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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3691–3695

Design, synthesis, and biological evaluation of novel T-Type calcium channel antagonists

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Received 28 April 2004; revised 7 May 2004; accepted 7 May 2004

Abstract—This paper describes the synthesis of several novel T-type calcium channel antagonists that inhibit calcium influx into the cell, which in turn regulates unknown aspects of the cell cycle pathway that are responsible for cellular proliferation. A library of compounds was synthesized and a brief structure–activity relationship will be described. From these studies we have identified a compound (**1**) that displays anti-proliferative activity in the low micromolar range across a variety of cancer cell lines.

Calcium is ubiquitous in all living organisms and requisite for a variety of physiological processes.[1–5] Calcium has been linked to cell cycle regulation[6–8] and hence to cellular proliferation via increases in intracellular calcium concentrations.[9,10] For instance, calcium is essential for the activation of early genes, which leads the cell into G1 phase; a rise in intracellular calcium is also thought to initiate the replication of DNA at the G1/S interphase.[10] It has been demonstrated that depletion of intracellular calcium arrests the cell cycle in the G0/G1 and S interphases.[11] Regulation of the changes in intracellular calcium has been proposed to be via a T-type calcium channel.[12] This may be an important tool to control the cell cycle signaling pathway in order to manage certain disease states, such as cancer, where the cell cycle is aberrant.

We have recently demonstrated that by blocking calcium uptake, cellular proliferation is inhibited.[13] In light of these findings; we have set out to rationally design a library of compounds that could regulate certain aspects of this calcium influx pathway, specifically those related to the T-type calcium channel, and thereby control cellular proliferation.

We first examined a variety of known L-type calcium channel blockers for their ability to inhibit the calcium influx pathway (Fig. 1). These initial studies have illuminated common structural motifs that were observed in some of the more active compounds (Fig. 2). Common areas of importance include heterocyclic rings that contain a tertiary nitrogen, aromatic domains, and ether linkages. By combining these common structural motifs we were able to envision a series of compounds to synthesize. This new series of molecules would be tested for their potential to block T-type calcium channels and ultimately cellular proliferation. The data for these studies are presented within the following text.

Synthesis of all the compounds shown in Table 1 are described in Scheme 1. As outlined in Scheme 1, a sodium borohydride reduction of any benzophenone derivative afforded the corresponding benzhydrol (Scheme 1).[14,15] Subsequently (L)-proline methyl ester ($n = 1$), or the *o*-, *m*-, or *p*-piperidine ethyl ester isomers[16] ($n = 2$) were coupled to a variety of aryl acetic acid derivatives using either dicyclohexylcarbodiimide (DCC) or (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) to afford the

Keywords: Calcium channel blockers; Cancer; T-type calcium channels; Cellular proliferation.
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doi:10.1016/j.bmcl.2004.05.011

