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Towards a KCC2 blocker pharmacophore model

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ABSTRACT

A multi-disciplinary approach was used to identify the first pharmacophore model for KCC2 blockers: several physico-chemical studies such as XRD and NMR were combined to molecular modelling techniques, SAR analysis and synthesis of constrained analogues in order to determine a minimal conformational space regrouping few potential bioactive conformations. These conformations were further compared to the conformational space of a different series of KCC2 blockers in order to identify the common pharmacophoric features. The synthesis of more potent analogues in this second series confirmed the usefulness of this KCC2 blocker pharmacophore model.

KCC2 is a neuronal-specific electroneutral potassium-chloride co-transporter belonging to a larger family of cation-chloride co-transporters (CCC's) which play important roles in a variety of physiological processes. KCC2 acts as a key modulator of inhibitory neurotransmission in the brain and in the spinal cord. Its potential role in pathologies such as epilepsy or neuropathic pain triggered the interest of several groups over the last few years.[1–5] Loop diuretics, such as furosemide, constitute a major class of drugs known to interact with KCC2 but without the adequate selectivity allowing using them as relevant pharmacological tools.

Despite the recent identification of selective KCC2 blockers chemical series,[6,7] no information is available regarding the structural requirements for binding to KCC2. In this Letter, we wish to report our strategy to derive the bioactive conformation of our hit compound **1** as well as the first pharmacophore model for KCC2 blockers by taking advantage of various sources of structural information such as molecular modelling techniques, crystallographic or NMR data. The hypotheses formulated along the study were assessed through SAR analysis as well as the design and synthesis of constrained analogues.

High throughput screening (HTS) of our corporate compound collection using a Rb^+ flux assay[8] on the KCC2 co-transporter led to the identification of benzyl 1-acetyl-2-benzylprolinate (*R*)-**1**[9] as a hit with an IC_{50} of 0.3 μM (Fig. 1). Interestingly, compound (*S*)-**1** is 100-fold less potent on KCC2 (IC_{50} = 50 μM, Table 1). We made the assumption that, for all analogues in this series, the active enantiomer has the same configuration as (*R*)-**1**, although only racemic mixtures were prepared. We reported earlier[6] the structure–activity relationships (SAR) around our hit compound focusing on the modulation of the two aryls and the *N*-acyl moieties. In order to identify the pharmacophoric features required for binding to the KCC2 transporter, several analogues were prepared where the ester function is replaced or which contain structural rigidification.

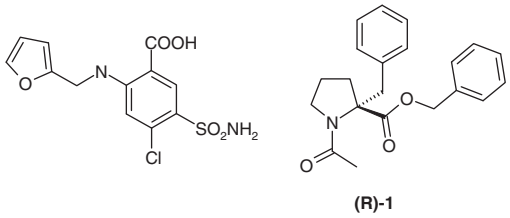

Figure 1. Furosemide reference compound and hit compound **1**.

An indoline-based analogue **2** was prepared by deprotonation with LiHMDS at −70 °C and alkylation with benzyl bromide (Scheme 1). The ketone analogues **4–5** were obtained following the synthetic route depicted in Scheme 2 from the methyl ester intermediate reported previously.[6] The aldehyde was prepared after a two-step procedure consisting of a $LiBH_4$ reduction of the ester followed by a Dess–Martin oxidation. The addition of a Grignard reagent then provided a primary alcohol that could be

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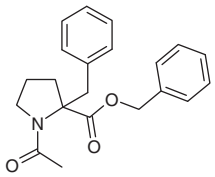
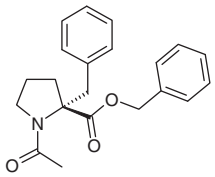
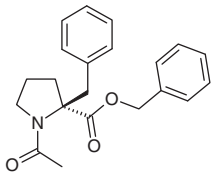
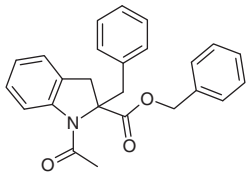
Table 1
KCC2 activities for ester compounds **1–2**

