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Graphical Abstract

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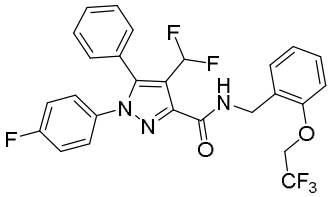

12d
$Ca_v3.1$: IC_{50} = 160 nM
$Ca_v3.2$: IC_{50} = 5000 nM
$Ca_v3.3$: IC_{50} > 10000 nM

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ABSTRACT

We identified and characterized a series of pyrazole amides as potent, selective $Ca_v3.1$-blockers. This series culminated with the identification of pyrazole amides **5a** and **12d,** with excellent potencies and/or selectivities toward the $Ca_v3.2$- and $Ca_v3.3$-channels. This compound displays poor DMPK properties, making its use difficult for *in vivo* applications. Nevertheless, this compound as well as analogous ones are well-suited for *in vitro* studies.

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A first generation of T-channel blockers was developed in the 1990s, and culminated with the discovery of mibefradil. This drug, developed at Roche for angina pectoris, and meanwhile withdrawn from the market due to unacceptable drug-drug interactions, is a rather potent, but unselective T-type calcium channel blocker.[1] A second generation of T-type calcium channel blocker arose during the last years, with two compounds, MK-8998[2] and Z-944,[3] entering clinical trials. These compounds are *selective* T-type calcium channel blockers, meaning that they block the $Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$ channels with similar potencies, while they do not block other channels.

We recently disclosed a series of dihydropyrazole derivatives as selective T-type calcium channel antagonists[4], and demonstrated their *in vivo* efficacy in two animal models of epilepsy, the WAG/Rij rat model for absence seizures. Simultaneously, we observed in spontaneously hypertensive rats that these compounds induced a prolongation of the PR interval on ECG. Due to the excellent PK/PD correlation and a strong parallelism with the desired antiepileptic effect, we hypothesized that this PR prolongation was directly linked to the blockade of all T-type calcium channel subtypes in the rodent heart. We conjectured that we might be able to dissociate both effects (i.e. desired antiepileptic effect without PR prolongation) by applying *subtype selective* T-type calcium channel blockers, i.e. compounds that would block one of the three T-type calcium channel only. A certain subtype selectivity was possible to achieve. In this paper, we describe the discovery and the study of $Ca_v3.1$-subtype selective blockers, whereas $Ca_v3.2$-subtype selective blockers were described in the previous paper. Furthermore, we described recently a compound with a moderate selectivity for the $Ca_v3.3$ channel.[5]

In the previous paper, we described a series of pyrrole-based $Ca_v3.2$-subtype selective blockers. In an effort to increase the polarity of such compounds, we investigated pyrazole analogues from this series. Indeed, compound **1**[6] (Fig. 1) had appeared as a weak hit in an HTS campaign (Table 1). Two other hits, compounds **2a** and **2b**, prepared via an amide coupling from commercially available starting material, proved to be moderately potent $Ca_v3.1$- and $Ca_v3.2$-blockers, with no measurable potency against $Ca_v3.3$, this in in the FLIPR® assay we had been using previously[4,5] (Table 1). In particular, an *ortho*-substituent seemed to confer a good subtype selectivity for the $Ca_v3.1$ channel. In a first SAR study at the amide position, we introduced an *ortho*-trifluoromethoxy substituent that had been successful in the development of $Ca_v3.2$-blockers, as described in the previous manuscript. Compound **2c** proved to be a slightly more potent and subtype selective $Ca_v3.1$-blocker. Compound **2c** was confirmed using patch-clamp (>99%, 83%, and 39% block of $Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$ at 10000 nM, respectively).

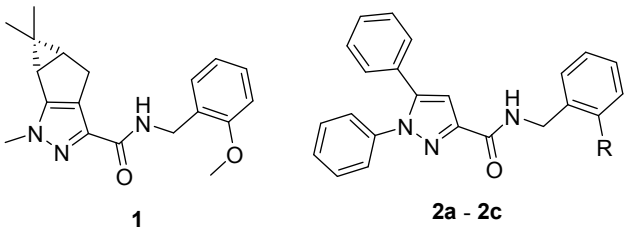


Fig 1. First pyrazole-based $Ca_v3.1$-blockers

Table 1. Potencies of a few T-channel blockers (FLIPR®)

