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SYNTHESIS AND "IN VITRO" CARDIOVASCULAR ACTIVITY OF 4-ARYL-2,3,3a,4-TETRAHYDRO-1H-PYRROLO[2,1-c][1,4]BENZOTHIAZIN-1-ONES AND 7-ACETOXY-6-PHENYL-7a,8,9,10-TETRAHYDROPYRROLO [2,1-d][1,5]BENZOTHIAZEPIN-10-ONE.

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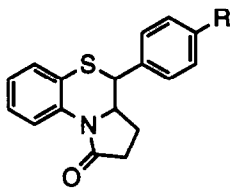
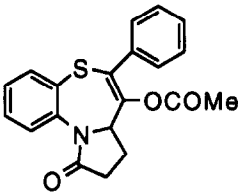
Abstract. The synthesis and the pharmacological characterization of novel heterocyclic systems **1** and **2** is described. On radioreceptor assay **1a** showed higher affinity for Calcium Channel Receptors (CCRs) and in functional studies higher potency and efficacy as negative inotropic agent than Diltiazem, without relevant calcium antagonist activity on vascular smooth muscle, revealing a clear-cut selectivity for cardiac over vascular tissue.

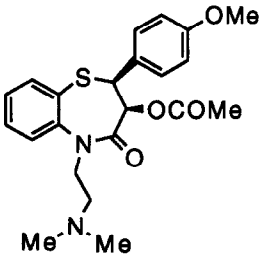
During exploration of synthetic methodologies to provide novel heterocycles and as part of our program intended to produce potential cardioactive agents[1], we synthesized pyrrolobenzothiazine and pyrrolobenzothiazepine derivatives **1a,b** and **2** related to Diltiazem **3** and Sesamodil[2] **4**. Nifedipine, Verapamil and Diltiazem are representative calcium entry blockers (CEBs) that reduce the magnitude of Ca^{++} current through the slow channels determining inhibition of the cardiac contractile apparatus.[3] Thus, blockade of the slow channels can result in a negative inotropic effect.[4a,b-8]



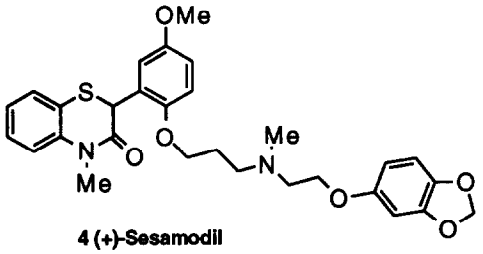
1 a R = H
b R = OMe



2



3 Diltiazem

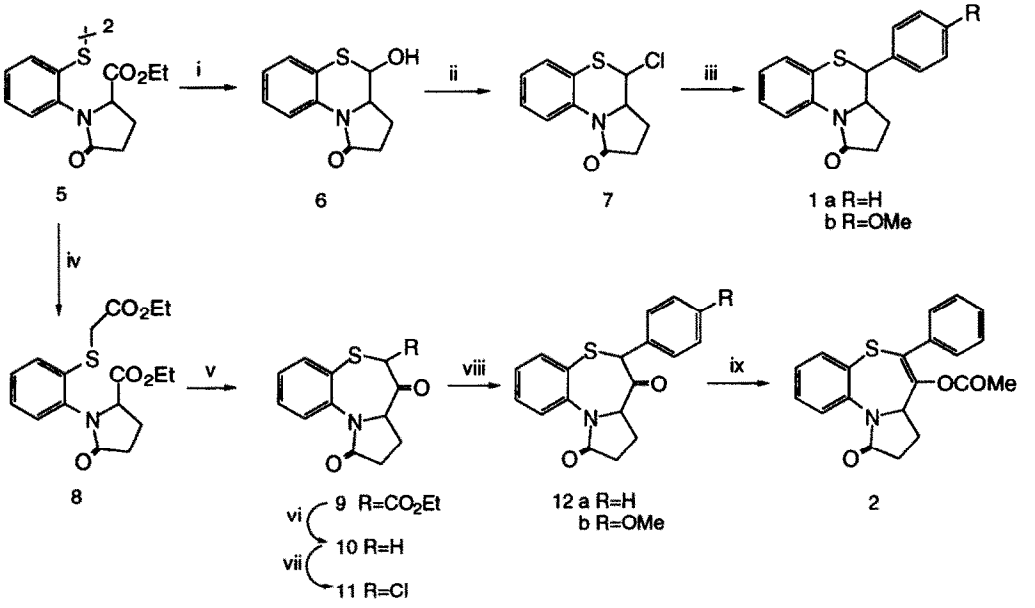


4 (+)-Sesamodil

Furthermore, Sesamodil, synthesized by Fujita and coworkers in 1990, is a prototype of a novel series of CEBs the pharmacological profile of which was found to be superior than that of Verapamil and Diltiazem. In this series of compounds is the side chain that plays an important role in the potent calcium antagonist activity,