Compound	Structure	IC_{50}[a] (μM)
1		0.8
(*R*)-1		0.3
(*S*)-1		50
2		0.2

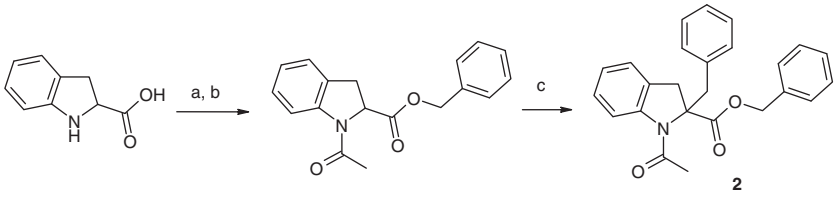
[a] Rb^+ flux assay on cloned rat KCC2.[8]

finally oxidized with the Dess–Martin reagent. The ether analogue **6** was easily obtained (Scheme 3) after alkylation of the previous alcohol intermediate with benzyl bromide. The oxazoline **7** was synthesized from the coupling of the 1-acetyl-2-benzylproline[6] with (*R*)-2-phenylglycinol followed by cyclisation in the presence of Deoxofluor™ (Scheme 4).[10] The oxazole **8** was prepared from the condensation of the primary amide with the 2-bromoacetophenone under microwave heating with poor yield (Scheme 5). A constrained analogue **9** was obtained from a known intermediate described by Duggan et al.[11] using a ring-closing metathesis followed by a catalytic hydrogenation (Scheme 6).

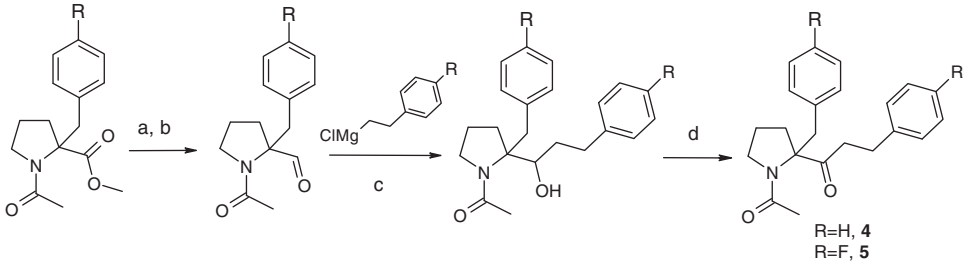
When compound **1** was identified, very little information was available on the pharmacophoric features required to observe binding to KCC2. As no other families of KCC2 blockers were known at the time, we decided to probe the bioactive conformation of compound **1** by systematically assessing each torsion angle of the molecule (Fig. 2) using molecular modelling techniques, crystallographic as well as NMR data and confronting those to SAR data. As the project progressed, additional families were identified which were used to derive a KCC2 pharmacophore described in this Letter.

We first focused on torsion angle T1. Within the 2-benzylprolinate family, we observed a loss of KCC2 activity upon replacement of the ester moiety by an amidic group (compound **3**, Table 2). On the other hand, compound **4**, in which the ester is replaced by $C(O)CH_2$ was synthesized and displays a potency of 0.3 μM. In addition, compound **6**, in which the ester was replaced by CH_2O only provides an activity of 20 μM. These data suggest that the carbonyl of the ester moiety is critical for activity and that the loss of potency of the amide **3** is not due to the loss of an H-bond acceptor but may rather be due to a conformational constraint. Indeed, the conformational space of compounds **1** and **3** were computed[12] and showed the presence of an intramolecular H-bond between the NH of the amide and the carbonyl of the acetamide in the case of compound **3** that constrains its conformational space compared to compound **1**. Some regions occupied by the benzyl ester group of **1** which was previously described as critical to the KCC2 activity[6] are not sampled by compound **3**. These regions are characterised by a torsion angle T1 of ~90°, 180° and 270°. Taking this feature into account, we designed several constrained bioisosteres of compound **1** in order to further explore T1. Compounds **7** and **8** were synthesized and tested against KCC2. Compound **7** in which T1 = 115° displays an IC_{50} of 1.3 μM which is in a comparable potency range as compound **1**. On the contrary, compound 8 which possess a torsion angle T1 ~ 180° is 10 times less potent. Torsion angle T1 ~ 90° was further considered as a potential pharmacophoric constraint.

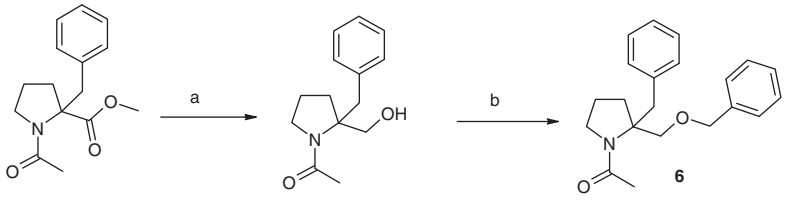
A search in the Cambridge Structural Database suggests that a torsion angle T3 of 180° is strongly favored. The *trans* conformation of the acetamide carbonyl is indeed described to be favored by an



