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Discovery of a class of potent gap-junction modifiers as novel antiarrhythmic agents

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ABSTRACT

In an effort to discover potent, orally bioavailable compounds for the treatment of atrial fibrillation (AF) and ventricular tachycardia (VT), we developed a class of gap-junction modifiers typified by GAP-134 (**1**, R^1 = OH, R^2 = NH_2), a compound currently under clinical evaluation. Selected compounds with the desired in-vitro profile demonstrated positive in vivo results in the mouse $CaCl_2$ arrhythmia model upon oral administration.

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The conduction of electrical impulses in the heart is established through gap-junctions. Targeting gap-junctions represents a distinct approach for the treatment of a variety of diseases involving dis-regulation of heart rhythm such as AF and VT.[1] Gap-junctions uncouple during ischemia leading to impulse slowing, unidirectional block and re-entry arrhythmias.[2] The molecular mechanism of action of gap-junction modulators including the clinical candidate GAP-134 (**1**, (2*S*,4*R*)-1-(2-aminoacetyl)-4-benzamidopyrrolidine-2-carboxylic acid, Fig. 1) remains unknown,[3] thus precluding the use of high through-put methods to screen for additional compounds. We chose to utilize an in vivo mouse $CaCl_2$ arryhythmia assay to screen analogs of GAP-134.[4]

Here, we report efficacious gap-junction modifiers that re-establish intercellular communications and delay time to cardiac conduction block in mice treated with intravenous $CaCl_2$. Starting with the glycine-proline dipeptide motif that is derived from compound **1**, we focused on modulating the lipophilic/hydrophilic balance by varying R^1 and R^2 groups (Fig. 1) to generate analogs with modified physicochemical properties and improved oral bioavailability. The effort to optimize the parameters and the activity of gap-junction modifiers had to be balanced by the requirement to establish in vivo activity for each synthetic target. The untraditional screening paradigm created limits on the scope of structure–activity relationship (SAR) studies we report in this manuscript.

The initial effort to study SAR around the clinical candidate **1** was focused on modifying hydrophilic areas of the molecule. We reasoned that by altering the polar groups such as carboxylic acid and amino functionalities, we could modulate compound lipophilicity. Blocking the N- and/or C-terminus of compound **1** should afford analogs with potentially better in vivo absorption. The C-terminal

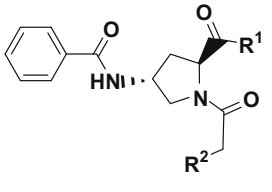

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Figure 1. GAP-134 dipeptide in clinical evaluation: R^1 = OH; R^2 = NH_2.

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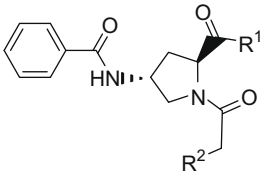
proline carboxylic acid group was converted to an amide functionality using simple coupling procedures.[5] The primary amide derivative **2** demonstrated significant in vivo efficacy in the mouse $CaCl_2$ model (Table 1). It was also discovered that amide **2** had poor stability in rat plasma. Therefore, despite potentially rapid clearance upon administration into mice, the primary amide analog **2** showed similar in vivo activity versus the parent compound **1**. This fact indicated that the substitution of the carboxylic acid group in **1** with an amide functionality does not lead to the loss of efficacy. This observation encouraged us to further investigate amide derivatives of compound **1**. Initially, it became our goal to identify the reason for the low stability of **2** and, thus, improve the properties of amide analogs. A drastic improvement in stability was observed when unsubstituted amide **2** was followed up with methyl amide **3** and ethyl amide **4**. Mass spectroscopy analysis of the plasma incubation samples of amides **2** and **3** suggested the formation of a single degradation product. Amide **3** was subjected to basic media over a 24 h time period to generate piperazine-dione **5** (Fig. 2) that was further confirmed to be identical to the degradation product in all plasma samples. Piperazine-dione **5** demonstrated no in vivo activity at the 10^{-11} mol/kg dose tested. Thus, amides **2** and **3** can have somewhat reduced potency due to their partial decomposition. The loss of activity for the ethyl amide compound **4**, however, cannot be attributed to stability issues since it showed no signs of decomposition. Structural considerations may have played a role in the reduced activity. Full plasma stability was achieved with bulkier amide groups in analogs **6** and **7**, where larger substituents prevented intramolecular cyclization. Stable amide derivatives showed in vivo activity that was comparable to that of parent compound **1**.

