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SYNTHESIS AND PRELIMINARY BIOLOGICAL EVALUATION OF 1-AMINOMETHYL-4-SUBSTITUTED-4H-PYRROLO[2,1-C][1,4] BENZOTHIAZINES, A NEW CLASS OF CALCIUM ANTAGONISTS.

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Abstract. The preparation of 4a-g, conformationally rigid calcium channel blockers related to Diltiazem, is described starting from tricyclic compounds 5a-c. On radioreceptor assay and preliminary biochemical tests some compounds show high affinity for Calcium Channel Receptors (CCRs) and calcium antagonistic activity comparable or superior to that of Diltiazem.

After the introduction of calcium channel blockers (CCBs) into the clinical practice for the treatment of cardiovascular diseases[1], several new compounds with calcium antagonistic activity have been described, most of them being structurally related to the dihydropyridines Nifedipine 1 and Nicardipine 2[2-4]. On the other hand, very few effective calcium antagonists related to Diltiazem 3 have been reported until today[5]. Therefore, little information is available concerning structure-activity relationships in this class of CCBs[6a-e].

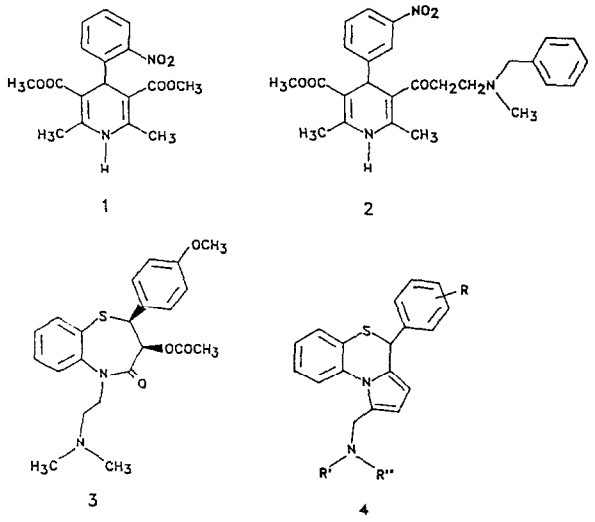
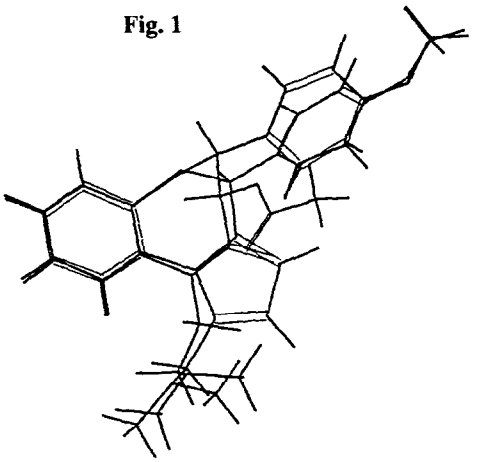