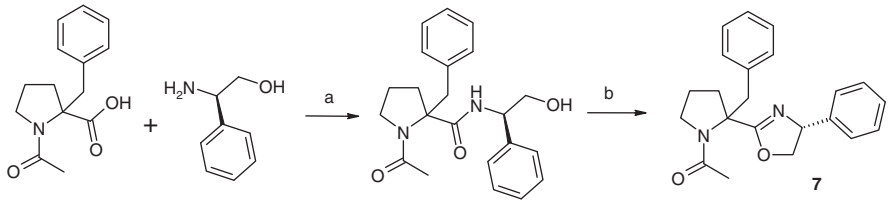
Scheme 1. Reagents and conditions: (a) CH_3COCl, K_2CO_3, CH_3CN, rt, 35%; (b) benzylbromide, K_2CO_3, KI cat., CH_3CN, 80 °C, 70%; (c) LiHMDS, THF, −70 °C, benzylbromide, 64%.



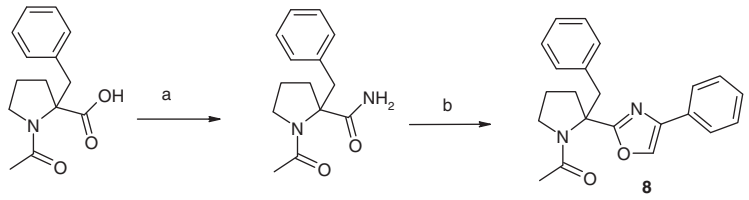
Scheme 2. Reagents and conditions: (a) $LiBH_4$, THF, 70 °C, 65%; (b) Dess–Martin reagent, CH_2Cl_2, rt, quant; (c) THF, 0 °C, 70% for R = H, 74% for R = F; (d) Dess–Martin reagent, CH_2Cl_2, rt, 82% for R = H, 80% for R = F.



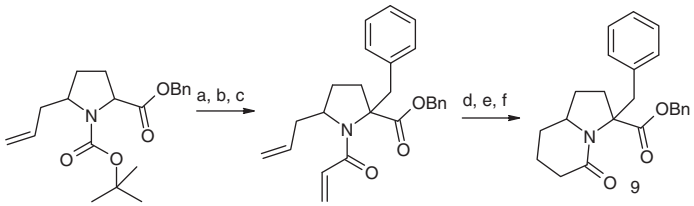
Scheme 3. Reagents and conditions: (a) $LiBH_4$, THF, RT, 62%; (b) benzylbromide, NaH, nBu$_4$NI cat., THF, rt, 30%.



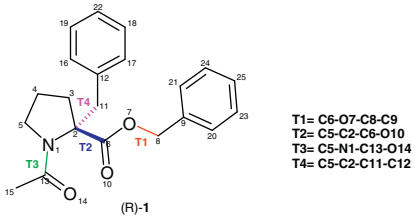
Scheme 4. Reagents and conditions: (a) EDCI, HOBt, DIPEA, CH_2Cl_2, rt, 65%; (b) deoxofluor™, CH_2Cl_2, −20 °C, 60%.



Scheme 5. Reagents and conditions: (a) EDCI, HOBt, NH_4OH aq, THF, 92%; (b) 2-bromoacetophenone, propionitrile, 120 °C, microwaves, 3 h, 2%.



Scheme 6. Reagents and conditions: (a) LiHMDS, THF, −70 °C, benzylbromide, 22%; (b) TFA, CH_2Cl_2, 86%; (c) acryloyl chloride, NEt_3, CH_2Cl_2, 95%; (d) Hoveyda-Grubbs catalyst 2nd gen. (10 mol %), CH_2Cl_2, 0 °C, 83%; (e) H_2, Pd/C, EtOH, 95%; (f) benzylbromide, DIPEA, CH_3CN, reflux, 65%.



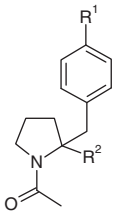
T1= C6-O7-C8-C9
T2= C5-C2-C6-O10
T3= C5-N1-C13-O14
T4= C5-C2-C11-C12

Figure 2. Chemical structure of benzyl *N*-acetyl-2-benzylprolinate (*R*)-**1** featuring the torsion angles considered for the study.

