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Design, syntheses, and SAR of 2,8-diazaspiro[4.5]decanones as T-type calcium channel antagonists

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ABSTRACT

It was hypothesized that an appropriately substituted 2,8-diazaspiro[4.5]decan-1-one could effectively approximate a 5-feature T-type pharmacophore model published in the literature. Compounds were designed and synthesized to test our hypothesis and were found to be potent T-type calcium channel inhibitors with modest selectivity over L-type calcium channels. The synthesis and SAR of the series is described.

Calcium is an important signaling molecule for many physiological processes in the human body. These include electrical signaling in the nervous system, as well as controlling heart and smooth muscle contraction and hormone release. The entry of calcium into cells is regulated by a diverse set of proteins called calcium channels. A fundamental role of Ca^{2+} channels is to translate an electrical signal on the surface membrane into a chemical signal within the cytoplasm, which, in turn, activates many important intracellular processes including contraction, secretion, neurotransmission, regulation of enzymatic activities, and gene expression, as well as cell death.[1]

Voltage-gated calcium channels are divided into two primary groups based on electrophysiological characteristics: low voltage activated (LVA or T-type) and high voltage activated (HVA).[2] The HVA group includes the L-, N-, P-, Q-, and R-types and requires a relatively large membrane depolarization to open and displays slow inactivation kinetics. In contrast, the LVA or T-type channels require a smaller membrane depolarization for activation. These channels are so named because they carry a transient current with a low voltage of activation and rapid inactivation.

The T-type channels are further subdivided into three subtypes based on the amino acid sequences of their pore-forming α_1 subunits.[3] Three genes that encode these subunits have been identified: CACNA1G for $Ca_V3.1$ (α_{1G}), CACNA1H for $Ca_V3.2$ (α_{1H}), and CACNA1I for $Ca_V3.3$ (α_{1I}). These three α_1 subunits are differentially and widely expressed in the central and peripheral nervous systems, cardiac, and vascular smooth muscle, kidneys, sperm, adrenal, and pituitary glands, as well the pancreas.[3]

Rapid gating kinetics and smaller membrane depolarization thresholds make T-type channels well suited to control neuronal excitability.[3,4] For example, the roles of T-type channels in regulating sleep rhythms and in certain forms of epilepsy have been established.[5] More recent evidence of a link between T-type channels and weight maintenance is intriguing and may suggest a potential for treatment of obesity.[6]

The role of T-type channels in nociception is less established, but evidence has been accumulating. It has been demonstrated that T-type channel-dependent, low-threshold Ca^{2+} spikes regulate excitability in DRG neurons. Furthermore, T-type activity is upregulated in neuropathic animals resulting in spontaneous burst firing of action potentials.[7]

The analgesic nitrous oxide selectively blocks DRG T-type currents with no effect on HVA currents at clinically relevant concentrations. A number of anesthetics including isoflurane were found to block T-type currents.[8]

The modestly selective T-type/L-type calcium channel antagonist mibefradil, briefly marketed as Posicor™ for the treatment of hypertension and angina pectoris, was withdrawn from the market less than a year after its introduction due to problems associated with inhibition of cytochrome P450 enzymes.[9] Nevertheless, mibefradil has been a useful tool for investigating the role of T-type calcium channels in in vitro and in vivo models.

Mibefradil and the T-type antagonist ethosuximide reduce in vivo hyperalgesic responses to thermal or mechanical stimuli induced by chemical agents or experimental nerve injury. Moreover, selective T-type knock-out animals provide further evidence for the role of T-type calcium channels in peripheral nerve pain signaling.[10]

The desire to find novel mechanisms of action and therapeutic targets for the treatment of hypertension, epilepsy, insomnia, obesity, and neuropathic pain has spurred considerable interest in the

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development of novel and selective antagonists to T-type channels.[11,12] The 5-feature T-type calcium channel pharmacophore model described by Pae and co-workers, consisting of three hydrophobic regions, one hydrogen bond acceptor and one positive ionizable region, captured our attention.[11b] It was recognized that 2,8-diazaspiro[4.5]decan-1-one, which contains a hydrogen bond acceptor and a positive ionizable atom could serve as a semi-rigid core from which to attach hydrophobic moieties. Furthermore, the spatial arrangement of the attached hydrophobic moieties, hydrogen bond acceptor, and positive ionizable atom appeared to approximate the model described by Pae and co-workers. It was hypothesized that compounds derived from such a scaffold would be potent inhibitors of the α1H T-type calcium channel.