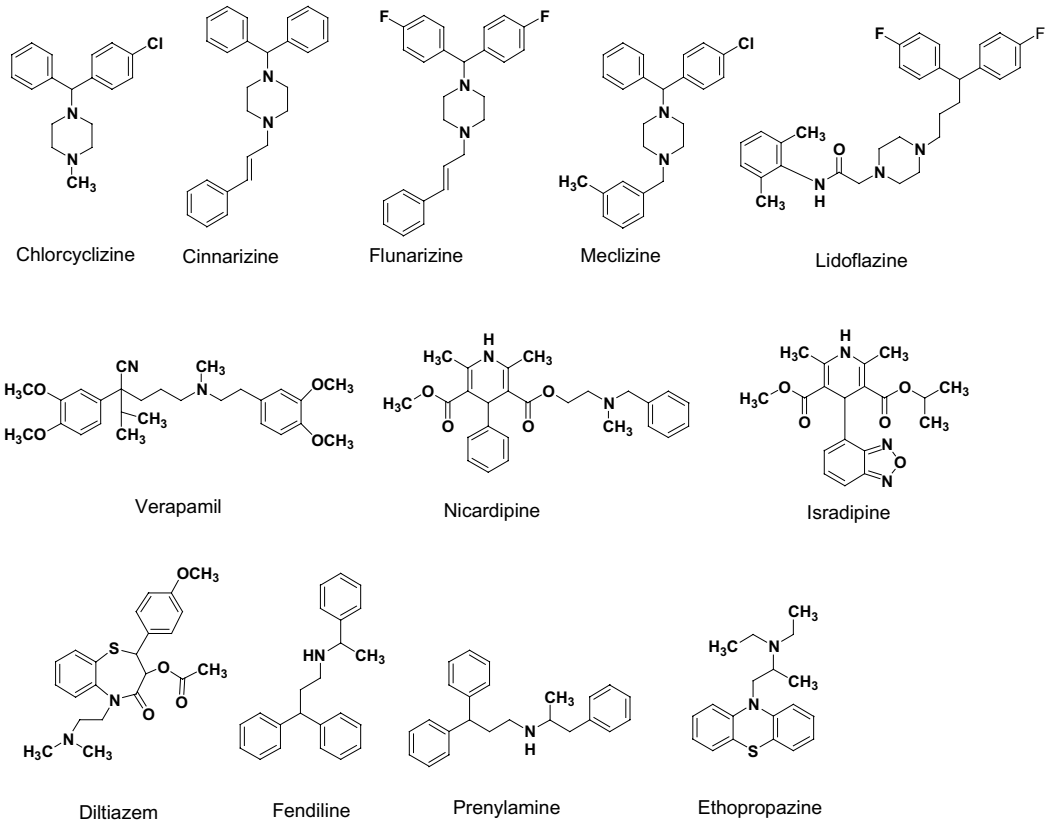


Figure 1. Initial L-type calcium channel blockers studied in anti-proliferation assay.

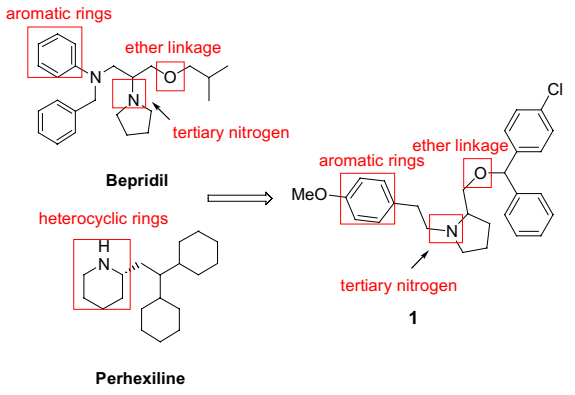


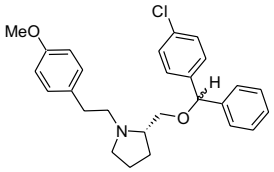
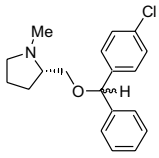
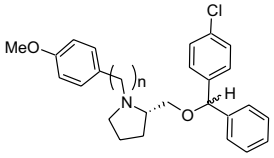
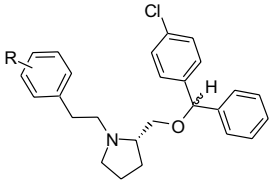
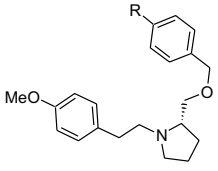
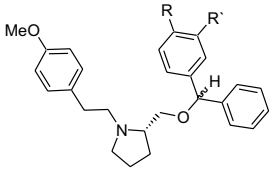
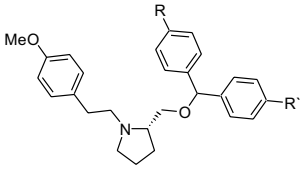
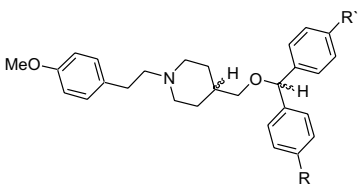
Figure 2. Bepridil and perhexiline, two of the initially screened compounds with areas of importance indicated with red boxes lead to the development of the structure shown, which is a T-type calcium channel antagonist, VCP11177 (**1**).

amides **6**. A dual reduction of both the amide and ester functionalities using lithium aluminum hydride afforded the amino alcohols **7**. The final step used *p*-toluenesulfonic acid to achieve the condensation of the benzhydrol and the alcohol, thus affording the final products **8** ($n = 1$),[17] and **9** ($n = 2$).[18] In the case of compound **10**, in Table 1, the N-methyl amine was afforded by a reductive amination using proline methyl ester, formaldehyde, and sodium cyanoborohydride. The synthesis after this point was performed according to Scheme 1. Synthesis of compounds **9a–c** and **9d–f** in Table 1, followed the same synthesis pathway as depicted in Scheme 1, however, *p*-piperidine ethyl ester and *m*-piperidine ethyl ester, respectively, were used in place of *o*-piperidine ethyl ester.

Measurement of cellular proliferation was carried out using Jurkat (T-cell), LNCaP (prostate), or MDA-361 (breast) tumor cells. All assays were carried out in triplicate in a standard flat bottom 96 well tissue culture plate in the presence of drug or vehicle (DMSO). Cells were grown for 48 h at 37 °C in a CO_2 incubator. Relative cell growth was determined with the CellTiter 96 aqueous cell proliferation assay (Promega, Madison WI), as described by the manufacturer using an automated plate reader. Results were calculated in a blinded fashion and are the means of triplicate determinations.

