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IK_{Ca}-channel blockers. Part 2: Discovery of cyclohexadienes

Klaus Urbahns,[a,*] Siegfried Goldmann,[a] Jochen Krüger,[a] Ervin Horváth,[b] Joachim Schuhmacher,[c] Rolf Grosser,[a] Volker Hinz[b] and Frank Mauler[b]

[a]*Institute of Medicinal Chemistry, Pharma Research Center, Bayer Health Care, D-42096 Wuppertal, FRG*
[b]*Institute of CNS Research, Pharma Research Center, Bayer Health Care, D-42096 Wuppertal, FRG*
[c]*Institute of Pharmakokinetics, Pharma Research Center, Bayer Health Care, D-42096 Wuppertal, FRG*

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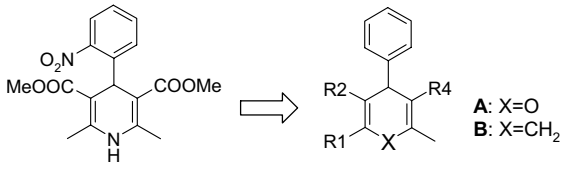
Abstract—Novel cyclohexadienes have been identified as potent and specific IK_{Ca}-channel blockers. In this communication we describe their synthesis as well as their chemical and biological properties. A selected derivative is being enriched in rat brain and reduces the infarct volume, intracranial pressure as well as the water content 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 involved in inflammatory cell activation and abundantly expressed in human brain.[1] In an earlier communication we described a novel 4-phenyl pyran dicarboxylic ester lead structure (**A**) derived from the dihydropyridine Nifedipine as nanomolar IK_{Ca} inhibitors. These compounds resulted from exchanging the dihyropyridine NH group with an isoelectronic oxygen atom and reduced the infarct volume in a rat subdural hematoma model of traumatic brain injury without cardiovascular side effects (Scheme 1).[2]



Scheme 1. Design of cyclohexadienes as IK_{Ca}-channel blockers starting from the lead structure Nifedipine. Isoelectronic replacement of Nifedipine's NH fragment leads to pyrans (**A**) and cyclohexadienes (**B**).

Keywords: IK_{Ca} channel; Potassium channel; Cyclohexadiene; Brain edema; Water content; Cerebral infarction; Subdural hematoma; Rat.
* Corresponding author at present address: AstraZeneca, R&D, Lund, Sweden. Tel.: +46 46 33 78 25; fax: +46 46 33 71 19; e-mail: klaus.urbahns@astrazeneca.com

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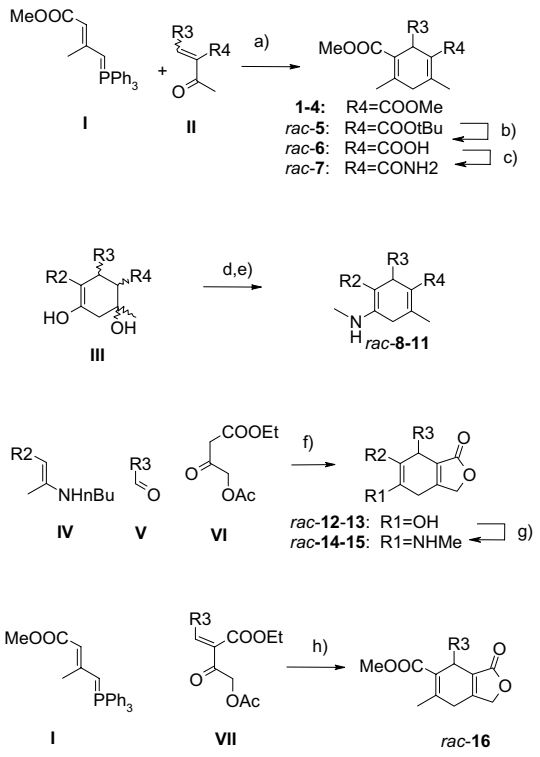
Whereas the NH/O exchange furnished potent and specific IK_{Ca} blockers, we were wondering whether a corresponding NH/CH_2 exchange would give similar results. In this report we describe the synthesis and pharmacological properties of a novel class of cyclohexadienes (**B**).