Compound	R-substituent	$Ca_v3.1$ IC_{50} (nM)[a]	$Ca_v3.2$ IC_{50} (nM)[a]	$Ca_v3.3$ IC_{50} (nM)[a]	Ratio $Ca_v3.2$/ $Ca_v3.1$
1	-	2100	5800	9000	-
2a	-H	200	510	>10000	2.6
2b	-F	130	1900	>10000	15
2c	CF_3CH_2O-	59	1000	>10000	17

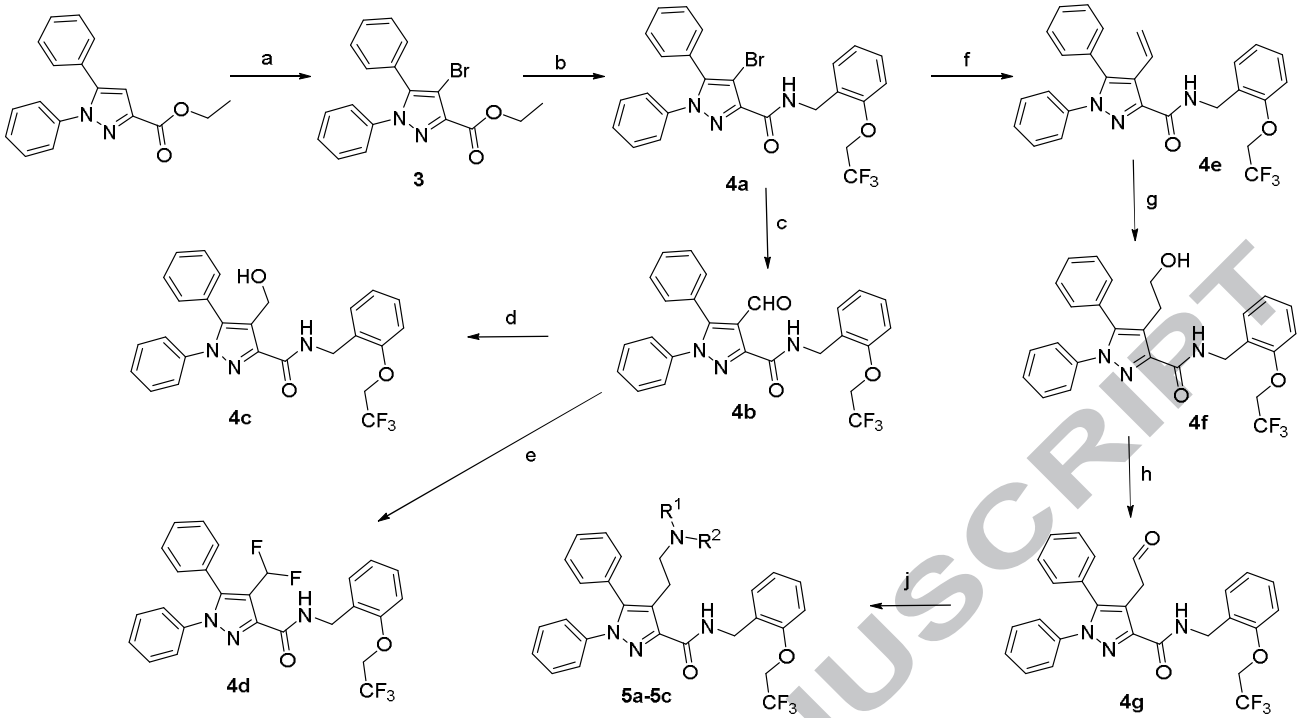
[a]Geometric mean of at least two measurements

In a next step, we explored at first the position 4 of the pyrazole ring. From commercial starting material, a bromination led to compound **3** (Scheme 1). Saponification and amide coupling led to a first derivative **4a**. Formylation, reduction, and fluorination led to compounds **4b**, **4c**, and **4d**, respectively. Vinylation of compound **4a** led to derivative **4e**, which was transformed into compounds **4f** and **4g** via hydroboration and oxidation. Eventually, reductive aminations of compounds **4g** led to secondary and tertiary amines **5a** – **5c**.