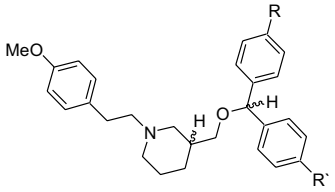
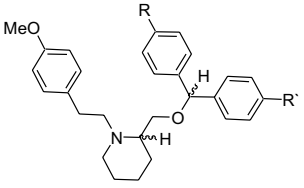
Measurement of changes in intracellular calcium ($[Ca^{2+}]_i$) concentration: Cells were incubated in growth media containing 1 μM of the acetoxy-methyl ester of the Ca^{2+} sensitive fluorescent dye indo-1 (indo-1/AM; Molecular Probes, Eugene OR) for 1 h at 37 °C. Cells were washed three times in buffer A [10 mM HEPES (pH 7.4) 1 mM $MgCl_2$, 3 mM KCl, 1 mM $CaCl_2$, 140 mM NaCl, 0.1% glucose, and 1% fetal bovine serum] and suspended to a final concentration of 10^6 cells/ mL. Before stimulation with thapsigargin,[12] cells were warmed to 37 °C. Changes in $[Ca^{2+}]_i$ were monitored in an SLM 8100C spectrofluorometer (SLM/Aminco; Urbana IL).

Table 1. Structure and biological data for all compounds

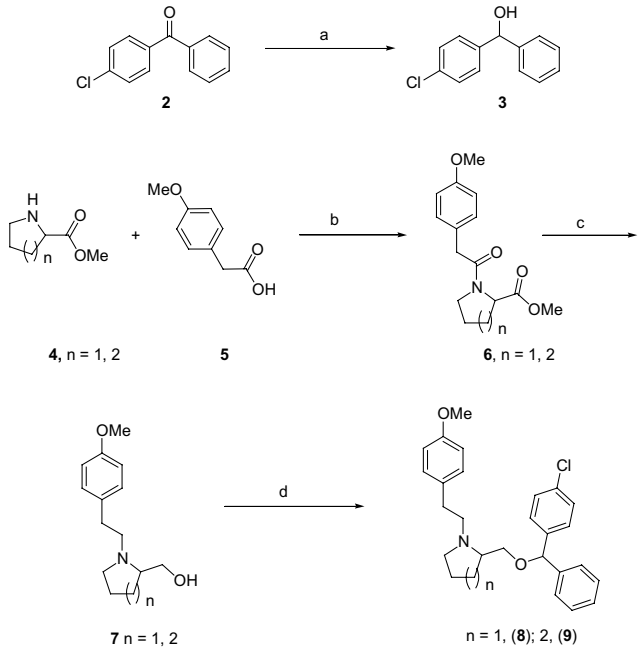
Compound		IC_{50} (μM)					
		Jurkat		LNCaP		MDA-361	
		Proliferation	Calcium Influx	Proliferation	Calcium Influx	Proliferation	Calcium Influx
	**1**	6	7	4	4	10	10
	**10**	60	30	17	50	47	100
	**8a**, $n = 1$	10	2	7.7	NE	30	30
	8b, $n = 3$	4	1	3.5	30	11	10
	**8c**, R = H	11	<1	8	30	27	3
	8d, R = 4-hydroxy	6	10	3	10	11	30
	8e, R = 2-methoxy	3.8	1	2.4	12	13	10
	8f, R = 3-methoxy	5	3	3.2	10	29	30
	**8g**, R = H	108	100	33	100	83	20
	8h, R = chloro	18	25	14	10	15	100
	**8i**, R = R′ = H	12	10	10	30	29	30
	8j, R = fluoro, R′ = H	13	3	2	10	14	20
	8k, R = bromo, R′ = H	40	30	15	15	1200	100
	8l, R = R′ = chloro	6	3	4	10	11	10
	**8m**, R = R′ = fluoro	5	3	4	10	14	30
	8n, R = R′ = chloro	3.6	5	3	4	11	25
	8o, R = R′ = methyl	11	3	6	5	40	20
	8p, R = R′ = methoxy	13	15	5.1	10	15	20
	**9a**, R = R′ = H	8.3	20	5	15	10	30
	9b, R = H, R′ = chloro	8.4	7	3.3	5	10.4	25
	9c, R = R′ = chloro	11.1	10	3.4	10	7.1	20

(*continued on next page*)

Table 1 (*continued*)

Compound		IC_{50} (μM)					
		Jurkat		LNCaP		MDA-361	
		Prolifer-ation	Calcium Influx	Prolifera-tion	Calcium Influx	Prolifera-tion	Calcium Influx
	**9d**, R = R′ = H	13.8	8	9.2	15	17.7	30
	9e, R = H, R′ = chloro	8.8	10	6.8	12	13	30
	9f, R = R′ = chloro	9.6	10	5	5	13	30
	**9g**, R = H, R′ = chloro	11	3	5	10	25	30
	9h, R = R′ = chloro	9	15	4	7	22	30

IC_{50} values are in μM concentrations; NE, no effect.