In order to get synthetic access to cyclohexadienes **1**–**4** and *rac*-**5**–**7**, we used a Carba-Hantzsch reaction recently developed in our laboratories.[3] This reaction uses phosphora **I** and provides cyclohexadienes as mixtures of two double bond isomers each of which can be isolated and/or interconverted (cf. Scheme 2).

The synthesis of *N*-methyl amino-substituted cyclohexadienes *rac*-**8**–**11** was accomplished starting from the known carbocycles **III** as a mixture of diastereomers and subsequent functional group interconversion. The final dehydration step yields the corresponding cyclohexadienes as mixtures of 3,6- (i.e., *rac*-**8**–**11**) and 3,5-double bond isomers, which were separated and isolated.[4]

The bicyclic derivative *rac*-**16** was obtained in a two step sequence in close analogy to the Carba-Hantzsch protocol. First **I** was reacted with **VII** in a Michael type reaction using NaHMDS in toluene at −78 °C. For this transformation toluene was the solvent of choice to suppress side reactions such as Diels–Alder dimerization of **VII**. Subsequently, NaOMe/MeOH was added to affect formation of the cyclohexadiene core and lactone ring closure. Using these optimized conditions, an alternative



Scheme 2. Synthesis of cyclohexadienes. (a) 3 equiv NaOMe, MeOH, 12 h, rt, 49–98% (mixture of double bond isomers, ratios: see[3]); (b) 5% CF_3COOH, CH_2Cl_2, 2 h, rt, 67%; (c) $SOCl_2$, 2 h, reflux, evaporate, then THF/aq NH_3 (1+2), 0 °C, 40% (+19% biphenyl amide); (d) MeOH/$MeNH_2$ (11 N), cat. *p*-Tos-OH, 2 h, reflux, 70–80%; (e) cat. *p*-Tos-OH, toluene, reflux, 2 h, 7–33%; (f) EtOH, reflux, 6 h, then: 10% aq HCl (33%), reflux, 2 h, 48–66%; (g) 10 equiv *N*-methyl ammonium formate, EtOH, reflux, 85–90%; (h) 1 equiv NaHMDS, toluene, −78 °C to rt, 3 days, then: 3 equiv NaOMe, MeOH, 0 °C to rt, 1 h, 21%.

3,5-double bond isomer could not be detected. The overall yield for this process is 21%.

A coupling reaction of *N*-butyl substituted aminocroton esters **IV** with benzylidene-type Michael acceptors was used to synthesize hetero-substituted bicyclic cyclohexadienes. In this case the large butyl amine substituent affects the reactivity of the aminocrotonic ester such that it turns the adjacent methyl group into a better nucleophile than nitrogen, leading to carbocycles rather than dihydropyridines. The resulting carbocyclic enamines were hydrolyzed under concomitant cyclization of the lactone ring to yield *rac*-**12**–**13**. Subsequent treatment with *N*-methyl ammonium formate furnished *rac*-**14**–**15**.[5]

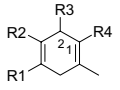
By and large, the SAR of cyclohexadienes follows trends similar to those observed in the 4-phenyl pyran series.[2] Direct comparison of **4** (1.5 nM) and the corresponding isolectronic pyran (24 nM) indicates however the superiority of cyclohexadienes in terms of potency. Whereas the cyclohexyl and pyridine derivatives were inactive (**1**, **2**), electron withdrawing substituents in *meta* position clearly enhanced potency (**3**).