Table 1
Effect of GAP-134 analogs on time to cardiac conduction block (ΔT) in mice receiving an intravenous infusion of calcium chloride



Compound	R^1	R^2	Increase in time to conduction block, ΔT (% of response in control)[a,b]	Plasma stability (% remaining)[c]
1	OH	NH_2	206.6 ± 21.0	93
2	NH_2	NH_2	193.2 ± 10.9[d]	27
3	NH–CH_3	NH_2	145.0 ± 13.1[d]	87
4	NH–C_2H_5	NH_2	137.2 ± 8.8	100
6	NH–*i*-Pr	NH_2	162.0 ± 20.8	100
7	NH–*cyclic*-Pr	NH_2	196.0 ± 16.0	96
8	NH–*cyclic*-pentyl	NH_2	110.6 ± 13.7	90
9	NH–*t*-Bu	NH_2	135.7 ± 11.0	99
10	(*R*)–NH–CH(CH_3)(*i*-Pr)	NH_2	120.1 ± 7.3[d]	89
11	NH-tetrahydro-2H-pyran-4-yl	NH_2	99.4 ± 8.3[d]	81
12	(*R*)–NH-tetrahydrofuran-3-yl	NH_2	143.5 ± 16.1	100
13	OCH_3	NH–Boc	166.3 ± 12.7[d]	0
14	OH	NH–Boc	156.2 ± 15.7	100
15	OH	NH–CH_3	120.7 ± 22.7	100
16	OH	NH–$(CH_3)_2$	118.9 ± 12.3	100
17	OH	NH–$COCH_3$	165.7 ± 14.0	100
18	OH	NH–CHO	170.4 ± 16.1	100
19	OH	NH–$COCF_3$	132.1 ± 17.4[d]	34

Compound stability in plasma.
[a] Values are means of at least three experiments, standard deviation is given.
[b] Measurements were performed at 10^{-11} mol/kg compound concentration.
[c] Rat plasma stability -% remaining after 3 h incubation.
[d] Data may reflect lower concentration due to compound instability.

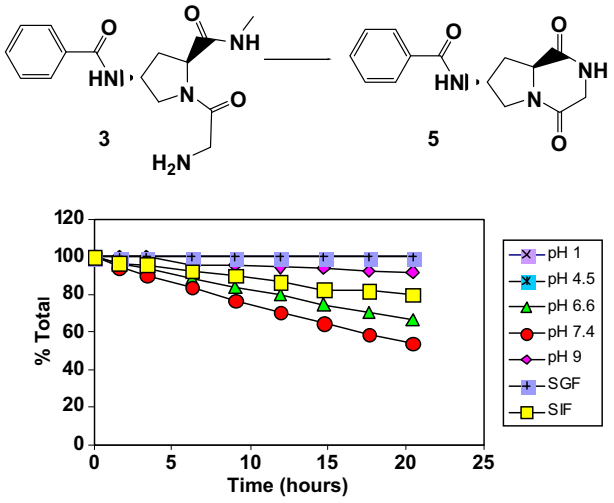


Figure 2. Degradation profile of **3** in buffers at 37 °C. SGF-simulated gastric fluid; SIF-simulated intestinal fluid.

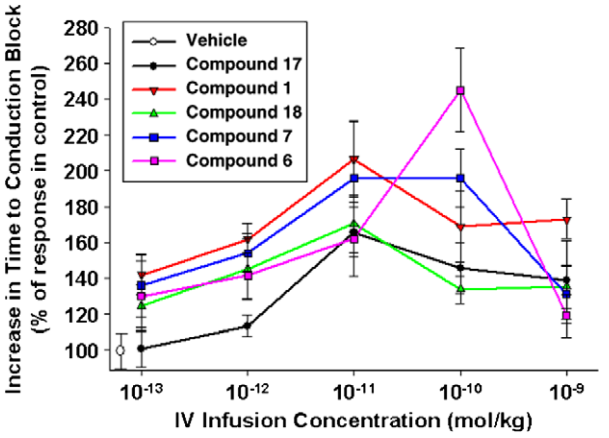


Figure 3. Mouse $CaCl_2$ data for **1**, **6**, **7**, **17** and **18** after IV administration.

These compounds were further investigated in dose response experiments. Other amide compounds at the C-terminus (**8**–**12**) demonstrated various degrees of activity and stability. It proved that large substituents (**12**) are tolerated to some degree and are suited for further studies. Methyl ester compound **13** showed activity in the mouse model despite having poor stability. It was determined that **13** undergoes hydrolysis to afford Boc derivative **14** with a similar activity as **13**.

