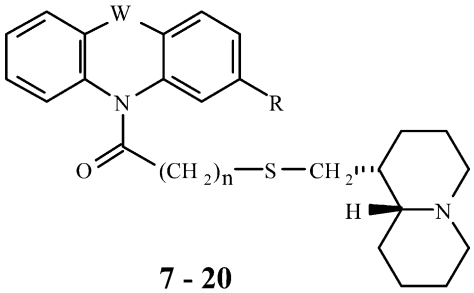
A solution of *N*-(ω-bromoalkanoyl)-2-substituted phenothiazine (3.2–3.5 mmol) in 2–3 ml of acetonitrile, which was followed by a solution of thiolupinine (3.2–3.5 mmol) in 2 ml of the same solvent, was introduced into an Aldrich pressure tube, flushed with nitrogen.

The tube was sealed and heated at 80 °C for 6 h (butyryl derivatives), or at 100° C for 16–22 h (for the remaining compounds). After cooling, the tube content was diluted with acidic water and the unreacted bromoalkanoylphenothiazine was removed by filtration and extraction with ether. The acid solution was basified and extracted with dichloromethane. After removing the solvent, the residue was chromatographed on alumina (1:25), eluting with ether (butyryl derivatives) or dichloromethane (the remaining compounds). Dichloromethane cannot be used to remove the unreacted

Table 1
Characteristics of novel compounds **1-epi** and **7**–**20** of Chart 1



1 - epi



7 - 20

Comp.	W	*n*	R	Formula [a]	m.p. (°C) [b]	Yield (%) [c]
1-epi				$C_{23}H_{26}N_2OS$, $C_{23}H_{26}N_2OS \cdot HCl \cdot 0.5H_2O$	121.5–122.5	70
7	CH_2CH_2	1	H	$C_{26}H_{32}N_2OS$, $C_{26}H_{32}N_2OS \cdot HCl \cdot 1.75H_2O$	104–105	73
8	CH_2CH_2	1	Cl	$C_{26}H_{31}ClN_2OS$ [d], $C_{26}H_{31}ClN_2OS \cdot HCl \cdot H_2O$	76–78	70
9	S	1	H	$C_{24}H_{28}N_2OS_2$ [e], $C_{24}H_{28}N_2OS_2 \cdot HCl \cdot 0.5H_2O$ [e]	131–132	67
10	S	1	Cl	$C_{24}H_{27}ClN_2OS_2$, $C_{24}H_{27}ClN_2OS_2 \cdot HCl \cdot 2H_2O$	127–128	61
11	S	1	CF_3	$C_{25}H_{27}F_3N_2OS_2$, $C_{25}H_{27}F_3N_2OS_2 \cdot HCl \cdot 1.75H_2O$	96–97	61
12	S	1	OCH_3	$C_{25}H_{30}N_2O_2S_2$ [e], $C_{25}H_{30}N_2O_2S_2 \cdot HCl \cdot H_2O$	Oil	40
13	S	2	H	$C_{25}H_{30}N_2OS_2$ [d], $C_{25}H_{30}N_2OS_2 \cdot HCl \cdot 0.75H_2O$	Oil	43
14	S	2	OCH_3	$C_{26}H_{32}N_2O_2S_2$, $C_{26}H_{32}N_2O_2S_2 \cdot HCl \cdot 0.5H_2O$ [e]	106–107	50
15	S	3	H	$C_{26}H_{32}N_2OS_2$ [d], $C_{26}H_{32}N_2OS_2 \cdot HCl \cdot 1.25H_2O$ [e]	Oil	40
16	S	3	OCH_3	$C_{27}H_{34}N_2O_2S_2$ [d], $C_{27}H_{34}N_2O_2S_2 \cdot HCl \cdot H_2O$ [e]	Oil	44
17	S	4	H	$C_{27}H_{34}N_2OS_2$, $C_{27}H_{34}N_2OS_2 \cdot HCl \cdot H_2O$ [e]	Oil	45
18	S	4	OCH_3	$C_{28}H_{36}N_2O_2S_2$ [d], $C_{28}H_{36}N_2O_2S_2 \cdot HCl \cdot 0.5H_2O$ [e]	Oil	41
19	S	5	H	$C_{28}H_{36}N_2OS_2$, $C_{28}H_{36}N_2OS_2 \cdot HCl \cdot 1.5H_2O$ [e]	Oil	52
20	S	5	OCH_3	$C_{29}H_{38}N_2O_2S_2$ [e], $C_{29}H_{38}N_2O_2S_2 \cdot HCl \cdot 0.75H_2O$ [e]	Oil	43