The most potent aromatic substitution pattern was the 4-Cl, 3-CF_3 motif, already identified in the pyran series. The presence and nature of the ester side chain was also important for biological activity. In contrast to its methyl counterpart, the bulky ester *rac*-**5** was inactive as well as the carboxylic acid *rac*-**6**. Interestingly, the corresponding amide *rac*-**7** showed retained activity. The corresponding *N*-methyl amino-substituted cyclohexadienes *rac*-**8**–**11** have also been investigated and showed potencies comparable to their symmetric counterparts. The methyl ester *rac*-**11** was more potent than its ethyl counterpart *rac*-**10**, again confirming the importance of a small ester side chain (Table 1).

The corresponding 3,5-cyclohexadienes, which were isolated as side products, have also been investigated, but did not show activity below 1000 nM (data not shown).

Although we considered the high potency of **4** as clearly promising, we realized that this class of monocylic cyclohexadienes still shows a remaining trend to 3,6/3,5 double bond isomerization, prohibitive for further development.

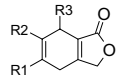
Table 1. IK_{Ca}-channel inhibition of cyclohexadiene derivatives **1**–**11**



Compound	R1	R2	R3	R4	IC_{50} (nM)
1	Me	COOMe	C_6H_{11}	COOMe	>1000
2	Me	COOMe	3-Pyridyl	COOMe	>1000
3	Me	COOMe	3,5-Cl_2Ph	COOMe	100
4	Me	COOMe	4-Cl, 3-CF_3Ph	COOMe	1.5
rac-**5**	Me	COO-*t*-Bu	4-Cl, 3-CF_3Ph	COOMe	>1000
rac-**6**	Me	COOH	4-Cl, 3-CF_3Ph	COOMe	>1000
rac-**7**	Me	$CONH_2$	4-Cl, 3-CF_3Ph	COOMe	30
rac-**8**	NHMe	COOMe	4-CF_3Ph	COOMe	200
rac-**9**	NHMe	COOMe	4-NO_2Ph	COOMe	20
rac-**10**	NHMe	COOEt	3-NO_2Ph	COOEt	70
rac-**11**	NHMe	COOMe	3-NO_2Ph	COOMe	25

Given is the mean of the IC_{50} (inhibition constant) of at least two experiments each performed in triplicates.

Table 2. IK_{Ca}-channel inhibition of bicyclic hexadiene lactones *rac*-**12**–**16**



Compound	R1	R2	R3	IC_{50} [nM]
rac-**12**	HO	COOEt	2-ClPh	200
rac-**13**	HO	COOEt	4-Cl, 3-CF_3Ph	40
rac-**14**	NHMe	COOMe	2,3-Cl_2Ph	400
rac-**15**	NHMe	COOMe	3-NO_2Ph	200
rac-**16**	Me	COOMe	4-Cl, 3-CF_3Ph	8

Given is the mean of the IC_{50} (inhibition constant) of at least two experiments each performed in triplicates.

The bicyclic cyclohexadiene lactone series had the clear advantage to show a lower tendency of isomerization, possibly due to ring strain imposed by the lactone moiety on the cyclohexadiene core. Comparing the potency of *rac*-**11**/*rac*-**15** and **4**/*rac*-**16** revealed that bicyclic lactones are generally by one order of magnitude less potent than their monocyclic counterparts (Table 2).

We identified *rac*-**16** as a potent and stable cyclohexadiene and therefore selected this compound for further pharmacological investigations.[6–8] After iv administration to rats, *rac*-**16** showed a more than 10-fold enrichment in brain tissue, allowing for meaningful investigations of *rac*-**16** in CNS-related animal models (Fig. 1). In a rat animal model of subdural hematoma (SDH), *rac*-**16** significantly and dose-dependently reduced intracranial pressure (ICP). Concomitantly, the agent also reduced the content of water in brain tissue, as determined 24 h after SDH. In a separate experiment, *rac*-**16** also reduced the infarct volume as determined 7d post SDH, indicating a sustained therapeutic effect (Fig. 2).

In conclusion, we have demonstrated the development of novel cyclohexadienes as potent low molecular weight IK_{Ca}-channel blockers starting from the dihydropyridine Nifedipine as a weak lead. The in vivo activity of *rac*-**16,** as a novel, pyran-unrelated structural class confirms a potential role of IK_{Ca} blockade for the treatment of traumatic brain injury.

