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4-Phenyl-4H-pyrans as IK_{Ca} Channel Blockers

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Abstract—4-Phenyl-4H-pyrans have been identified as potent and specific IK_{Ca} channel blockers. Their synthesis and structure–activity relationships are described. A selected derivative, *rac*-**11**, reduces the infarct volume in a rat subdural hematoma model of traumatic brain injury after iv administration.

The intermediate conductance Ca^{2+}-activated potassium ion channel (IK_{Ca}) is a tetrameric transmembrane protein which confers potassium ion conductivity to cellular membranes at increased intracellular Ca^{2+} concentrations. Cloned in 1997, IK_{Ca} has been implicated in several disease states including sickle cell anaemia, immune system associated disorders and central ischemic events.[1–3]

Traumatic brain injury, as a consequence of externally inflicted trauma, is a major health care problem associated with severe adverse effects on cognitive functions and high social economic burden.[4] An effective pharmacotherapy is not available for this indication. IK_{Ca} blockade has been hypothesised to play a beneficial role in the prevention of microglial respiratory burst following central nervous system damage. Furthermore, *Schwab* has demonstrated a critical role of IK_{Ca} in cellular migration.[5,6]

The characterisation of IK_{Ca} channels in brain injury settings has, however, so far been hampered by the lack of potent and specific low molecular weight blockers of this ion channel. Recently, Nifedipine and related dihydropyridines have been described as weak inhibitors of this ion channel in the micromolar range.[7] However, Nifedipine also inhibits L-type Ca^{2+}-selective ion channels and is therefore not suited for the study of IK_{Ca}'s in vivo due to its strong cardiovascular effects. Diligent SAR-studies have identified the central NH–structure of the dihydropyridine ring to be an important prerequisite of Nifedipine's Ca^{2+}-antagonistic activity.[8] We therefore rationalised that an isoelectronic replacement from NH to O could lead to more potent and specific IK_{Ca} channel blockers (Scheme 1).

The biological activity of 4-phenyl-4H-pyrans has not been reported hitherto. We herein describe the synthesis and SAR of novel pyran derivatives and report on their in vivo activity in a rat model of traumatic brain injury.

Chemistry

The pyrans have been synthesised based on the method of Wolinsky following reaction Scheme 2.[9] $Zn(OAc)_2$-mediated condensation of 1,3-dicarbonyl compounds with aromatic aldehydes delivered 4-phenyl-pyrans in high yields. Compound *rac*-**7** and *rac*-**11** were first obtained as side products (10%) of the syntheses of **5** and **10**, respectively, possibly due to the strong acetylating reaction conditions. However, *rac*-**7** or *rac*-**11** can also be obtained in a directed synthesis.[10,11]

Biology

IK_{Ca} channel inhibition was determined measuring ionomycin-induced Rb^+ efflux of pre-loaded rat C6BU1 glioma cells. IK_{Ca} channel expression as well as

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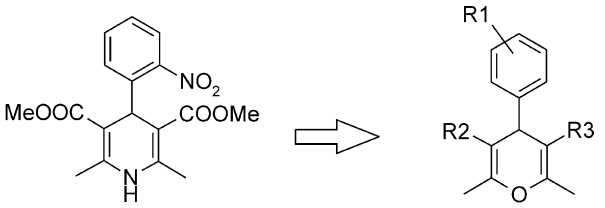
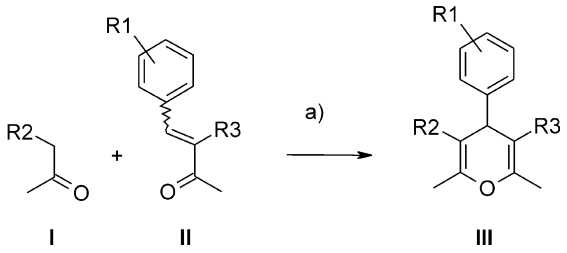


Scheme 1. Design of IK_{Ca} channel blockers using Nifedipine as a weak lead. Isoelectronic replacement of Nifedipine's NH fragment leads to pyrans.



Scheme 2. Synthesis of pyran-dicarboxylic esters: (a) $Zn(Cl)_2$, Ac_2O, heat.