Fig. 1

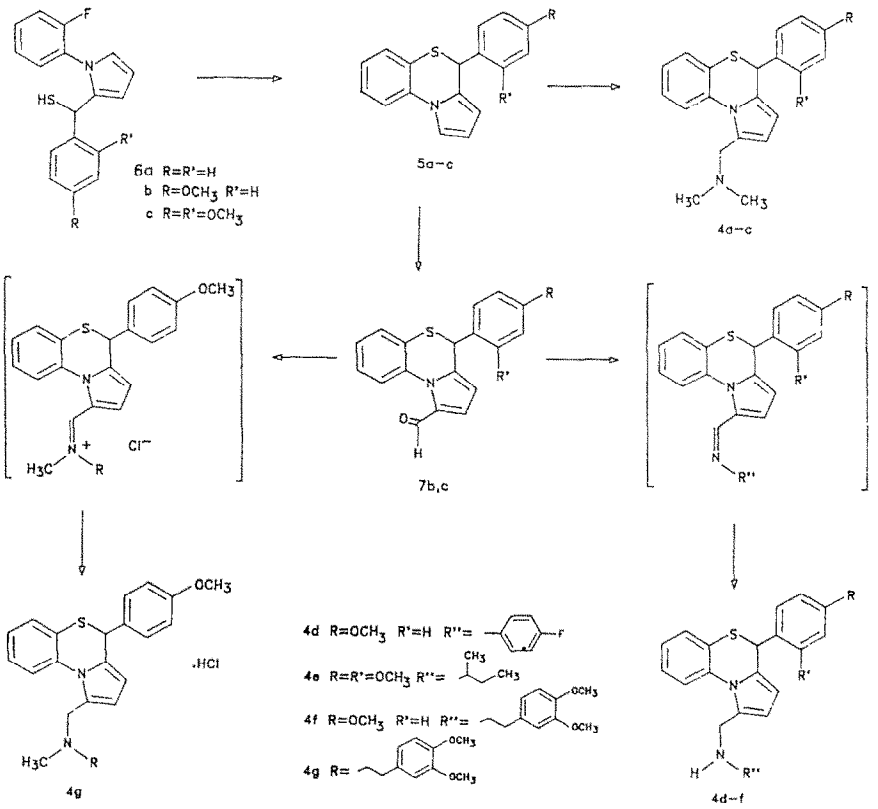


Only recently, Fujita and coworkers[7] have reported that some 2-[2-[(aminoalkyl)oxy]-5-methoxyphenyl]-3,4-dihydro-4-methyl-3-oxo-2H-1,4-benzothiazines possess potent calcium antagonistic activity associated with

weak cardiac suppression. We wish now to report the results concerning design, synthesis and preliminary biological evaluation of N-substituted 1-aminomethyl-4-aryl-4H-pyrrolo[2,1-c][1,4]benzothiazines **4** as new CCBs.

Molecular modeling studies[8] showed a very good superimposition between the minimum energy conformations[9] of Diltiazem **3** and the prototype **4b** of our class of compounds (Fig. 1). The replacement of the amide linkage of **3** with a bioisosteric[10] pyrrole ring as well as the contraction of the central ring from seven- to six-membered leads to the more rigid compound **4b**, but doesn't substantially alter the mutual distances and spatial disposition of two of the pharmacophoric groups, namely the 4-methoxyphenyl substituent and the dimethylaminomethyl side chain.

SCHEME



Therefore, in order to assess their calcium antagonistic properties, we synthesized the family of products **4a-g**. Following a general procedure previously described[11], we prepared the new 4-aryl-4H-pyrrolo[2,1-c][1,4]benzothiazines **5a-c** involving cyclization of the thiol derivatives **6a-c** *via* intramolecular nucleophilic displacement of the fluorine atom, as a key step. The transformation of **5a-c** into the target compounds **4a-g** was accomplished as outlined in the Scheme.

Mannich reaction on **5a-c** gave derivatives **4a-c** (83-88% yield), while compounds **4d-g** were prepared *via* aldehydes **7b,c** obtained in turn by Vilsmeier-Haack formylation (DMF, $POCl_3$) of **5b,c** (88, 93% yield).

Reductive amination of 7b,c with primary amines in the presence of $NaBH_3CN/ZnCl_2$ afforded the aryl and alkylaminomethyl derivatives 4d-f (78-82% yield). Finally, compound 4g was prepared by $NaBH_4$ reduction of the immonium salt, obtained in turn by reaction of N-methyl-2-(3,4-dimethoxyphenyl)ethylamine with aldehyde 7b in HCl/MeOH (85% yield) (See Table 1).

Biochemistry. Compounds 4a-g were subjected to radioreceptor assay[12,13] (RRA) to evaluate their ability to displace [^{3}H]-nitrendipine from CCRs[14]. Biochemical data are reported in Table 1. While 4a,c,d,f,g are less or equally potent, 4b,e display higher affinity than that of Diltiazem.

Table 1. Chemical and biological data of test compounds

compound	formula[a]	mol. weight	mp(°C)	recrist. solvent	IC_{50}(nM)	K_i(nM)
4a	$C_{20}H_{20}N_2S$	320	141-42	EtOAc	315	118
4b	$C_{21}H_{22}N_2OS$	350	174-75	EtOAc	0.42	0.16
4c	$C_{22}H_{24}N_2O_2S$	380	171-72	EtOAc	150	56
4d	$C_{25}H_{21}FN_2OS$	416	176-77	i-PrOH	5870	2200
4e	$C_{24}H_{28}N_2O_2S$	408	119-20	MeOH	14.8	5.55
4f	$C_{29}H_{30}N_2O_3S$	487	132-33	EtOAc	534	200
4g	$C_{30}H_{33}ClN_2O_3S$[b]	537	--	--	53	19
Diltiazem					42	16

[a] Elemental analyses were within ±0.4% of the theoretical values; N.M.R. and MS spectra confirmed the assigned structures

[b] As hydrochloride

The ability of the above described compounds to block calcium channels was tested using electrophysiological techniques[18]. Fig.2 shows traces of transmembrane calcium currents recorded under voltage-clamp stimulations in mouse oocytes[19]. These cells are known to possess voltage-dependent Ca^{2+} channels similar to the T-type[20], although they are also partially inhibited by dihydropyridines[21]. Panel A and A' show the blocking action of 4c (10 μM) on the currents elicited by the voltage stimulations as drawn in panel C. For comparison, panel B and B' show a similar effect caused by Diltiazem (10 μM) on another oocyte. Table 2 summarizes the results obtained with compounds 4a-c at 10 μM and 100 μM.