Herein we describe the design, syntheses, and structure–activity relationship (SAR) of a series of novel 2,8-diazaspiro[4.5]decanone analogs. To test our hypothesis, we coupled commercially available reagents 2-(4-chlorobenzyl)-2,8-diazospiro[4.5]decan-1-one **1** and 4,4-difluorobenzhydryl chloride to form 2-(4-chlorobenzyl)-8-(4,4-difluorobenzhydryl)-2,8-diazaspiro[4.5]decan-1-one **2** (Scheme 1). Compound **2** was found to inhibit the α1H T-type calcium channel subtype 58 ± 1% at a concentration of 30 nM in patch clamp electrophysiology assays, thus validating our hypothesis (Table 1).[13] Compound **2** displayed modest (~10-fold) selectivity (T-type/L-type Ca channels) as it inhibited the α1C L-type calcium channel 31 ± 5% at 0.1 μM in our patch clamp assay. The potency and T-type/L-type selectivity of **2** was similar to that of mibefradil.[14] The unsubstituted 2-(4-chlorobenzyl)-2,8-diazospiro[4.5]decan-1-one, **1**, was not active at 3 μM concentration in the α1H T-type assay.

Next the amide carbonyl hydrogen bond acceptor was removed by treating compound **2** with lithium aluminum hydride to produce the bis-amine **3**. This compound inhibited α1H (66 ± 8% at 1 μM) but was far less potent than **2**, indicating the importance of the hydrogen bond acceptor for potent T-type activity. Reductive amination of diazaspirodecanone **1** with aldehydes by treatment with sodium triacetoxyborohydride in 1,2-dichloroethane afforded analogs **4–7**. Compound **4** demonstrated that removal of one of the three hydrophobic regions led to a large decrease in α1H potency (65 ± 3% at 3 μM). Inserting a methylene spacer between the amine and the benzhydryl moiety (compound **5**) led to a modest decrease in α1H potency (59 ± 4% at 0.1 μM).

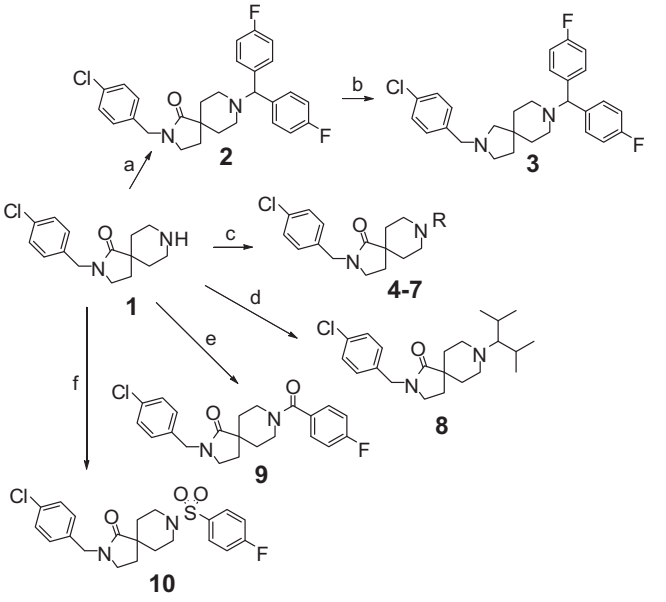
Table 1
Percent Inhibition of α1H and α1C at concentration in μM[a]

Compd	R	α1H% inhib @ (μM)	α1C% inhib @ (μM)
1		NA[b] @ 3	
2		58 ± 1 @ 0.03	31 ± 5 @ 0.1
3		66 ± 8 @ 1	
4	4-FPhCH$_2$	65 ± 3 @ 3	59 ± 7 @ 3
5	(4-FPh)$_2$CHCH$_2$	59 ± 4 @ 0.1	
6	2-EtOPhCH$_2$	67 ± 6 @ 3	
7	3-PhOPhCH$_2$	56 ± 14 @ 0.3	
8		40 ± 4 @ 0.3	
9		NA @ 1	
10		45 ± 4 @ 3	54 ± 8 @ 3
11	4-ClPhCH$_2$CH$_2$	30 ± 11 @ 0.03	
12	3-Cl PhCH$_2$	91 ± 1 @ 0.3	
		NA @ 0.03	
13	3-MeOPhCH$_2$	52 ± 1 @ 0.03	67 ± 14 @ 0.3
14	MeOCH$_2$CH$_2$	28 ± 2 @ 1	
15	MeOCH$_2$CH$_2$CH$_2$	44 ± 9 @ 1	58 ± 8 @ 10
16	*i*PrOCH$_2$CH$_2$CH$_2$	76 ± 3 @ 1	56 ± 5 @ 3
17	O(CH$_2$CH$_2$)$_2$NCH$_2$CH$_2$CH$_2$	35 ± 2 @ 1	
18	*i*Pr	57 ± 6 @ 3	52 ± 3 @ 3
19	*t*-BuCH$_2$CH$_2$	44 ± 13 @ 0.3	60 ± 13 @ 0.3
20	H	NA @ 3	
21		68 ± 3 @ 1	42 ± 0 @ 0.1
22		59 ± 4 @ 0.3	44 ± 2 @ 0.1

