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QUINOLIZIDINYL DERIVATIVES OF 5,11-DIHYDRO-6H-PYRIDO[2,3-b][1,4]BENZODIAZEPIN-6-ONE AS LIGANDS FOR MUSCARINIC RECEPTORS

Federica Novelli [a], Anna Sparatore [b], Bruno Tasso [a], Fabio Sparatore [a] *

a Dipartimento di Scienze Farmaceutiche - Università di Genova
Viale Benedetto XV, 3 - 16132 Genoa, Italy

b Istituto Chimico Farmaceutico e Tossicologico - Università di Milano
Viale Abruzzi, 42 - 20131 Milan, Italy

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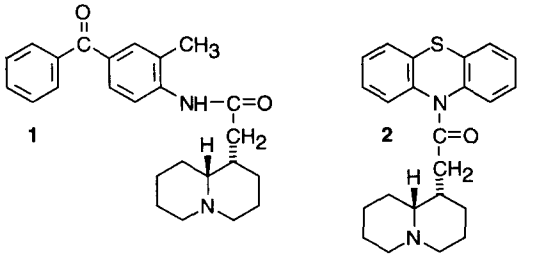
Abstract: Quinolizidinyl derivatives of the tricyclic systems characterizing pirenzepine and nuvenzepine, were prepared and tested as ligands for muscarinic M_1, M_2 and M_3 receptors; 5,11-dihydro-11-[(S-lupinyl)-thioacetyl]-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one exhibited IC_{50} = 10 nM for M_1 and 760 nM for both M_2 and M_3 subtypes. During the synthesis some interesting side compounds were isolated and characterized.

A few years ago we showed [1] that 3-methyl-4-(N-homolupinanoyl)aminobenzophenone **1** was able to displace [^{3}H]-pirenzepine from muscarinic M_1 receptors with IC_{50} = 8 nM, while much higher concentrations of this compound were required to displace [^{3}H]-methylscopolamine from M_2 and M_3 receptors, with IC_{50} respectively 2400 and 1300 nM. Thus compound **1** exhibited a selectivity for M_1 versus M_2 and M_3 muscarinic receptor subtypes quite superior to that shown by pirenzepine.

The N-homolupinanoylphenothiazine **2** behaved similarly [2], suggesting that the rigid and bulky quinolizidine nucleus, characterizing the homolupinanoyl moiety, could play an important role for such a selectivity on M_1 receptors.



Therefore we deemed worthy to explore the effect of combining in the same molecule the quinolizidine nucleus with the tricyclic moiety 5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one, which characterizes pirenzepine, AF-DX-116, AQ-RA741 and other potent and selective muscarinic ligands [3-5].

Fax number: 39 010 3538358; E-mail: sparator@unige.it

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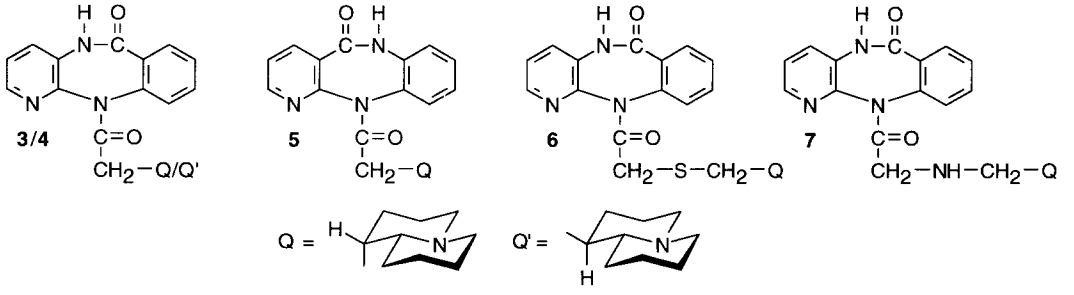
As pointed out by Eberlein *et al.* [6], among the pyridobenzodiazepinone derivatives, the muscarinic subtype recognition is fundamentally determined by the nature of the basic side chain and the spatial placement of its terminal protonated nitrogen in relation to the tricyclic backbone.

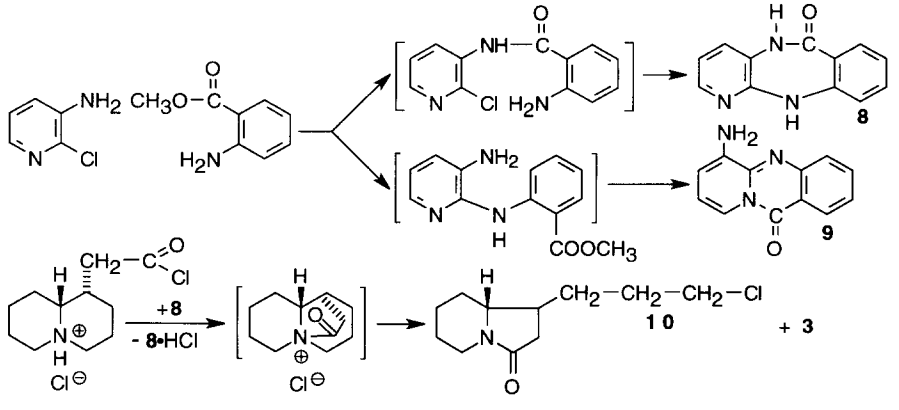
Thus, in order to somehow vary the spatial location of the quinolizidine nitrogen relative to the tricyclic system the set of compounds **3-7** was prepared.



