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Synthesis and Anticalcium Activity of New Compounds Containing the 2,3-Dihydro-1,4-dioxino[2,3-*b*]pyridine System

I. Sánchez,[1] MD. Pujol,[1,*] G. Guillaumet,[2] R. Massingham,[3] A. Monteil,[4] G. Dureng,[4] E. Winslow[4]

[1]Laboratori de Química Farmacèutica, Fac. Farmàcia, Universitat de Barcelona, Avda. Diagonal 643, 08028 Barcelona (Spain). Telephone: 934024534; Fax: 934035941; E-mail: mdpujol@farmacia.far.ub.es.

[2]Institut de Chimie Organique et Analytique associé au CNRS, Université d'Orléans, BP-6957, 45067 Orléans, Cedex 2 (France).

[3]UCB Pharma, Pharmaceutical Sector, Chemin du Foriest, B-1420 Braine-L'Alleud (Belgique).

[4]Laboratoire RL-CERM, ZI La Varenne, rue H. Goudier, 63203 Riom, Cedex (France).

Summary: New compounds (**3** and **4**) possesing the 2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine group were synthesized and tested as calcium antagonist agents. Both of them showed moderate anticalcium activity.

Key words: 1,4-dioxino[2,3-*b*]pyridine, 1,4-benzodioxin, anticalcium activity.

The calcium antagonists, represented by calcium channel blockers and calmoduline antagonists, are a well-established, important class of drugs widely used in the treatment of cardiovascular diseases.[1,2] This kind of agents constitutes a very heterogeneous group with different structural and pharmacological properties.[1,2] In particular, we have recently described the synthesis and pharmacological activity of a series of 1,4-benzoxazine,[3] and 1,4-benzodioxin[4] derivatives with anticalcium properties. Our initial results showed that compounds **1** and **2** presented an anticalcium activity, in the tests assessed, comparable with those of the patterns tested. In order to extend this work, we have now reported the synthesis and biological activity of new compounds (**3** and **4**) containing the 2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine heterocyclic system with the aim to identify and establish

additional structural features and structure-activity relation-ship on this group of compounds (Fig. 1).

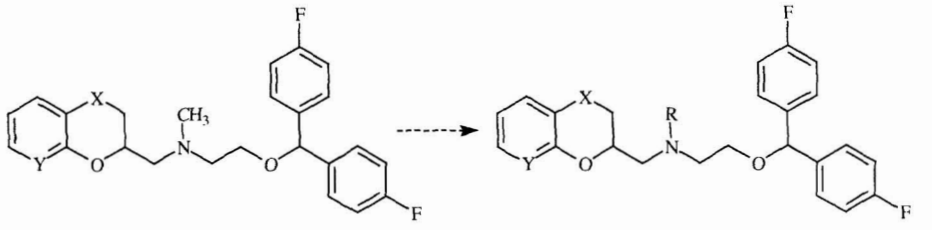
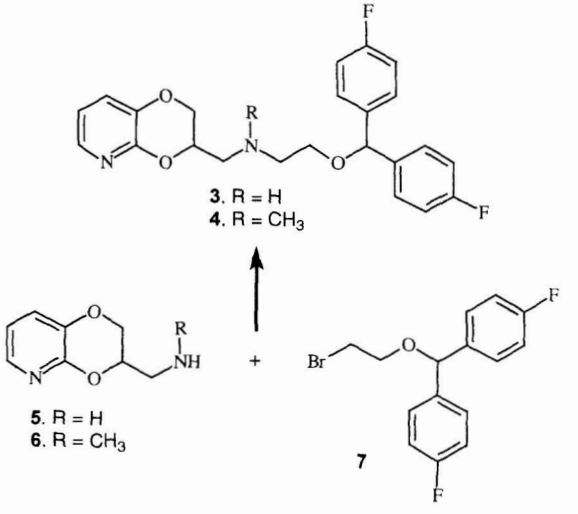