[a] All compounds were analyzed for C, N, H, S and results were within ±0.3% of the calculated values, unless otherwise stated.
[b] m.p. of hydrochlorides are not indicated because they melt in a wide range of temperature.
[c] The indicated yields refer to the free bases; the conversion to hydrochlorides was practically quantitative.
[d] Not analyzed.
[e] The sulfur content resulted lower than the calculated value for more than 0.3%.

bromoalkanoylphenothiazine since it also dissolves certain amount of lupinylthioalkanoylphenothiazine hydrochloride.

Characteristics of compounds **15**–**20** are collected in Table 1.

3.5. Binding assays

Binding assays were performed at the Receptology Department of CEREP, Celle L'Evescault, France, or, for central nicotinic receptor, at MDS Panlabs, Inc., Bothell, WA.

Membranes were prepared by standard techniques and incubated in the conditions indicated below for each receptor subtype. After incubation, membranes were rapidly filtered under vacuum through glass-fiber filters (GF/B, Packard). Filters were washed three times with ice-cold buffer and bound radioactivity was measured with a scintillation counter (Topcount, Packard or Betaplate), using a liquid scintillation cocktail (Microscint 0, Packard).

Compounds were tested at four or five concentrations in the range 10 μM–1 nM. K_i values are the means of duplicate experiments.

3.5.1. Muscarinic M_1 binding

Membranes from rat brain cortices were incubated with 1 nM [^{3}H]pirenzepine ($K_d = 6.7$ nM) for 60 min at 22 °C. Non-specific binding was determined in the presence of 1 μM atropine.

3.5.2. Muscarinic M_2 binding

Membranes from rat heart were incubated with 2 nM [^{3}H]AF-DX 384 ($K_d = 2.1$ nM) for 60 min at 22 °C. Non-specific binding was determined in the presence of 1 μM atropine.

3.5.3. Central nicotinic acetylcholine receptor binding

Membranes from rat brain cortices were incubated with [^{3}H]cytisine ($K_d = 3.2$ nM) at a concentration of 2 nM for 75 min at 4 °C. Non-specific binding was estimated in the presence of 100 μM nicotine.

3.6. Inhibition of human acetylcholinesterase

Compound **1** was tested as an acetylcholinesterase inhibitor with the method described by Ellman et al. [25] by MDS Panlabs, Inc. Human recombinant acetylcholinesterase (Sigma, C-1682) was used.

Test compound and/or vehicle was incubated with acetylthiocholine iodide (700 μM) and 5,5-dithiobis-2-nitrobenzoic acid in sodium phosphate buffer (pH 7.4) at 37 °C. The reaction was initiated by the addition of 2 ng acetylcholinesterase and the thiocholine generated reacted continuously with dithiobis(nitrobenzoic)acid to produce a yellow anion (5-thio-2-nitrobenzoic acid) proportional to enzymatic activity. This was determined after 20 min by spectrophotometry at 405 nm. The compound was initially screened at 10 μM. Physostigmine was used as reference compound, with $IC_{50} = 0.12$ μM.