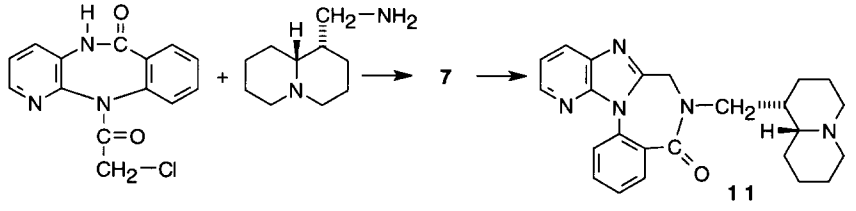
5,11-Dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one [5] or the isomeric 6,11-dihydro-5H-pyrido[2,3-b]-[1,5]benzodiazepin-5-one [7] (8 mmol) was reacted with homolupinanoyl chloride hydrochloride or *epi*-homolupinanoyl chloride hydrochloride [1,8,9] (4.8 mmol) in chloroform/dioxane to obtain compounds **3-5**. 5,11-Dihydro-6H-pyrido[2,3-b]-[1,4]benzodiazepin-6-one was also reacted with chloroacetyl chloride and the corresponding chloroacetyl derivative [10], in ethanol solution, was treated with thiolupinine [11] and amino-lupinane [12] respectively to have compounds **6** and **7**.

During the synthesis of the required 5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one (**8**) from 3-amino-2-chloropyridine and methyl anthranilate, according to the method of Engel *et al.* [5], we isolated (by crystallization of crude **8** from methoxy-ethanol) a side product not found by the Authors and identified as 6-amino-11H-pyrido[2,1-b]quinazolin-11-one (**9**), which was previously obtained by Kovac *et al.* [13] through a different synthetic sequence. The formation of the last compound should follow the collateral chloro atom displacement by the anthranilate amino group, reaffirming the difficulty to form 7-membered rings when 6-member rings are also possible.

When the homolupinanoyl chloride hydrochloride was reacted with the basic pyridobenzodiazepinone **8** (or the isomeric trycicle), besides a small amount of the expected homolupinanoyl derivative **3** (or **5**), a lactam of structure **10** (1-[(3-chloro)-propyl]-3-oxooctahydroindolizine) was mainly formed through a preliminary deprotonation and intramolecular acylation.



Another interesting side reaction was observed during the preparation of compound **7**, which progressively rearranged to compound **11** through a reaction of the side chain secondary amino group with the seven-membered lactam function. Compound **11** represents the 6-lupinyl derivative of a novel heterocyclic ring system (6,7-dihydro-6,8,12,12b-tetraazadibenzo[a,h]azulen-5-one).



Compounds **3-7**, together with the side product **11,** were tested for displacement of [^{3}H]pirenzepine from muscarinic M_1 receptors (brain cortex of rat) and [^{3}H]methylscopolamine from muscarinic M_2 (rat heart) and M_3 (submaxillary salivary glands of rats) receptors. The results of the binding assays are collected in **Table 1**.

Table 1 - Results of binding assays on muscarinic receptor subtypes *

	IC_{50} (nM)			ratio IC_{50}	
Compound	M_1	M_2	M_3	M_2/M_1	M_3/M_1
3	110	6000	>10000	54.5	>91
4	40	1900	3500	47.5	87.5
5	650	6800	>10000	10.5	>15.4
6	10	760	760	76	76
7	28	1000	800	35.7	28.6
11	1200	12000	>10000	10	>8.3
pirenzepine	3.8	690	300	181.6	78.9

* IC_{50} values are the mean of duplicate experiments

Conforming to the expectations, compounds **3**, **4**, **6** and **7** exhibited a good affinity for M_1 muscarinic receptors, which was quite superior to that for M_2 and M_3 subtypes. Compound **6** was the most active, resulting just a little inferior to pirenzepine concerning both the affinity for M_1 receptor and selectivity for M_1 versus M_2 subtypes. Compound **4**, bearing the quinolizidine nucleus linked equatorially, exhibited higher affinity to muscarinic receptors than its axial epimer **3**.