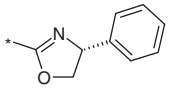
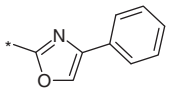
n→π* electronic effect with the proximal ester group as described by Hinderaker and Raines.[13] In order to test this hypothesis a constrained analogue, compound **9**, in which T3 equals 180° was synthesized and was shown to display an IC_{50} comparable to compound **1** (Table 3). This amide *trans* conformation was further confirmed by the ROESY NMR data generated on compound **5**. Indeed, the spectra obtained for this compound only displays one set of signals that could be attributed to the *trans* rotamer (Table 4, correlation 15–5). Moreover, the crystal structure[14] of compound **2**, a close analogue of **1** with an indoline core scaffold, was obtained, this compound being one of the only analogue of this series that could be crystallized in our hands. In this structure, the *trans* conformation of the amide carbonyl was also observed (Fig. 3).

In order to assess torsion angles T2 and T4, the ROESY NMR data obtained for compound **5** was further exploited, this ketone analogue was chosen because it affords more protons useful for measuring atom interdistances than the ester analogues. Correlations observed for compound **5** were converted into three distance intervals based on the relative intensities of the signals (Table 4). Intramolecular distances were calculated for the 42 low energy conformers of the conformational space of compound **5**. A set of 9 conformers complied with NMR constraints and presented torsion angles T1 and T3 of respectively 90° and 180°. These conformers display torsion angle values for T2 and T4 in the following ranges: 180° < *T2* < 299° and T4 ∼ 20° or ∼140°. In the crystal structure of compound **2**, these torsion angles are T2 = 189.5° and T4 = 21.4°, close to a low energy NMR and SAR compliant conformer of **5** (Fig. 3). At this stage of the project, using molecular modelling as well as spectroscopic studies on the only KCC2 blockers series known to us at the time, we were able to restrict the bioactive conformations of our series to less than a dozen conformers where T1 ∼ 90°, T3 ∼ 180°, 180° < T2 < 299° and T4 ∼ 20° or

Table 2
KCC2 activities for bioisoster compounds **3**–**8**

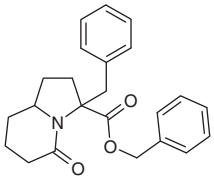


3-8

Compound	R^1	R^2	IC_{50}[a] (μM)
3[6]	H	$CONHCH_2Ph$	100
4	H	$COCH_2CH_2Ph$	0.3
5	F	$COCH_2CH_2Ph$ (p-F)	0.2
6	H	CH_2OCH_2Ph	20
7	H		1.3
8	H		12.6

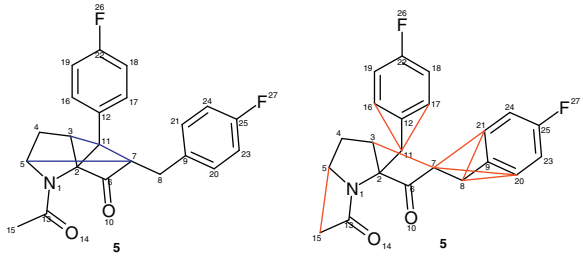
[a] Rb^+ flux assay on cloned rat KCC2.[8]

Table 3
KCC2 activities for constrained analogue **9**

Compound	Structure	IC_{50}[a] (μM)
9		1

[a] Rb^+ flux assay on cloned rat KCC2.[8]

Table 4
ROESY NMR data for compound **5**



Protons	Relative intensities[a] (ROESY)	H–H interatomic distances (Å)
5a–7	0.6	3.7–5
11a–7	0.6	3.7–5
3a–11a	0.3	3.7–5
11b–3b	0.11[b]	3.7–5
15–5a	0.9	2.5–3.7
15–5b	1.1	2.5–3.7
3a–11b	0.8	2.5–3.7
3b–7	1	2.5–3.7
7–20/21	1	2.5–3.7
8–20/21	1 (ref)	2.5–3.7
11a–16/17	1.1	2.5–3.7
11b–16/17	1.4	<2.5

[a] 200 ms Spinlock;measured in aceton-d_6.
[b] Measured in $CDCl_3$.

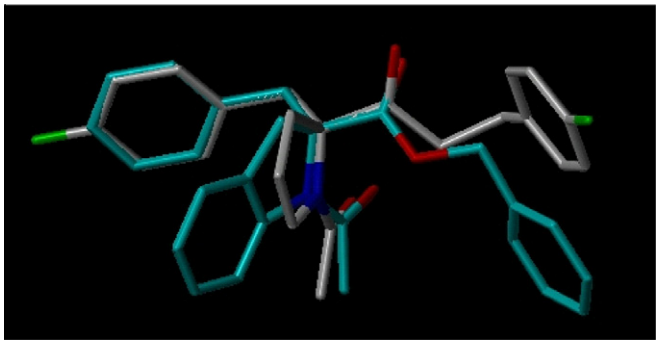
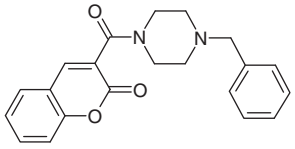
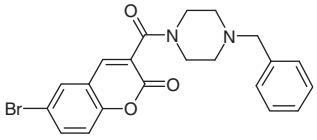