although they exibit lower cardioselectivity compared to **3** and Verapamil. Previously, we reported our studies on the synthesis and the pharmacological characterization of conformationally rigid pyrrolobenzothiazines, structurally related to Diltiazem, where the amide moiety was substituted by a bioisosteric pyrrole ring.[1] Continuing our efforts, we describe herein the synthesis and the unexpected biological results associated with heterocycles **1** and **2** where the pyrrole ring has been replaced by a pyrrolidinone ring without the dimethylaminomethyl side chain: the alteration of the bicyclic system of Diltiazem to tricyclic tetrahydro-1H-pyrrolobenzothiazine resulted in potent calcium antagonist activity and in selectivity for cardiac over vascular tissue.[9] Thus, we synthesized tetrahydro-1H-pyrrolobenzothiazines **1a,b** and tetrahydropyrrolobenzothiazepine **2** following the route outlined in the Scheme, using previously described disulphide **5**[10] as starting material.

SCHEME



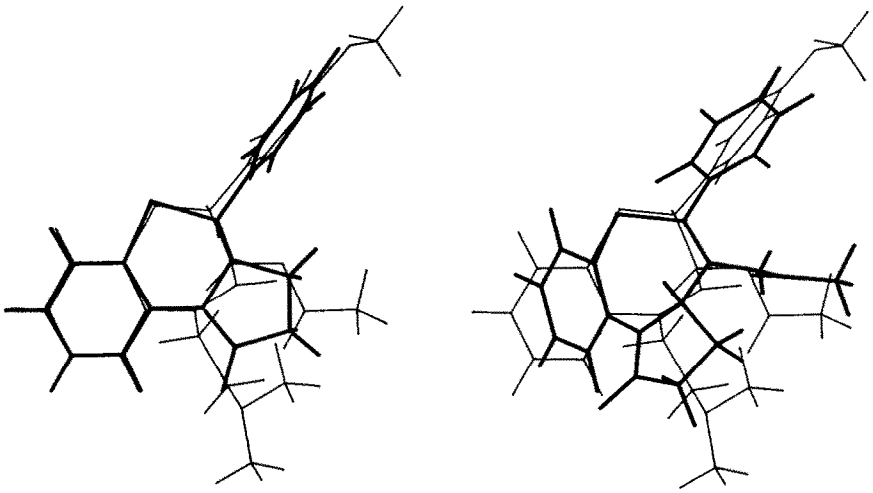
Reagents and conditions : i, Lithium diethylborohydride, THF, -40 → -20 °C; ii, $SOCl_2$, CH_2Cl_2; iii, a: PhH, $AlCl_3$, reflux; b: $PhOCH_3$, $AlCl_3$, CH_3NO_2, $(CH_2Cl)_2$, reflux; iv, $NaBH_4$, $BrCH_2CO_2Et$, THF, rt; v, Na, toluene, reflux; vi, CH_3CO_2H, HCl 6N, reflux; vii, SO_2Cl_2, CH_2Cl_2; viii, a: PhH, $AlCl_3$, reflux; b: $PhOCH_3$, $AlCl_3$, CH_3NO_2, $(CH_2Cl)_2$ 80 °C; ix, KH, THF, CH_3COCl.

Diastereoselective reductive cyclization of compound **5** with lithium diethylborohydride afforded the cyclic hemithioacetal **6** in 79% yield (85% de, diastereoisomers can be separated by fractional crystallization). Halogenation of **6** with thionyl chloride (84% yield) followed by Friedel-Crafts reaction provided the target compounds **1a,b** (83 and 67% yield, respectively) (also at this stage the diastereoisomers can be separated by

fractional crystallization; on the basis of ^{1}H NMR data and molecular mechanics calculations the major isomers **1a,b** show a *trans* configuration for protons at C3a and C4). The Dieckman reaction of diester **8**, obtained *via* reductive alkylation of disulphide **5** (87% yield), afforded cyclic β-keto ester **9** (78% yield) which after saponification, decarboxylation and halogenation with sulfuryl chloride was transformed into compound **11** (59% overall yield from **9**). Aluminium chloride catalyzed Friedel-Crafts reaction of **11** provided ketones **12a,b** in 80% and 23% yield, respectively. Finally compound **2** was prepared by enolization and acylation of ketone **12a** in 82% yield (see Table 1).