L-type Ca^{2+} channel absence was ensured via PCR on the m-RNA level using gene-specific primers. Charybdotoxin was used as an internal standard ($IC_{50} = 9$ nM).[12]

Activity on other ion channels was measured on the basis of affinities to known binding sites.[13] Blood pressure and heart rate measurements in rats were performed as described. [14]

Neuroprotective potential in traumatic brain injury was investigated in an acute rat subdural hematoma model.[15] Briefly, non-heparinised autologous blood was collected by puncture of the tail vein and injected directly into the subdural space. The compound was administered for 4 h as a continous iv infusion started directly after surgical induction of subdural hematoma. After 7 days, cortical infarct volumes were determined by a computer-assisted image analysis system using histological sections of the infarcted brain area. IK_{Ca} channel expression was demonstrated 7 days after injury by PCR (data not shown).

Results

The encouraging submicromolar activity of the simple dimethyl ester **2** ($IC_{50} = 160$ nM) clearly indicated that isoelectronic replacement of Nifedipine's (**1**) NH group is a suitable strategy to separate Ca^{2+} channel and IK_{Ca} channel antagonist SAR. The activity of **2** could be further enhanced by one order of magnitude on introducing an electron-withdrawing substituent such as Cl into the 4-position of the phenyl ring leading to **3**. Whereas the 3-Cl derivative (**4**) exhibited comparable activity, the 2-Cl derivative (**5**) was less active. This trend is directly opposite to what is known from Nifedipine's SAR versus Ca^{2+} channels and provides a further means of selectively inhibiting IK_{Ca}.

Investigation of the ester chain length in **5** showed decreased activity on aliphatic chain prolongation and consequent clogP increase (*rac*-**6**, **8**, **12**, **13**).[16] This trend is again in contrast to the nature of the DHP/Ca^{2+} channel interaction, where increased activity has been described on ester side chain prolongation. Furthermore, this finding suggests different binding site characteristics of Nifedipine and derivatives to IK_{Ca}- (water accessible) and L-type Ca^{2+} channels (lipid bilayer accessible), respectively (Table 1).

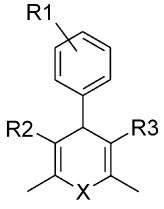
Further investigation of the pyran SAR led us to *rac*-**11**, with 100-fold improvement of IK_{Ca} channel inhibition compared to our starting point Nifedipine. This material was equipotent to the non-specific IK_{Ca} channel blocker charybdotoxin, and was therefore selected for further pharmacological characterization.

As expected, *rac*-**11** did not display any binding affinity to other ion channels at 100 nM.[13] In particular, the Nifedipine binding site of the L-type Ca^{2+} channel is not addressed by this compound. Consistently, *rac*-**11** does not show significant effects on heart rate or blood pressure when administered at doses up to 3 mg/kg iv (Figure 1). In addition, *rac*-**11** shows no binding affinity to K_v channels and is therefore more specific than charybdotoxin (K_v channel: $K_i = 10$ nM).

When administered to rats as a 4 h continous iv infusion started directly after subdural hematoma induction, *rac*-**11** showed clear neuroprotective efficacy, reducing infarct volume by up to 40% after 7 days (Figure 2).

In conclusion, we have demonstrated the development of novel pyrans as potent and specific low molecular

Table 1. IK_{Ca}-channel inhibition of pyran derivatives **2**–**5**, *rac*-**6**-**7**, **8**–**10**, *rac*-**11** and **12**–**13** in comparison with Nifedipine (**1**)



Compd	X	R^1	R^2	R^3	IC_{50}[nM]
1	NH	2-NO_2	COOMe	COOMe	1000
2	O	H	COOMe	COOMe	160
3	O	4-Cl	COOMe	COOMe	24
4	O	3-Cl	COOMe	COOMe	26
5	O	2-Cl	COOMe	COOMe	100
rac-**6**	O	4-Cl	COOEt	COOMe	35
rac-**7**	O	4-Cl	COOMe	COMe	75
8	O	4-Cl	COOEt	COOEt	150
9	O	4-Cl	COMe	COMe	220
10	O	4-Cl,3-CF_3	COOMe	COOMe	24
rac-**11**	O	4-Cl,3-CF_3	COOMe	COMe	8
12	O	4-Cl	COO*n*Pr	COO*n*Pr	[a]
13	O	4-Cl	COO*n*Bu	COO*n*Bu	[b]

Given is the mean of the IC_{50} (inhibition constant) of at least two experiments each performed in triplicates.
[a]72% residual Rb efflux at 1000 nM inhibitor concentration.
[b]Inactive at 1000 nM.