Fig. 2

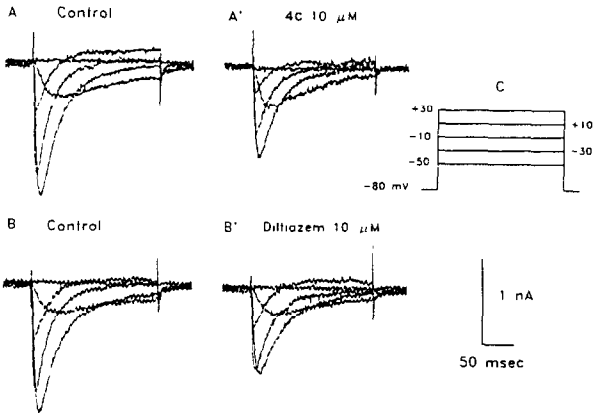


Table 2. Electrophysiological data

Compounds	10 μM		100 μM	
Diltiazem	24%	(13)	62%	(5)
4a	40%	(7)	65%	(11)
4b	20%	(11)	62%	(8)
4c	24%	(8)	74%	(5)

Percent reduction of the peak Ca^{2+} current elicited by a test pulse to -10mV following application of Diltiazem and the test compounds at two different concentrations (number of cells in brackets).

In conclusion, we have prepared some tricyclic amines **4a-g**, structurally related to Diltiazem, as potential calcium channel blockers. Some of them show high affinity and potent calcium blocking properties in biochemical and electrophysiological tests.

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References and Notes

1) Godfraind, T.; Miller, R.; Wibo, M. Pharmacological Reviews **1986**, 38, 321.
2) Fleckenstein, A. Cir. Res. Suppl. 1 **1983**, 52, 3.
3) Singh, B.; Baky, S.; Nademanee, K. Am. J. Cardiol. **1985**, 55, 214B.
4) Millard, R.W.; Lathrop, D.A.; Grupp, G.; Ashraf, M.; Grupp,I.L.; Schwartz, A. Am. J. Cardiol. **1982**, 49, 499.
5) Hirozumi, I.; Mikihiko, K.; Tomiki, H.; Hisao, O.;Kaoru, T.; Mitsunori, G.; Tadamasa, D.; Keiichi, A.; Mikio, T.;Sakae, M.; Hiroshi, N.; Nagau, T. J. Med. Chem. **1991**, 34, 675.
6) (a) Nagao, T.; Sato, M.; Nakajima, H.; Kiyomoto, A. Chem. Pharm. Bull. **1973**, 21, 92. (b) Kugita, H.; Inoue,H.; Ikezaki, M.; Konda, M.; Takeo, S. Chem. Pharm. Bull. **1971**, 19, 595. (c) Floyd, D.; Kimball, D.; Krapcho, J.; Das, J.; Turk, C.; Moquin, R.; Lago, M.; Duff, K.; Lee, V.; White, R.; Ridgewell, R.; Moreland, S.; Brittain, R.; Normandine, D.; Hedberg, A.; Cucinotta, G. J. Med. Chem. **1992**, 35, 756. (d) Das, J.; Floyd, D.; Kimball, D.; Duff, K.; Chi Vu, T.; Lago, M.; Moquin, R.; Lee, V.; Gougoutas, J.; Malley, M.; Moreland, S.; Brittain, R.; Hedberg, S.; Cucinotta, G. ibidem, **1992**, 35, 773. (e) Kimball, D.; Floyd, D.; Das, J.; Hunt, J.; Krapcho, J.; Rovnyak, G.; Duff, K.; Lee, V.; Moquin, R.; Turk, C.; Hedberg, S.; Moreland, S.; Brittain, R.; MacMullen, D.; Normandin, D.; Cucinotta, G. ibidem, **1992**, 35, 780.
7) Fujita, M.; Ito, S.; Ota, A.; Kato, N.; Yamamoto, K.; Kawashima, I.; Yamauchi, H.; Iwao, J. J. Med. Chem. **1990**, 33, 1898.
8) Molecular mechanics calculations were performed on our prototype **4b** and on Diltiazem with use of molecular modeling programs PCMODEL/BKM (version 4.0, by Serena Software, Bloomington, In.) and Sybyl (version 5.3, Trypos Associated, St. Louis, MO) on a Silicon Graphics Personal Iris 4D/35.
9) X-ray crystal coordinates (Kojic-Prodic, B.; Ruzic-Toros, Z.; Sunjic, V.; Decorte, E.; Moimas, F. Helv. Chim. Acta, **1984**, 67, 916) were used as input geometry for the Diltiazem molecule. The prototype structure was 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 the prototype energy minimized conformer and Diltiazem X-ray structure by using the BKM grid method. The final conformationally minimized structures were transferred to Sybyl