Table 2. Potencies of first $Ca_v3.1$-subtype selective blockers (FLIPR®)

Compound	R^1R^2N-substituent	$Ca_v3.1$ IC_{50} (nM)[a]	$Ca_v3.2$ IC_{50} (nM)[a]	$Ca_v3.3$ IC_{50} (nM)[a]	Ratio $Ca_v3.2$/ $Ca_v3.1$
4a	-	20	350	>10000	17
4c	-	55	410	1370	7.4
4d	-	49	820	>10000	17
4e	-	71	850	>10000	12
4f	-	46	310	650	6.8
5a	EtNH-	52	280	1900	5.3
5b	Cyclopropyl-NH	200	590	900	2.9
5c	Et_2N-	240	2150	4370	9.0

[a]Geometric mean of at least two measurements



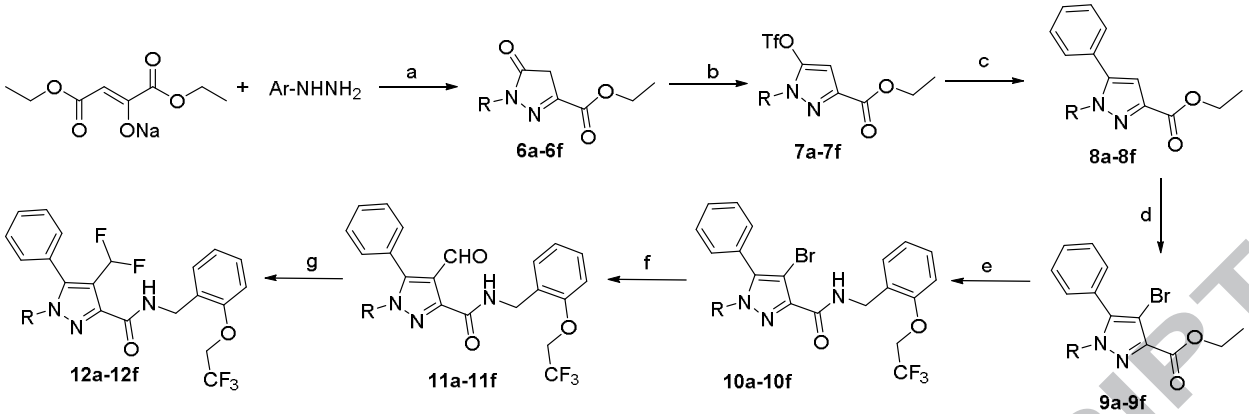
Scheme 1: Preparation of $Ca_v3.1$-subtype selective blockers **4a**-**4g** and **5a**-**5c**. (a) NBS, AcOH, microwave, 150 °C, 10 min, 87%; (b) (i) 2.5M aq. NaOH, EtOH, reflux, 1 h; (ii) *ortho*-trifluoroethoxybenzylamine, EDC·HCl, HOBt, DMAP, DIPEA, CH_2Cl_2, rt, overnight, 50% over two steps; (c) 3M MeMgCl in THF, 1.6M ^{t}BuLi in pentanes, DMF, THF, -78°C to rt, 2 h, 46%; (d) $NaBH_4$, MeOH, rt, 1 h, 73%; (e) DAST, CH_2Cl_2, rt, 2 weeks, 39%; (f) vinylboronic anhydride pyridine complex, $Pd(^tBu_3P)_2$, K_2CO_3, H_2O, dioxane, 80 °C, overnight, 85%; (g) 0.5M 9-BBN in THF, 70 °C, overnight, 6M aq. NaOH, 30% H_2O_2, 50 °C, 1 h, 93%; (h) Dess-Martin periodinane, CH_2Cl_2, 0 °C to rt, 3 h, 64%; (j) amine, $NaBH(OAc)_3$, CH_2Cl_2, rt, 6 h, 71-94%.

The bromine substituent (compound **4a**) increased slightly the potency on the $Ca_v3.1$- and $Ca_v3.2$-channel compared to compound **2c**, while no potency was measurable on the $Ca_v3.3$-channel. Overall, subtype selectivity toward the $Ca_v3.2$-channel, the most difficult one to achieve, was maintained. From compounds **4c** – **4f**, we concluded that polarity at this position is not an important factor for the absolute potency on the $Ca_v3.1$-channel. On the other hand, a polar primary hydroxyl substituent tended to decrease selectivity toward the $Ca_v3.3$ channel. In a further effort to improve polarity, we introduced primary and secondary amines (compounds **5a** – **5c**). Here again, a polar ethyl amine (**5a**) retained the potency toward $Ca_v3.1$ with a low selectivity toward $Ca_v3.2$. The other amines, (**5b** and **5c**) led to a loss in potency.