Comparing compounds **6** and **7** with **3** and **4**, it is observed that affinity for M_1 receptors is increasing with the increase of the distance between the quinolizidine nucleus and the tryciclic system.

Finally compound **5** and **11**, containing a modified heteropolycyclic system, showed only poor affinity for all tested muscarinic receptors.

On the whole these results, while supporting the observation of Eberlein *et al.* [6] on the importance of the nature and spatial placement of the basic side chain relatively to the polycyclic system, warrant further investigation to verify the observed trend of increasing affinity with the increase of the side chain length.

Moreover it is worth noting that compound **6,** as hydrochloride, is soluble in dichloromethane and therefore it is more lipophilic than pirenzepine and likely able to pass the blood brain barrier; thus the pharmacological profile of **6** and similar compounds should be also investigated, since activity on CNS could be expected.

Experimental

Melting points were measured in a vacuum sealed capillary on a Büchi apparatus and are uncorrected. Elemental analyses were performed with CE EA 1110 CHNS-O instrument and the results (CHN for free bases, CHNCl for hydrochlorides) were within ±0.3% of calculated values. UV and IR spectra were recorded, respectively, on Perkin Elmer mod. 550S and Paragon 1000 PC spectrophotometers. ^{1}H NMR spectra were taken on a Varian Gemini 200 spectrometer using $CDCl_3$ as solvent; chemical shifts are reported in ppm.
^{1}H NMR spectra of compounds **3**, **4**, **5**, **6** and **7** were conforming to the expected without any particular feature.

General procedure

The reaction mixtures were either heated, for 18-20 h, at 120°C in a closed tube (**3-5**), or refluxed for the same time under nitrogen (**6-7**). After removing the solvent, the residue was taken up with acidic water, filtering in the case of **3-5** the unreacted tricycle, or extracting with ether the unreacted chloroacetyl-tricycle (**6-7**). The acid solution was made alkaline and extracted with dichloromethane. The crude basic product was either chromatographed on alumina (**3**, **4**, **5**), using dichloromethane followed by dichloromethane plus 0.5% methanol as eluents, or just washed with dry ether (**6**). In the preparation of **7**, the alkalization produced first the precipitation of the side product **11** and then the separation of **7**, which was extracted as usual. The latter compound was purified by chromatography on silica gel eluting with ethanol plus 3% diethylamine.

3: m.p. 240-243°C; $C_{23}H_{26}N_4O_2$.

4: m.p. 184-185°C; $C_{23}H_{26}N_4O_2$. Hydrochloride: $C_{23}H_{26}N_4O_2 \cdot HCl \cdot H_2O$

5: m.p. 223-225°C; $C_{23}H_{26}N_4O_2$. Hydrochloride: $C_{23}H_{26}N_4O_2 \cdot HCl \cdot 0.75H_2O$.

6: m.p. 197-199°C; $C_{24}H_{28}N_4O_2S$. Hydrochloride: m.p. 230-231°C; $C_{24}H_{28}N_4O_2S \cdot HCl \cdot H_2O$.

7: m. p. undefined (progressive conversion to **11**); $C_{24}H_{29}N_5O_2$. Hydrochloride $C_{24}H_{29}N_5O_2 \cdot HCl \cdot 1.75H_2O$.

11: m.p. 186-188°C (ethanol); $C_{24}H_{27}N_5O$; ^{1}H NMR: δ 8.70 (dd, J = 8.08, 1.62, 1H arom.), 8.34 (dd, J = 7.92, 0.96, 1H arom.), 8.11 (dd, J = 4.76, 1.60, 1H arom.), 7.90-7.60 (m, 2H arom.), 7.60-7.40 (m, 1H arom.), 6.83 (dd, J = 8.09, 4.80, 1H arom.), 4.31 and 4.23 (quart AB, J = 14.64, 2H, CH_2-N), 4.00-3.80 (dd, J = 13.50, 9.10, 1H, CH_2-Q), 3.80-3.60 (dd, J = 13.50, 4.50, 1H, CH_2-Q), 3.00-2.70 (m, 2H, Q), 2.30-1.00 (m, 14H, Q).

10: oil (chromatographed on neutral alumina activity III, with CH_2Cl_2); $C_{11}H_{18}ClNO$; IR: 1688 cm^{-1} (C=O in a five membered lactam); ^{1}H NMR: δ 4.20-4.00 (dm, 1H, CH_2-N); 3.54 (t, J = 6.20, 2H, CH_2-Cl), 3.10-2.90 (m, 1H, CH-N), 2.70-2.40 (m, 1H, CH_2-N; δ 2.54, dd, J = 16.23, 8.35, 1H, CH_2-CO), 2.07 (dd, J = 8.65, 1.65, 1H, CH_2-CO), 2.00-1.00 (m, 11H).

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