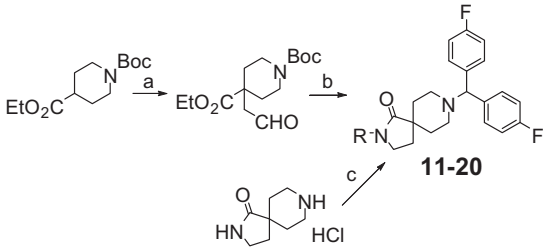
[a] For assay conditions see references.
[b] NA (not active) denotes <20% inhibition.

Analogs **6** and **7** attempted to fill the third hydrophobic region by attaching a moiety to the benzyl substituent; α1H potency increased in relation to the size of the moiety off the benzyl group (**6**, EtO, 67 ± 6 at 3 μM and **7**, PhO, 56 ± 14 at 0.3 μM). A Strecker and Bruylants reaction sequence with diazaspirodecanone **1** afforded analog **8**,[15] which replaced the two phenyl rings of the benzhydryl moiety with two isopropyl groups; this analog inhibited α1H (40 ± 4% at 0.3 μM) but was less potent than **2**. Acylation and sulfonylation of **1** afforded compounds **9** and **10**, which were inactive and weakly active, respectively.

Compounds **11–20** were prepared to explore changes to the hydrophobic region on the amide side of the spirocycle. Alkylation of ethyl 1-*tert*-butoxycarbonylpiperidine-4-carboxylate with allyl bromide followed by oxidative cleavage afforded the ethyl ester aldehyde (Scheme 2). Reductive aminations and concomitant cyclization afforded Boc-protected diazaspirodecanones. Acid catalyzed deprotections and alkylations with 4,4-difluorobenzhydryl chloride afforded compounds **11–19**. Insertion of a methylene spacer into the 4-chlorobenzyl moiety (**11**, 30 ± 11% at 0.03 μM) or moving the chloro from the ortho to the *meta* position (**12**, 91 ± 1% at 0.3 μM) reduced α1H potency, however the 3-methoxy-



Scheme 1. Reagents and conditions: (a) 4,4-difluorobenzhydryl chloride, Cs_2CO_3, DMF, 70 °C, 16 h, 17%; (b) $LiAlH_4$, THF, rt, 16 h, 7%; (c) RCHO, $NaBH(OAc)_3$, DCE, 5–16 h, 77–99%; (d) (i) isobutyraldehyde, $(CH_3)_3SiCN$, CH_3CN, rt, 16 h, 51%; (ii) *i*PrMgBr, THF, rt, 16 h, 21%; (e) 4-fluorobenzoyl chloride, NaOH, THF, H_2O, rt, 1 h, 81%; (f) 4-fluorophenylsulfonyl chloride, NaOH, THF, H_2O, rt, 12 h, 69%.



Scheme 2. Reagents and conditions: (a) (i) $((CH_3)_3Si)_2NLi$, allyl bromide, THF, −70–0 °C, 4 h, 97%; (ii) $NaIO_4$, OsO_4(cat), dioxane, H_2O, rt, 16 h, 76%; (b) (i) RNH_2, $NaBH(OAc)_3$, DCE, rt, 1 h then reflux, 16 h, 45–86%; (ii) CF_3CO_2H, DCM, rt, 1 h, 100%; (iii) 4,4-difluorobenzhydryl chloride, K_2CO_3, DMF, 80 °C, 16 h, 11–21%; (c) 4,4-difluorobenzhydryl chloride, K_2CO_3, DMF, 80 °C, 16 h, 10%.