N-Terminal analogs (Table 1) generally showed weaker activity in the mouse $CaCl_2$ model. Mono- or di-methylation of the amino group (**15**, **16**) led to the reduced efficacy. Acyl derivatives **17** and **18** were more potent than alkyl analogs **15** and **16**. Hence, compounds **17** and **18** were further investigated in full dose response studies. It was assumed that the acyl groups should significantly change the lipophilicity of the molecules to warrant further PK studies despite their relatively weaker potency. Compounds **6**, **7**, **17** and **18** were compared to the clinical candidate **1** with respect to in vivo efficacy after IV and oral administrations (Fig. 3 and Figure 4, respectively). Dose dependency was observed for all studied analogs of **1** with ΔT values after IV administration reaching over 200% versus control (Fig. 3). The bell shaped dose response curve is specific to the mouse $CaCl_2$ model, and has been previously observed with other antiarrhythmic compounds by Zealand Pharma's research group.[6] Compounds **6** and **7** showed effi-

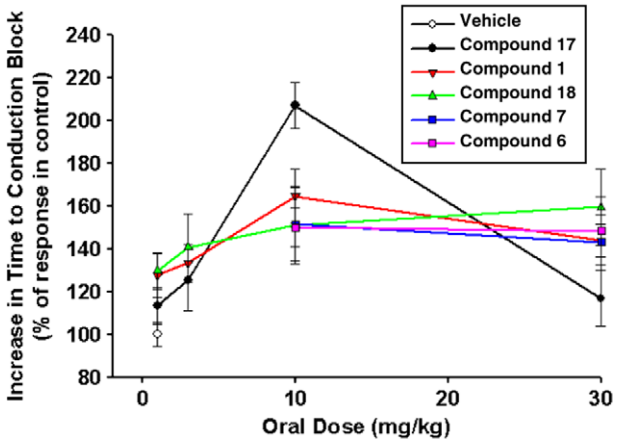


Figure 4. Mouse $CaCl_2$ data for **1**, **6**, **7**, **17** and **18** after oral administration.

cacy that is either similar to the activity of compound **1** or exceeds it at some doses. Oral administration of compounds **6**, **7**, **17** and **18** with doses 1–30 mg/kg significantly prolonged the time to conduction block in mice after infusion of $CaCl_2$ (Fig. 4), thus, establishing oral activity of the compound class. The oral dose response curve for **17** showed differences with the other compounds' curves where much higher efficacy was observed at 10 mg/kg, while the efficacy at the highest dose was lower than for compounds **1**, **6**, **7** and **18**.

Additional structure–activity relationship points were obtained in the course of the initial medium through-put screening of the Zealand Pharma small pepetide library via IV administration at 2×10^{-11} mol/kg ($n = 7$–11). The lack of robust screening capabilities precluded us from reproducing multiple compounds in dose response measurements. Nevertheless, activity was unambiguously established for multiple analogs of compound **1** that demonstrated ΔT value of 149.8% of saline control at 2×10^{-11} mol/kg dose. It was found that the presence of electron-donating and electron-withdrawing *para*-substituents is tolerated. For example, the ΔT values for *p*-nitro-group and *p*-methyl-group analogs are 166.0% and 168.3% of control, respectively. The enantiomer of compound **1**, (2*R*,4*S*)-1-(2-aminoacetyl)-4-benzamidopyrrolidine-2-carboxylic acid, showed similar in vivo activity in the mouse $CaCl_2$ model (134.9%). One of the diastereomers of compound **1**, (2*S*,4*S*)1-(2-amino-acetyl)-4-benzoylamino-pyrrolidine-2-carboxylic acid, has a $\Delta T = 168.3\%$ of control.

Having established the oral activity of compounds **6**, **7**, **17** and **18**, our efforts focused on the evaluation of their PK properties compared to clinical candidate **1**. The aforementioned amides have either the C- or N-terminus modified with a more lipophilic functionality than present in molecule **1**. All analogs were dosed orally and IV in CD-1 mice (Table 2) since the mouse model was a primary screening tool for the program. However, clinical candidate **1** was previously evaluated in a dog atrial fibrillation model.[7] Therefore, all advanced compounds were tested in dog PK both oral and IV routes to compare them to **1** (Table 3).