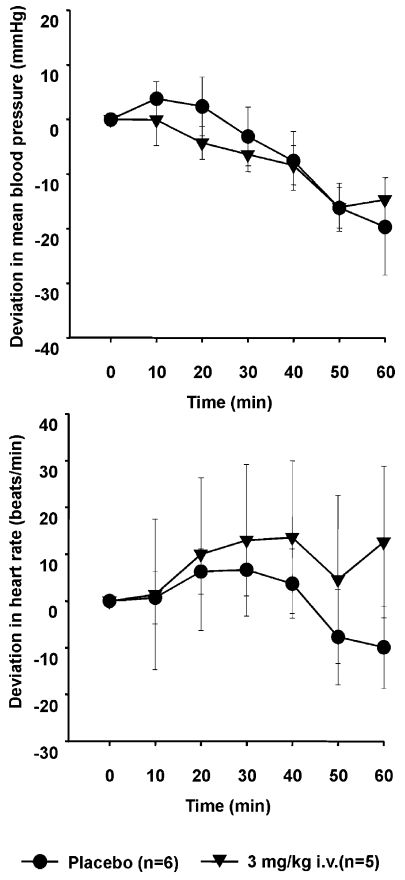


Figure 1. *rac*-**11** has no significant effect on blood pressure (above) or heart rate (below) after iv administration of 3 mg/kg to anaesthetized rats.

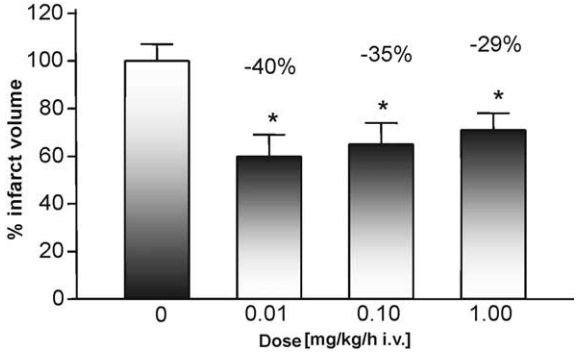


Figure 2. Efficacy of *rac*-**11** on infarct volume in a rat model of traumatic brain injury. The compound was administered via a 4 h continous iv infusion started directly after subdural hematoma induction. Data are mean ± SEM. * $p > 0.05$.

weight IK_{Ca} channel blockers starting from the dihydropyridine Nifedipine as a weak lead. The in vivo activity of *rac*-**11** suggests a major role of IK_{Ca} blockade for the treatment of traumatic brain injury.

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References and Notes

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10. All compounds were characterised by ^1H NMR spectroscopy and mass spectroscopy. Synthesis of *rac*-**11**: A mixture of acetic acid anhydride (20 mL), anhydrous zinc chloride (8.4 g, 60 mmol), 2-acetyl-3-(4-chloro-3-triflouro-methylphenyl)-acrylic acid methyl ester (10.0 g, 33 mmol) and 2,4-pentanedione (7.0 g, 50 mmol) was heated (60 °C) for 3 h. The resulting yellow solution is poured into ice water and extracted with CH_2Cl_2. The combined extracts are washed and purified via column chromatography (toluene/AcOEt = 25 + 1); mp: 96 °C; analysis calcd for $C_{18}H_{18}ClO_4F_3$ (388.7): C: 55.61%; H: 4.15%; O: 16.46% found: C: 55.55%; H: 4.17%; O: 16.32%, ^1H NMR ($CDCl_3$): δ 2.20 (s, 3H, $COCH_3$) 2.36 (s, 6H, C(2,6)-CH_3); 3.69 (s, 3H, $COOCH_3$); 4.82 (s, 1H, H-C(4)); 7.48–7.51 (m, 3H).
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16. The calculated log P values were obtained using the ACD Lab web service, Advanced Chemistry Development, Inc., Toronto, Canada. The following clogP values were obtained for **5**: 4.25, *rac*-**6**: 4.78; **8**: 5.31; **12**: 6.38; **13**: 7.44.