Scheme 1. Synthesis of **8a**–**q** and **9a**–**h**. Reagents and conditions: (a) $NaBH_4$, THF; (b) DCC, CH_2Cl_2, NEt_3; or PyBOP, NEt_3, CH_2Cl_2; (c) $LiAlH_4$, THF; (d) **3**, *p*-toluenesulfonic acid, toluene.

Table 1 provides the results of assays used to measure the inhibition of calcium influx and cellular proliferation by the compounds synthesized as described in Scheme 1. From this data, a brief structure–activity profile emerged as follows: The compound that has a methyl pyrrolidine is less active than compounds with more bulkier groups attached to the nitrogen (**10** compared to **8a**–**f**). The benzhydrol moiety is crucial for activity as seen from the loss in activity for compounds **8g** and **h**. Substitutions on one of the phenyl rings of the benzhydrol seem to be important for imparting biological activity, this can be seen from examples **8i**–**l**. The 4-chlorobenzhydrol compound (**1**) showed the best activity across all of the cell lines in the series. The 4,4′-dichlorobenzhydrol moiety (**8n**) was made to deduce if stereochemistry at the tertiary benzhydrol carbon played a role in whether the compound exhibited biological activity. Surprisingly **8n** showed no appreciable increase in biological activity to that of the mono-chloro derivative (**1**) indicating that the stereochemistry at this center may have an overall effect on the biological activity of the compounds tested. The six membered piperidine ring systems (**9a**–**h**) exhibited no overall increase in biological activity in all of the cell lines to that of the five membered pyrrolidine ring systems.

In conclusion, we have presented strong data indicating that cellular proliferation can indeed be regulated by inhibiting calcium influx via chemical means. By using a variety of L-type calcium channel blockers we were able to formulate an active chemical structure that inhibits T-type calcium channels with greater potency than the original L-type calcium channel blockers tested. Refinement of this chemical structure has led to a greater understanding of the structure–activity relationship required to inhibit calcium influx and, consequently, cellular proliferation.

Acknowledgements

We thank Dr. Mahendra Chordia and Brian Heasley for all their help and for reviewing this manuscript.

References and notes

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13. Haverstick, D. M.; Heady, T. N.; Macdonald, T. L.; Gray, L. S. *Cancer Res.* **2000**, *60*, 1002–1008.
14. The convergent synthesis of library of compounds started from commercially available starting materials such as substituted benzophenones, (L)-proline methyl ester and the appropriate phenylacetic acids. The routine synthetic steps such as reduction of benzophenone with sodium borohydride afforded benzhydrols. The coupling of a variety of aryl acetic acid derivatives with proline methyl ester using either dicyclohexylcarbodiimide or (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate yielded proline amides, or piperidine amides subsequent double reduction of amide and ester functionalities furnished amino-alcohols. Final synthesis of ethers from benzhydrols and N-substituted prolinols or piperidinols was achieved with *p*-toluene sulfonic acid condensation, using a Dean–Stark.
15. The standard reductions employed on the benzophenone would in no way induce a stereochemical preference on the forming benzhydrol. Therefore, these compounds were synthesized as the racemate and were subsequently submitted for biological assaying as the two possible diasteriomers.
16. All piperidine isomers were purchased as the racemic mixture and no further chiral resolutions or chiral separations were employed to increase the enantiomeric mixture.
17. Chloro-phenyl)-phenyl-methoxymethyl]-1-[2-(4-methoxyphenyl)-ethyl]-pyrrolidine (**1**): ^{1}H NMR ($CDCl_3$): δ 7.21–7.33 (m, 9H), 6.95–7.01 (d, 2H), 6.74–6.80 (d, 2H), 5.37 (s, 1H), 3.79 (s, 3H), 3.45–3.56 (m, 2H), 3.11–3.23 (m, 2H), 2.65–2.88 (m, 3H), 2.48–2.62 (m, 1H), 2.26–2.40 (q, 1H), 1.88–2.04 (m, 1H), 1.71–1.86 (m, 2H), 1.56–1.71 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 158.3, 141.6, 133.1, 130.0, 128.9, 128.8, 128.1, 128.0, 127.5, 127.4, 114.2, 83.8, 73.7, 63.9, 58.4, 55.4, 55.1, 35.0, 29.1, 23.6; MS (*m*/*z*): 436.2 $(M)^+$; Anal. Calcd for $C_{27}H_{30}ClNO_2$: C, 74.38; H, 6.94; N, 3.21. Found: C, 74.64; H, 6.88; N, 3.24.
18. The piperidine analogs, in each case, were submitted as a mixture of the four possible diasteriomers.