With these results in hand, we investigated the SAR at position 1 of the pyrazole ring. For this, we prepared numerous derivatives as depicted in Scheme 2[7]. Cyclization between ethyl oxaloacetate and the corresponding hydrazine led to compounds **6a**-**6f**. Triflation (**7a**-**7f**), Suzuki couplings (**8a**-**8f**) and bromination led to fully substituted pyrazoles **9a**-**9f**.

Saponification and amide couplings delivered amides **10a**-**10f**, which were subsequently formylated (**11a**-**11f**) and reductively aminated to compounds **12a**-**12f**. Unfortunately, we were not able to develop a chemistry that would allow the introduction of diversity at position 1 at a later stage of the synthesis.

Considering the SAR, we focused our efforts by fixing the substituent at position 4 to a bromine or to a difluoromethyl, since the highest level of selectivity was achieved with them. Introduction of a benzyl group at position 1 led to a loss of potency (Table 3, compound **10a**) and the presence of the small methyl group led to compound **10e**, with very similar potencies as was measured with the benzyl group. On the other hand, introducing a somewhat larger trifluoroethyl group (compound **10f**) led to an increase in potency and selectivity. Overall, most potent and selective compounds were obtained from an *ortho*-fluorophenyl substituent (compound **10b**). Other substitution pattern led to a loss in potency. Generally, the difluoromethyl sub-series (compounds **12a**-**12f**) were less potent than their bromo analogues (compounds **10a**-**10f**). Finally, the excellent selectivity ratio of compound **12d** should be noted.



Scheme 2: Preparation of compounds **10a**-**10f** and **12a**-**12f**. (a) $RNHNH_2$, AcOH, H_2O, toluene, reflux, overnight, 58-92%; (b) Tf_2NPh, Et_3N, THF, 0 °C to rt, 1 h, 58-82%; (c) $PhB(OH)_2$, $Pd(PPh_3)_4$, 2M aq. Na_2CO_3, toluene, 95 °C, overnight, 54-85%; (d) NBS, AcOH, microwave, 150 °C, 10 min, 59-86%; (e) (i) 2.5M aq. NaOH, EtOH, reflux, 1 h, (ii) *ortho*-trifluoroethoxybenzyyl amine, EDC·HCl, HOBt, DMAP, DIPEA, CH_2Cl_2, rt, overnight, 30-50%; (f) 3M MeMgCl in THF, 1.6 M ^tBuLi in pentanes, DMF, THF, -78°C to rt, 2 h, 35-58%; (g) DAST, CH_2Cl_2, rt, 2 weeks, 25-56%.

Table 3. Pyrazole derivatives **10a-f** and **12a-f**: SAR (FLIPR®)

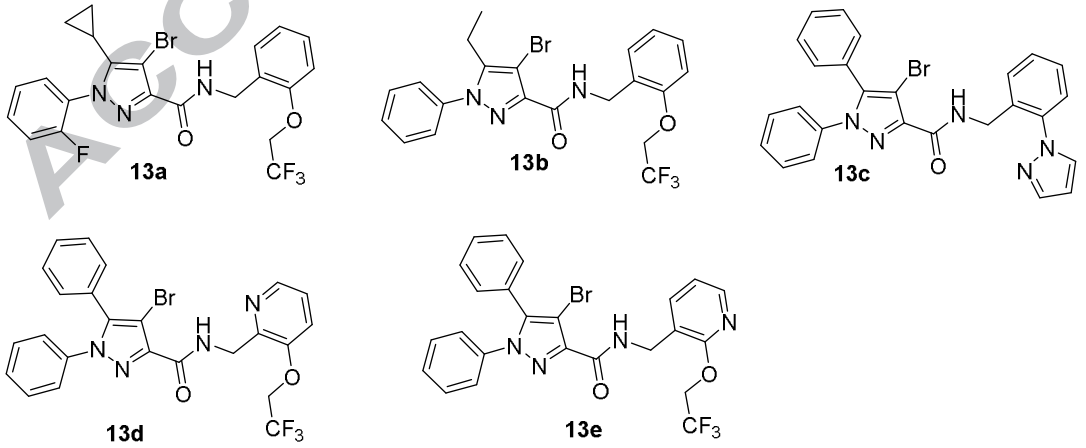
Compound	R-substituent	$Ca_v3.1$ IC_{50} (nM)[a]	$Ca_v3.2$ IC_{50} (nM)[a]	$Ca_v3.3$ IC_{50} (nM)[a]	Ratio $Ca_v3.2$/ $Ca_v3.1$
10a	Bn-	335	1450	>10000	4.3
10b	*o*-F-Ph-	28	300	>10000	11
10c	*m*-F-Ph-	220	580	>10000	2.6
10d	*p*-F-Ph-	88	410	>10000	4.7
10e	Me-	280	220	2850	0.8
10f	CF_3CH_2-	88	890	1500	10
12a	Bn-	250	170	>10000	0.7
12b	*o*-F-Ph-	190	1100	8600	5.8
12c	*m*-F-Ph-	360	1500	-	4.2
12d	*p*-F-Ph-	160	5000	>10000	31
12e	Me-	250	370	890	1.5
12f	CF_3CH_2-	410	600	1400	1.5