As some PK parameters of compounds **6**, **7**, **17** and **18** were found to be similar to GAP-134 (**1**), the stability of these analogs required further investigation. All compounds showed $T_{1/2}$ >30 min upon incubation with human and rat microsomes. The stability in aqueous solutions of various pH values, simulated intestinal fluid and simulated gastric fluid was also established for these four compounds. However, compound **18** underwent in vivo metabolism resulting in formation of small amount of GAP-134 (**1**). As a result, further development of **18** was stopped. Absorption limited PK profile suggests that additional efforts to modulate compounds

Table 2
CD-1 mice pharmacokinetic parameters for compounds **1**, **6**, **7**, **17** and **18**[a]

	1	**6**	**7**	**17**	**18**[c]
IV (1 mg/kg) CD-1 mice					
Clearance (mL/min/kg)	65[b]	58	20	24	12
V_{ss} (L/kg)	2.7	2	0.6	0.3	0.2
$T_{1/2}$ (h)	1.6	0.9	2.1	0.3	0.4
PO (5 mg/kg) CD-1 mice					
Mouse% F	6	6	7	2	2
$T_{1/2}$ (h)	1.9	1.6	1.2	1.2	1.4
C_{max} (ng/mL)	26	44	87	38	89
T_{max} (h)	0.5	0.25	0.25	0.25	0.25
AUC (ng h/mL)	73	90	305	66	144

[a] Values are obtained from the mean drug concentration. ($N = 3$ animals/time point).
[b] Actual dose was 0.77 mg/kg for compound **1**.
[c] IV Dose for compound **18** was 1.6 mg/kg.

Table 3
Dog pharmacokinetic parameters for compounds **1**, **6**, **7**, **17** and **18**[a]

	1	**6**	**7**[b]	**17**	**18**[c]
IV (1 mg/kg) dogs					
Clearance (mL/min/kg)	5 ± 0.5	5 ± 0.4	4 ± 0.5	8 ± 2.2	5 ± 0.9
V_{ss} (L/kg)	8 ± 2.2	1.1 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
$T_{1/2}$ (h)	6.3 ± 0.4	8.6 ± 0.5	6.9 ± 0.4	1.2 ± 0.2	1.5 ± 0.2
PO (5 mg/kg) dogs					
Dog% F	14	16	18	8	13
$T_{1/2}$ (h)	4.0 ± 0.5	8.3 ± 1.8	6.3	4.5 ± 2.8	6.2 ± 3.6
C_{max} (ng/mL)	799 ± 392	650 ± 201	658	258 ± 117	737 ± 136
T_{max} (h)	1.1 ± 0.6	1.2 ± 0.8	2.0	0.8 ± 0.3	1.4 ± 0.8
AUC (ng h/mL)	4791 ± 0.5	2849 ± 385	4471	972 ± 197	3820 ± 461

[a] Values are means of at least three animals.
[b] Where there is no SD, the number of animals is two.
[c] For compound **18**, IV dose administered was 2.3 mg/kg and oral was 7.8 mg/kg.

lipophilicity may be necessary. The oral bioavailability of compounds **6** and **7** was found to be similar to **1**, suggesting that modification of only one terminus of the de-peptide motif is not sufficient to improve bioavailability of the scaffold.

In summary, we report the development of the first orally bioavailable scaffold of small molecule gap-junction modifiers. Selected compounds prolonged the time to AV conduction block in the mouse model upon oral administration. Compounds **6**, **7**, **17** and **18** showed no hERG or CYP450 inhibition. Further modifications of this unique dipeptide scaffold to improve PK parameters may be of interest for future studies.

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5. (2*S*,4*R*)-4-benzamido-1-(2-(*tert*-butoxycarbonylamino)acetyl)pyrrolidine-2-carboxylic acid (0.05 g, 0.1 mmol), 1-hydroxybenzotriazole monohydrate (Aldrich, 0.021 g, 0.15 mmol, 1.2 equiv) and 1-(3,3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Aldrich, 0.029 g, 0.15 mmol, 1.2 equiv) were dissolved in acetonitrile (15 mL) under a nitrogen atmosphere with ice cooling. The temperature was gradually increased to room temperature over 2 h time period, and the mixture was then stirred at room temperature overnight. The reaction solution was again cooled to 0 °C, a 25–30% aqueous solution of the corresponding amine (prepared from a pure reagent obtained from Aldrich)

(0.1 mL) was added, and stirring was continued with cooling for 30 min and then at room temperature for 2 h. Acetonitrile (5 mL) was added to the reaction mixture, and the volatiles were removed in vacuo. The semi-solid residue was purified by silica-gel (EMD, 0.040–0.063 mm) chromatography (developing solvent: 3–5% gradient methanol–dichloromethane) to afford the corresponding amides in 80–87% yield. The products from the previous step were dissolved in dry dichloromethane (10 mL) under a nitrogen atmosphere, and a 1 M ethereal solution of hydrogen chloride (Aldrich) (1 mL) was added while keeping the temperature below 30 °C. The reaction mixture was stirred overnight under a nitrogen atmosphere. The precipitate was filtered, washed with dichloromethane (2 mL) and diethyl ether (2 mL) and dried under high vacuum to afford a hydrochloride salt of the corresponding compounds in 75–84% yield and at least 98% purity.

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