and the chosen features were overlapped using the MULTIFIT routine method.
10) Wijngaarden, I.; Kruse, C.; van Hes, R.; van der Heynden, J.; Tulp, M. J. Med. Chem. **1987**, 30, 2099.
11) Nacci, V.; Campiani, G.; Garofalo, A. Synth. Commun. **1990**, 20(19), 3019.
12) Jacqmin, P.; Lesne, M. 2nd European Congress of Biopharmaceutics and Pharmacokinetics, Salamanca, 24-27 Apr. **1984**, Abs. p. 355.
13) Dorow, R.; Schneider, H. Br. J. Clin. Pharmacol. **1982**, 13, 561.
14) Method: tissue homogenate of cerebral cortex containing calcium channel receptors was prepared according to Ehlert F.J. et al.[15]. Binding determination (RRA): the receptor binding assay was determined as follow: 200 µl of tissue homogenate were incubated for 90 min in a dark room at O°C with 100 µl of [^{3}H]-nitrendipine $3x10^{-10}$ M (87.0 Ci/mmol) (NEN) and 100 µl of the test compound (dissolved in DMSO 5%) in 50 mM of Na/HEPES buffer pH 7,4 (total vol 2ml). The incubations were stopped by adding 4ml of cold buffer followed by rapid filtration through glass fiber filter disks. The samples were subsequently washed 3 times with 4.5 ml of the same buffer and placed into scintillation vials; 10ml of Filter-Count liquid scintillation cocktail was then added to each vial and counting was carried out by a scintillation spectrometer (Packard T.-C. 300C). Non-specific binding was defined as non-displaceable binding in the presence of $1x10^{-4}$ of unlabeled diltiazem and specific binding as the difference between total and non-specific binding. Inhibition of [^{3}H]-nitrendipine by Diltiazem is 70% approximatively, according with reference 15. Blank experiments were carried out to determine the effect of the solvent DMSO (5%) on the binding. The concentration of the test compounds that inhibited [^{3}H]-nitrendipine binding by 50% (IC_{50}) was determined by log-probit analysis with 6 concentrations of the displacers, each performed in duplicate. The IC50 values obtained were used to calculate apparent inhibition constants (K_i) by the method of Prusoff et al.[16], by the following equation: $K_i=IC_{50}/(1+S/K_d)$ where S represents the concentration of the ligand used and K_d is its receptor dissociation constant, obtained by Scatchard analysis[17], (K_d values of [3H]-nitrendipine was $2.58x10^{-10}$ M).
15) Ehlert, F.J.; Itoga, E.; Roeske, R.W.; Yamamura, H. Biochem. and Biophys. Res. Commun. **1982**, 104, 937.
16) Cheng, Y.; Prusoff, W. Biochem. Pharmacol. **1973**, 22, 3099.
17) Scatchard, G. Ann. NY Acad. Sci. **1949**, 51, 660.
18) Transmembrane Ca^{++} currents were measured using the whole-cell clamp technique[22]. The extracellular solution contained (in mM): NaCl 125, KCl 6, $MgCl_2$ 1.2, $CaCl_2$ 20, Hepes 20 (pH7.4); the intracellular solution contained (in mM): KCl 140, $MgCl_2$ 1, $CaCl_2$ 1, EGTA 10, Hepes 10 (pH 7.4). The holding potential was kept at -80 mV and 200 msec long depolarizations to various test

potential were administered to elicit Ca^{++} currents. Traces were recorded, analyzed and displayed using pClamp Software and Sigmaplot.

19) (A, A', B, B'): each panel shows five superimposed traces of Ca^{++} currents elicited by the voltage-clamp pulses illustrated in panel C. A and A' same oocyte before and after perfusion with 10 μM **4c**. B and B' another oocyte before and after perfusion with 10 μM Diltiazem.

20) Peres, A. J. Physiol. **1987**, 391, 573.

21) Peres, A.: our recent unpublished results indicate an average 60% reduction of the peak current by 10 μM Nifedipine, suggesting that the mouse oocyte Ca^{2+} channels may not be "pure" T-type but show properties common to L-type channels.

22) Hamill, O.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. Pflug. Arch. **1981**, 391, 85.