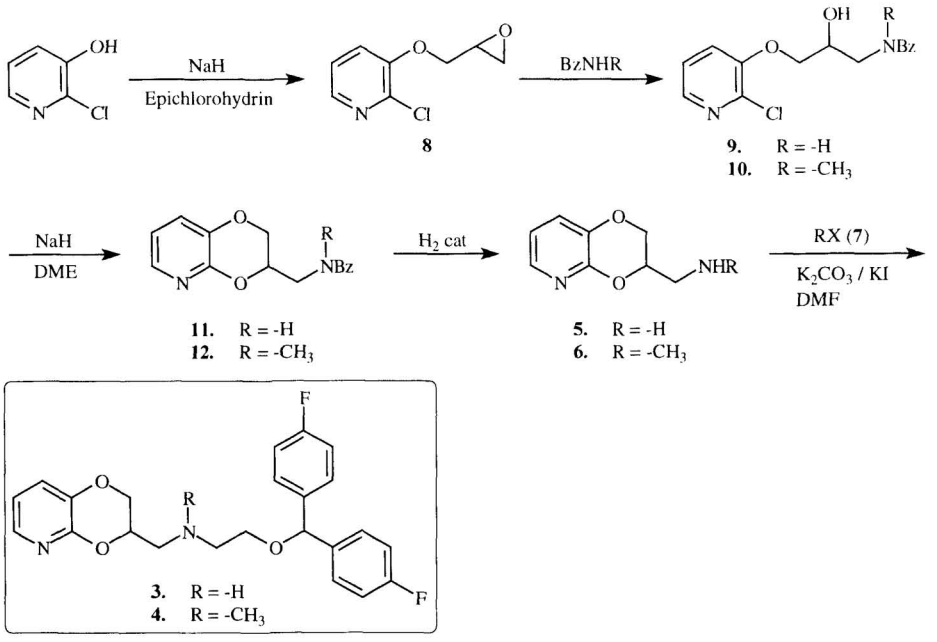
Figure 1. Bioisoster structures of the reported anticalcium agents.

The synthesis of compounds **3** and **4** was based on the alkylation of the corresponding primary (**5**) or secondary (**6**) amine with the bis(*p*-fluorophenyl)methoxyethyl bromide,[3] according to the scheme 1.



Scheme 1

The required key amines **5** and **6** were synthesized through a described procedure,[5,6,7] by treatment of 2-chloro-3-pyridinol with epichlorohydrin in basic media (scheme 2).



Scheme 2

The opening of the epoxide **8** with the appropriate amine (benzylamine or benzylmethylamine) provided the aminoalcohols **9** and **10**. The cyclization of these was carried out with NaH in DME affording the amines **11** and **12** which contain the 2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine group in their structure. The required primary or secondary amines **5** and **6** were generated by deprotection of **11** and **12** respectively under hydrogenation conditions in excellent yields. Alkylation of **5** and **6** by the alkyl bromide **7** in the presence of anhydrous K_2CO_3 and KI[3] gave the expected derivatives **3** and **4** in 56% and 71% yield respectively.

The anticalcium activity of the reported compounds was measured in terms of their capacity to inhibit the contractions induced by agonists in the vascular smooth muscle which modifie the concentration of free calcium. The compounds synthesized were subjected to two pharmacological tests and the results are listed in the table 1. The detailed testing procedures have been reported[8,9] previously. The tested compounds **3** and **4** exhibited a moderate activity as anticalcium agents.

The introduction of a pyridine ring in the place of the benzene nucleus impliy a weak decrease of the anticalcium activity, respect to the potassium test, however showed a comparable behavior with the patterns **1** and **2**, according to the caffeine test. The observed results showed that the anticalcium activity does not depend on the heterocyclic system, and the major basic character of the pyridine does not have an effect in the activity, respect to these biological tests. The presence of a tertiary amine in the lateral chain (compound **4**) has a relevant effect on the activity, in accordance with the potassium test. In comparing with the caffeine test, the effect of the tertiary amine is less significant and, on the contrary, compound **3** (secondary amine) is more active than compound **4** (tertiary amine).

In conclusion, we have established that the bioisoster changes related above led to compounds which showed a calcium antagonist activity equivalent to that seen, not only in their analogues **1** and **2**, but in important, well known anticalcium agents such as nifedipine, verapamil, diltiazem and flunarizine.

Table 1. Anticalcium activity of the **1-4** compounds

	Caffeine IC_{50} **(μM)**	**Potassium** IC_{50} **(μM)**
1	>30 (23%)	5.1
2	>30 (0%)	0.28±0.09
3	30	>10 (30%)
4	>30 (38%)	7.5
Diltiazem	>100 (10%)	0.64
Flunarizine	>30 (5%)	1.5
Nifedipine	>10 (4%)	0.0086
Verapamil	>100 (32%)	0.24±0.06

Experimental

General

NMR spectra were recorded on a Varian-200 instrument at 200 (^{1}H) or 50.3 (^{13}C) MHz using tetramethylsilane as an internal standard. The assignments of ^{13}C NMR signals were made with the aid of DEPT sequence. Elemental microanalyses were performed by Serveis Científico-Tècnics, Universitat de Barcelona; results obtained for C, H and N were within ± 0.4 % of the values calculated for the formula shown. Merck 60 (40-60 microns) and Merck 60 F_{254} silica gel were used for column chromatography and thin layer chromatography respectively. The organic extracts were dried over Na_2SO_4. Organic solvents were purified by standard procedures. All reagents were of commercial quality or were purified before use.