On the basis of our investigation on conformationally rigid CEBs related to Diltiazem, using **3** as a model for 3D-comparative studies, molecular modeling[11] indicated a very good structural similarity between **3**[12] and **1a** (*trans* isomer). Superimpositions were performed between all the conformers obtained by an extensive search executed in a 3 Kcal energetic window on the two molecules. For this pourpose 13 atoms have been chosen on each molecule (12 aromatic carbons and the heterocyclic nitrogen), and a RMS of 0.42 Å was found as the best value. The same procedure applied to compound **2** gave a poorer superimposition with **3** (RMS of 0.70 Å) (see Figure). This is in good agreement with the reported lower affinity and negative inotropic activity of compound **2** relatively to **3** and **1a** (Table 1 and 2). The relative position occupied in space by the phenyl linked to the α-carbon to the sulfur really differentiates the molecules **1**, **2** and **3**. In compound **2** this group lies in the plane of the condensed benzene ring while in compounds **1** and **3** it is out of this plane. This fact is substantially due to the different conformation of the heterocyclic ring system of the studied molecules **1**, **2** and **3**.

Figure



Left: superimposition between **1a** (bold, *trans* isomer) and **3**; **Right**: superimposition between **3** and **2** (bold).

Pharmacology. Compounds **1a,b** and **2** were subjected to radioreceptor assay[13-15] on rat cortex homogenate to evaluate their ability to displace [^{3}H]-nitrendipine from CCRs, following a procedure which has already been reported[1]. In this assay compound **1a** displayed higher affinity than that of Diltiazem (see Table 1). Furthermore, compounds **1a,b** and **2** were tested on guinea pig isolated atria and helicoidal aortic strips for inotropic,

chronotropic and vascular activities, respectively, following procedures which have already been reported.[16] Data are shown in Table 2.

Table 1. Chemical and binding data of compounds 1a,b and 2.

Compd	Formula[a]	Mol. Weight	mp (°C)	recryst. solvent[b]	IC_{50} (nM)[c]	Ki (nM)
1a	$C_{17}H_{15}NOS$	281	154-55	A	0.78	0.29
1b	$C_{18}H_{17}NO_2S$	311	149-50	B	1800	675
2	$C_{20}H_{17}NO_3S$	351	176-77	C	1550	581
Diltiazem					42	16

[a]Elemental analyses were within ±0.4% of the theoretical values; MS spectra confirmed the assigned structures. NMR spectral data of compounds **1a,b** and **2** are shown in note 17 .

[b]A = Ethyl ether; B = Diisopropyl ether; C = Ethyl acetate/Cyclohexane.

[c]The concentration of the tested compounds that inhibited [^{3}H]-nitrendipine binding on rat cortex homogenate by 50% (IC_{50}) was determined by log-probit analysis with 6 concentrations of the displacers, each performed in duplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (Ki) by the Prusoff's method.

Table 2. Cardiovascular activity of tested compounds 1a,b and 2.

	Negative inotropic activity on isolated guinea-pig left atrium[a] at 10^{-4} mol/L conc. (n=5-7)	Negative chronotropic activity on isolated guinea-pig spontaneously beating right atrium[b] at $5x10^{-5}$ mol/L conc. (n=5-7)	ED_{50} of inotropic negative potency on stimulated guinea-pig left atrium.		Calcium antagonist activity on K^+ depolarized guinea-pig aortic strips at 10^{-4} mol/L conc. (n=4-5)	IC_{50} of calcium antagonist potency on K^+ depolarized guinea pig aortic strips.	
Compd	% decrease (M±SEM)	% decrease (M±SEM)	ED_{50}[c] (μmol/L)	95% conf. lim. ($x10^{-6}$)	% inibition of Ca^{++} contraction[d] (M±SEM)	IC_{50}[c] (μmol/L)	95% conf.lim. ($x10^{-6}$)
Diltiazem	78±3.4[e]	94±5.6[f]	0.79	0.7-0.85	88±2.3	2.6	2.2-3.1
1a	66±2.3[e]	20±1.6	0.23	0.19-0.27	37±2.9	20.0	16-27
1b	60±0.7	39±2.9[g]	1.2	0.9-1.6	32±0.9	16.6	13-21
2	96±1.5	27±1.7[e]	1.1	0.9-1.4	26±1.7	32.4	28-39

[a] The left atria were driven at 1 Hz. The indicated conc. expressed the max. effect for each compound.