3.7. Muscarinic M_1 functional response

The field stimulated (95% of maximum voltage, 0.1 Hz, 0.5 ms) prostatic portion of rabbit vas deferens, bathed in physiological salt solution at 31 °C, was used [26] (MDS Panlabs, Inc.). Test substance-induced reduction of resultant neurogenic twitch responses (> 50% related to control 3 μM McN-A-343 response) indicated possible M_1 receptor agonism. At a test concentration where no significant agonistic activity was seen, ability to inhibit the McN-A-343-induced relaxation response indicated antagonist activity.

4. Results and discussion

Results of binding assay to M_1 and M_2 muscarine receptor subtypes are collected in Table 2.

All tested compounds were highly capable of displacing specific ligands ([^{3}H]pirenzepine and [^{3}H]AF-DX 384) from M_1 and M_2 receptor subtypes, with several of them exhibiting K_i values in the nanomolar range. However, only moderate selectivity for one or the other subtype was observed, with a ratio of K_iM_2/K_iM_1 in the range 10.4–0.36. The most selective compound was 10-(epi-homolupinanoyl)phenothiazine (**1-epi**).

These results might appear in conflict with the previous observation of a very high selectivity of compound **1** for M_1 versus M_2 receptor subtype [7].

Indeed, at that time affinity for M_2 subtype was determined through the displacement of [^{3}H]*N*-methylscopolamine, a non-selective ligand that binds to this subtype much more tightly than the selective ligand AF-DX 384.

Concerning the structure–activity relationships, the following comments are relevant.

On the whole, the *N*-homolupinanoyl derivatives **1**–**5** exhibited a lower affinity for both M_1 and M_2 subtypes in comparison to the *N*-[ω-(*S*-lupinylthio)alkanoyl]derivatives **6**–**20** and affinity was generally higher for M_1 than for M_2 subtype.

It is worth noting that affinity for M_1 and M_2 receptor subtypes was influenced in opposite directions (enhancing the former and decreasing the latter), when the connection of the chain to the quinolizidine ring was changed from axial (**1**) to equatorial (**1-epi**).

In the set of *N*-homolupinanoyl derivatives, the exchange of phenothiazine ring with the dihydropyridobenzodiazepinone moiety (**5**) reduced the affinity for both receptors. The introduction of substituents on position 2 of phenothiazine ring (**2** and **3**) did not modify significantly the affinity for both subtypes, with the exception of the polar ethoxycarboamido group (**4**) which resulted deleterious to the affinity for M_1 subtype.

The comparison of affinities of compounds **6**, **7** and **9**, bearing the same (*S*-lupinylthio)acetyl residue on the cyclic nitrogen and lacking any substituent on the aromatic nucleus indicates that the iminodibenzyl moiety is less suitable for binding to both M_1 and M_2 subtypes, while the phenothiazine and the dihydropyridobenzodiazepinone systems gave comparable results in spite of their different polarity and lipophilicity.

The 2-unsubstituted *N*-[ω-(*S*-lupinylthio)alkanoyl]phenothiazines **9**, **13** and **15** exhibited nanomolar affinity for both M_1 and M_2 receptor subtypes, with K_i comparable with those of pirenzepine (2.6 nM) and methoctramine (4.46 nM).

Thus, the importance of the length of the chain connecting the tricyclic system to the quinolizidine nucleus is confirmed. However, further increasing the number of carbon atoms of the alkanoyl residue (**17** and **19**), a moderate decrease of affinity was observed, which is somewhat stronger for M_1 than for M_2 subtype, with a consequent inversion of selectivity.

Also in the set of *N*-(lupinylthio)alkanoyl derivatives, the introduction of substituents on position 2 of the phenothiazine ring has only moderate effects on the affinity to receptors: chlorine and trifluoromethyl group reduced the affinity for both M_1 and M_2 subtypes, while the methoxy group generally reduced the affinity for M_2, but improved that for M_1 subtype.