Figure 3. Crystallographic structure of compound **2** (cyan), a close analogue of **5** from the indoline family. Torsion angles are T1 = 215.8°, T2 = 189.5°, T3 = 172.9° and T4 = 21.4°. These torsion angles are close to a low energy NMR and SAR compliant conformer (grey) of **5** where T1 = 85.9°, T2 = 223.4°, T3 = 178.8° and T4 = 22.4°.

Table 5
KCC2 activities for coumarin compounds **10**–**11**

Compound	Structure	IC_{50}[a] (μM)
10		4
11		0.6

[a] Rb^+ flux assay on cloned rat KCC2.[8]

~140°. Torsion angles T2 and T4 needed to be further refined in order to obtain a pharmacophore model for KCC2 blockers.

Following the identification of a novel coumarin-based chemical series of KCC2 blockers by HTS (Table 5), we chose the rather rigid compound **10** to deduce the common pharmacophoric features with compound **1**. A pharmacophore model was derived using DISCOtech implemented within the SYBYL 7.2 software package.[15] The conformational space of compound **10** was computed and used as input of DISCOtech, alongside the previously identified plausible bioactive conformations of compound **1**. Several pharmacophore hypotheses were generated around the possible compound superimpositions. Among them;one model was in agreement with the known SAR. It involves one of the lowest energy conformer of compound **1** (0.4 kcal/mol from the global minimum) characterised by the following torsion angles: T1 ~ 90°, T2 ~ 195°, T3 ~ 180° and T4 ~ 20°. Four common pharmacophoric features were pointed out: 2 H-bond acceptors and 2 aromatic rings. In this model, the amidic oxygen atom of compound **10** corresponds to the acetamide of compound **1** while the coumarin carbonyl oxygen atom is aligned on compound **1** ester function. The benzyl ring and the coumarin moiety in compound **10** are aligned respectively on the benzyl ester and the 2-benzyl group of compound **1** (Fig. 4).

Previously reported SAR[6] indicated that the KCC2 affinity is increased when the 2-benzyl group of compound **1** is substituted in para with a bulky substituent. This corresponds to the 7-position of the coumarin (yellow arrow) according to the pharmacophore model. This hypothesis was confirmed by compound **11** where adding a bromine substituent led to a seven-fold increase in potency.

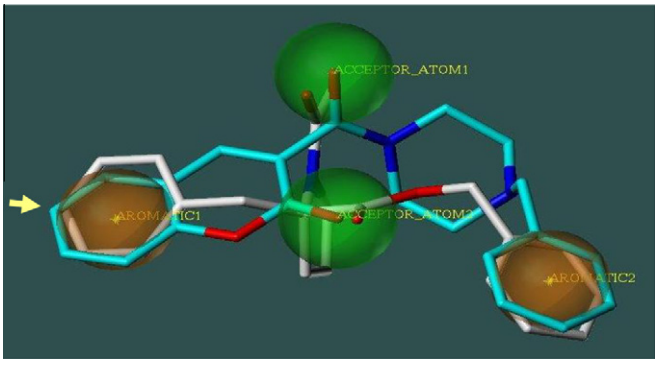


Figure 4. Superimposition of compound **1** (C atoms in white) and **10** (C atoms in cyan). The common pharmacophoric features are HB acceptors (green spheres) and aromatic rings (orange spheres).

In conclusion, a multi-disciplinary approach was used to identify the potential bioactive conformations of our lead series. Several constrained benzyl prolinate analogues were synthesized and tested in order to evaluate the importance of the torsion angles on KCC2 activity. T1 $\sim 90°$ and T3 $\sim 180°$ were rapidly considered as key pharmacophoric constraints. NMR ROESY experiments were then performed to further assess torsion angles T2 and T4. X-ray diffraction experiments on one analogue have further confirmed the NMR observations. Finally, the first KCC2 pharmacophore model was derived by the superimposition of a few plausible bioactive conformations of the benzyl prolinate compound **1** and a rigid ligand **10**, belonging to a structurally-unrelated chemical family.

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9. (*R*)-**1** was confirmed as the active enantiomer of **1** by a synthesis from commercially available (2R)-benzylproline.
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