General procedure of alkylation

Anhydrous K_2CO_3 (3 mmol) and a catalytic amount of KI were slowly added to a solution of the corresponding amine (1 mmol) and the alkyl bromide **7** (3 mmol) in 5 mL of DMF. The reaction mixture was stirred under an argon atmosphere at room temperature for 72 h. After removing the solvent, the crude product was dissolved in water and extracted with ether (3 x 40 mL). The organic layers were dried, filtered and concentrated obtaining a residue which was purified on silica gel column chromatography eluting with Hexane / AcOEt 70 / 30.

3-[N-bis(p-fluorophenyl)methoxyethylaminomethyl]-2,3-dihydro-1,4-dioxino[2,3-b]pyridine ***(3)***

Compound **3** was obtained as a yellow oil (640 mg, 73% yield) starting from the primary amine **5** (350 mg, 2.11 mmol), the alkyl bromide **7** (2.07 g, 6.33 mmol) and following the general procedure of alkylation described above. Anal. calc. for $C_{23}H_{22}N_2O_3F_2$ C 66.58%, H 5.37%, N 7.39% Found C 66.78%, H 5.27%, N 7.09%. ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.93 (m, 4H, **CH_2NCH_2**); 3.49 (m, 2H, CH_2O); 3.92 (dd, J_1 = 11.2 Hz, J_2 = 7.5 MHz, 1H, C2-H); 4.22 (dd, J_1 = 11.2 Hz, J_2 = 2.5 Hz, 1H, C2-H); 4.48 (m, 1H, C3-H); 5.27 (s, 1H, CHAr); 6.93 (m, 5H, C7-H, C3'-H, C5'-H); 7.20 (d, J = 6 Hz, C8-H); 7.25 (m, 4H, C2'-H, C6'-H); 7.82 (d, J = 5 Hz, C6-H).

3-[N-bis(p-fluorophenyl)methoxyethyl-N-methylaminomethyl]-2,3-dihydro-1,4-dioxino[2,3-b]pyridine ***(4)***

Compound **4** was obtained as a yellow oil (880 mg; 56% yield) starting from the secondary amine **6** (665 mg, 3.7 mmol), the alkyl bromide **7** (3.63 g, 11.1 mmol) and following the general procedure of alkylation. Anal. calc. for $C_{24}H_{24}N_2O_3F_2$ C 67.59%, H 5.67%, N 6.57% Found C 67.60%, H 5.65%, N 6.59%. ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.40 (s, 3H, CH_3N); 2.82 (m, 4H, $\mathbf{CH_2NCH_2}$); 3.52 (t, J = 5 Hz, 2H, CH_2O); 3.98 (dd, J_1 = 10 Hz, J_2 = 6 Hz, 1H, C2-H); 4.29 (dd, J_1 = 10 Hz, J_2 = 3 Hz, 1H, C2-H); 4.48 (m, 1H, C3-H); 5.32 (s, 1H, CHAr); 6.82 (dd, J_1 = 8 Hz, J_2 = 5 Hz, 1H, C7-H); 6.99 (m, 4H, C3'-H, C5'-H); 7.18 (d, J = 8 Hz, 1H, C8-H); 7.28 (m, 4H, C2'-H, C6'-H); 7.81 (d, J = 5 Hz, 1H, C6-H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 43.5 (CH_3, CH_3N); 57.1 (CH_2, $\mathbf{CH_2NCH_2}$); 66.0 and 67.0 (CH_2, CH_2O, C2); 72.5 (CH, C3); 82.3 (CH, CHAr); 115.1 (CH, J = 21 Hz, C3', C5'); 118.2 (CH, C7); 124.4 (CH, C8); 128.5 (CH, J = 8 Hz, C2', C6'); 137.8 (C, C1'); 139.0 (C, C8a); 139.8 (CH, C6); 151.8 (C, C4a); 162.2 (C, J = 247 Hz, C4').

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