In order to gain some insight on the functional characteristics of the relevant compounds which could be useful for the future development of this preliminary study, a few other assays were performed on single selected compounds.

Compound **1** was assayed for inhibition of acetylthiocholine hydrolysis produced by human recombinant acetylcholinesterase; at a 10-μM concentration, this compound failed to inhibit the enzyme.

Compound **6** was tested for affinity to central nicotinic receptor, but even at a 10-μM concentration,

Table 2
Affinity of compounds **1**–**20** (Chart 1) for rat muscarinic M_1 and M_2 receptor subtypes

No.	Comp.	*n*	R	K_i [a](nM)		Ratio K_i (M_2/M_1)
				M_1	M_2	
N-Homolupinanoyl and epi-homolupinanoyl						
1	phenothiazine		H	31	82	2.64
1-epi	phenothiazine		H	9.8	102	10.4
2	phenothiazine		Cl	30	65	2.16
3	phenothiazine		OCH_3	43	96.5	2.24
4	phenothiazine		NHCOOEt	150	107	0.71
5	dihydropyridobenzodiazepinone			76	107.6	1.41
N-(Lupinylthio)acetyl-						
6	dihydropyridobenzodiazepinone			6.9	4.6	0.67
7	iminodibenzyl		H	34	12.3	0.36
8	iminodibenzyl		Cl	11	14.8	1.34
9	phenothiazine		H	4.2	6.2	1.48
10	phenothiazine		Cl	6.1	13.3	2.18
11	phenothiazine		CF_3	4.7	15.2	3.23
12	phenothiazine		OCH_3	2.8	7.7	2.75
N-(ω-Lupinylthio)alkanoyl-						
13	phenothiazine	2	H	3.1	3.3	1.06
14	phenothiazine	2	OCH_3	2.3	4.5	1.96
15	phenothiazine	3	H	3.0	4.8	1.60
16	phenothiazine	3	OCH_3	4.0	2.5	0.62
17	phenothiazine	4	H	17.9	7.8	0.43
18	phenothiazine	4	OCH_3	10.7	10	0.93
19	phenothiazine	5	H	35.9	13	0.36
20	phenothiazine	5	OCH_3	23.3	19.5	0.84
	pirenzepine			2.6	206 [b]	79.2
	methoctramine			17.8 [b]	4.46	0.25

[a] Mean of duplicate experiments: each value differed from the mean by less than 10%.
[b] Historical K_i values from CEREP.

it was unable to displace [^{3}H]cytisine from a rat brain preparation.

Finally, M_1 antagonism was demonstrated for compound **5**, which inhibited the McN-A-343 (4-(3-chlorophenylcarbamoyloxy)-2-butyryltrimethylammonium chloride)-induced relaxation response of field stimulated prostatic portion of rabbit vas deferens, with $IC_{50} = 270$ nM (pirenzepine $IC_{50} = 120$ nM).

Therefore, even on the basis of the previously described [7] functional studies on isolated tissues (guinea pig ileum and left atria), it can be argued that our compounds behave as M_1 and M_2 antagonists, however, the muscarinic receptor antagonism will be unambiguously defined when the same functional investigations are carried out on the whole set of compounds.

5. Conclusion

The homolupinanoyl- and lupinylthioalkanoyl derivatives of some tricyclic systems (such as phenothiazine, iminodibenzyl and dihydropyridobenzodiazepinone) display good affinity to both M_1 and M_2 receptors, with nanomolar K_i's which, for the best compounds *N*-[(ω-lupinylthio)propionyl/butyryl]phenothiazines (**13**–**16**), were comparable to those of pirenzepine and methoctramine, respectively.

Only moderate selectivity for one or the other subtype was seen in rat receptors labeled with [^{3}H]pirenzepine and [^{3}H]AF-DX 384, respectively. The highest selectivity for M_1 versus M_2 receptor subtype was seen in compound **1-epi**, the only one bearing the quinolizidine moiety linked equatorially.

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