[a]Geometric mean of at least two measurements

Modifications at position 5 were possible applying Suzuki couplings in a similar manner as described in Scheme 2. Unfortunately, such modifications let only to limited improvement in terms of potency. The introduction of a cyclopropyl group (compound **13a**, Table 4), or of an ethyl group (compound **13b**) led to moderately potent derivatives with almost no subtype-selectivity.

Having optimized the substituents at the pyrazole ring, we reinvestigated the amide substituent, focusing on more polar heteroaryl systems (compounds **13c** – **13e** in Table 4). Again, with this approach we were unable to improve potency and selectivity with regard to the former compounds.

Table 4. Last pyrazole derivatives (FLIPR®)



Compound	$Ca_v3.1$ IC_{50} (nM)[a]	$Ca_v3.2$ IC_{50} (nM)[a]	$Ca_v3.3$ IC_{50} (nM)[a]	Ratio $Ca_v3.1/Ca_v3.2$
13a	100	290	110	2.8
13b	110	380	1700	3.4
13c	32	400	1800	12
13d	45	380	>10000	8.5
13e	58	600	>10000	10

[a] Geometric mean of at least two measurements.

With apolar substituents only being tolerated for potency, the compounds were bound with poor physicochemical and ADME properties. Most compounds were quasi-insoluble in aqueous medium at pH 7 (< 1mg/L). Compounds **5a** – **5c**, bearing an aliphatic amine, formed an exception. In particular, compound **5a** displayed a solubility of 429 mg/L in an aqueous medium at pH 7 and a rat liver microsomal intrinsic clearance (RLM) of 377 µL/min·mg protein. We decided to administer this compound in vivo to Wistar rats at 100 mg/kg po, and measured plasma and brain concentrations after 1 h. Under these conditions, we reached a plasma concentration of 810 nM ($C_{u,plasma}$ = 4.9 nM), and a total brain concentration of 80 ± 17 nM. Under these conditions the unbound plasmatic concentration remained below the IC_{50}-values, and the brain exposure was marginal, precluding any *in vivo* pharmacological experiment. Compound **5a** also suffered from selectivity issues toward other channels. IC_{50}-value for the $Ca_v1.2$-channel was measured at 860 nM, and this compound was strongly blocking sodium channels in rat cortical neurons at 10 µM (72% and >99% blockade at the first and last peak respectively). Under the same conditions, the slow- and fast potassium channels were blocked 49% and 44% respectively.

In conclusion, we designed potent $Ca_v3.1$-blockers with selectivity ratio up to 13 toward the $Ca_v3.2$ channel, and even better toward the $Ca_v3.3$ channel. These compounds also block partially sodium and potassium channels in organotypic slices. *In vivo* exposition of compound **5a** remains modest, nevertheless this class of compounds can be used for *in vitro* studies.

Conflict of interest

The authors declare no conflict of interest.

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Abbreviations

AcOH: acetic acid; 9-BBN: 9-borabicyclo(3.3.1)nonane; DAST: diethylaminosulfur trifluoride; DIPEA: *N*,*N*-diisopropylethylamine; DMAP: 4-(dimethylamino)pyridine; DMF: *N*,*N*-dimethylformamide; EDC·HCl: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt: Hydroxybenzotriazole; NBS: *N*-bromosuccinimide; ^{t}Bu: *tert*-butyl; Tf: trifluorosulfonyl; THF: tetrahydrofuran.

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