[b] Pretreatment ranged from 165-190 beats/min. The indicated conc. expressed the max. effect for each compound.

[c] Calculated from log conc.-response curves (Probit analysis by Litchfield and Wilcoxon with n=5-7).

[d] The 10^{-4} mol/L conc. gave the max effect for most compounds.

[e] At the 10^{-5} mol/L conc.; [f] At the 10^{-6} mol/L conc.; [g] At the 10^{-4} mol/L conc.

The pharmacological profile of compounds **1a,b** and **2** is particularly intriguing: as far as negative inotropic activity is concerned they all have efficacy and potency comparable to Diltiazem, the most potent being **1a** whose potency is about 4 times greater than that of the reference standard; in fact the rank order of potency is **1a**>Diltiazem>**2**>**1b**. On the other hand these novel compounds show a large drop both of negative chronotropic efficacy in spontaneously beating right atria and of calcium antagonist activity evaluated in aortic smooth muscle. Considering also that compound **1a** showed higher binding affinity for rat cortex slow calcium channels than **3**, it is well founded to suppose that the remarkable negative inotropic activity of this compound may be due to an inhibitory activity of the functional properties of L-type Ca^{++} channels present in myocardial tissue.

As these preliminary results suggest a clear-cut tissue selectivity (i. e. cardiac vs. smooth muscle), and the mechanism by which the presence of certain structural features in these Diltiazem related CEBs alters the tissue selectivity is not fully understood, our compounds seem to deserve further investigation.

Acknowledgment. This work was supported by a grant from MURST, Roma, and by CNR-Progetto Speciale "Meccanismi della Trasduzione del Segnale".

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11- Molecular mechanics calculation were performed on **1**, **2** and Diltiazem with use of molecular modeling programs PCMODEL/BKM (version 4.0, by Serena Software, Bloomington, IN.) and Sybyl (version 5.3, Tripos Associates, St. Louis, MO) on a Silicon Graphics Personal Iris 4D/35.

12- X-ray crystal coordinates (Kojic-Prodic, B. et Al. *Helv. Chim. Acta*, **1984**, *67*, 916) were used as input geometry for the Diltiazem molecule. The structure of **1** and **2** were generated by the DRAW option in PCMODEL program and initially energy minimized by the MMX routine of the same program until convergence. Systematic conformational analyses were performed on **1** (*trans* isomers) and **2** energy minimized conformers and on Diltiazem X-ray structure by using the BKM statistical method. The final conformationally minimized structures were transferred to Sybyl and the chosen features were overlapped using the MULTIFIT routine method.

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17- Spectral data of compounds **1a,b** and **2**:

1a: IR (KBr) 2935, 1705, 1460, 1200, 755 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.39 (m, 1 H), 7.40 (m, 5 H), 7.20-7.00 (m, 3 H), 4.16 (m, 2 H), 2.52 (m, 2 H), 2.03 (m, 1 H), 1.75 (m, 1 H); ^1H NMR (200 MHz, $CDCl_3$/DMSO-d6 5:3) δ 8.38 (d, 1 H, J = 5.6 Hz), 7.54-6.94 (m, 8 H), 4.35 (d, 1 H, J = 9.9 Hz), 4.14 (m, 1 H), 2.52 (m, 2 H), 2.15 (m, 1 H), 1.75 (m, 1 H);

1b: IR (KBr) 2985, 1710, 1620, 1485, 1265, 1125, 750 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.36 (d, 1 H, J = 8.1 Hz), 7.40-6.82 (m, 7 H), 4.12 (m, 2 H), 3.82 (s, 3 H), 2.54 (m, 2 H), 2.04 (m, 1 H), 1.75 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.97, 30.26, 48.66, 55.32, 60.88, 114.53, 122.02, 124.51, 124.77, 124.97, 125.53, 128.17, 129.79, 133.28, 159.83, 173.80; MS *m/z* 311 (M^+), 282, 268, 241, 190, 162.

2: IR (KBr) 2922, 1767, 1708, 1456, 1255, 758 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.64 (d, 1 H, J = 8.1 Hz), 7.52-7.22 (m, 8 H), 4.57 (dd, 1 H, J = 4.8, 7.2 Hz), 2.96-2.35 (m, 4 H), 1.75 (s, 3 H).

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