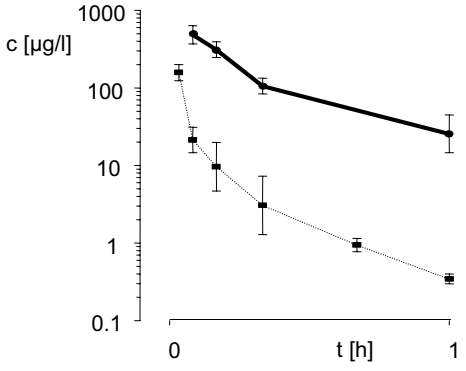


Figure 1. Blood (···) and brain (—) concentrations of *rac*-**16** after iv administration of 0.2 mg/kg to rats (5% ethanol/ 5% Solutol/ 90% saline).

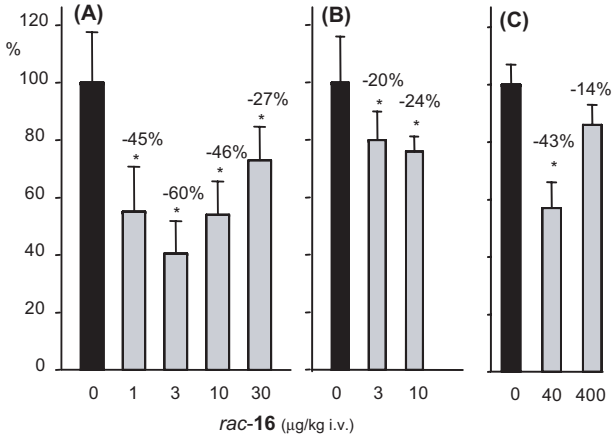


Figure 2. **A** and **B**: Efficacy of *rac*-**16** against developing brain edema in a 24 h rat SDH model as shown by (**A**) intracranial pressure and (**B**) brain water content. *rac*-**16** was administered as a continuous iv infusion for 4 h directly after induction of SDH. Values are given as percent change compared to controls (*$P < .05$, $n = 8$–12 per dose group). **C**: Neuroprotective efficacy of *rac*-**16** in a rat SDH model, administered immediately after induction of SDH. *rac*-**16** was administered as a continuous iv infusion for 4 h. Infarct volumes were determined 7 days after SDH. Infarct volumes were calculated as percentage of infarct volumes of the control group, which was set to 100%. Values above bars indicate the percent infarct volume reduction compared to controls (*$P < .05$, $n = 8$–12 per dose group).

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6. Synthesis of *rac*-**16**: In an argon atmosphere at −78 °C a solution of NaHMDS (22.0 mL, 22.0 mmol, 1 M solution in THF) was added to a suspension of **VII** (10.0 g, 22.0 mmol) in 250 mL of dry toluene and it was stirred for 60 min at −78 °C. Subsequently, a solution of **VIII** (12.5 g, 32.9 mmol) in 250 mL toluene was added, the reaction mixture was allowed to reach room temperature over night and was

maintained at this temperature for 4 days. At 0 °C a solution of NaOMe in methanol (2.5 mL of a 25% solution, 11.0 mmol) was added and the mixture turned deep red instantly. After 35 min another portion of NaOMe (2.51 mL of a 25% solution, 11.0 mmol) was added, the cooling bath was removed, and it was stirred at room temperature for 85 min. A saturated aqueous solution of NH_4Cl was added (250 mL) followed by four time extraction with ethyl acetate (250 mL each). The combined organic layers were dried (Na_2SO_4) and the solvents were distilled off in vacuo. The crude product was purified using a C_{18}-HPLC column (Kromasil 100, eluent: acetonitrile/water 15:85–90:10) to yield 1.78 g (21% yield) of *rac*-**16**. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.10 (s, 3H), 3.21 (m, 1H), 3.52 (s, 3H), 3.62 (m, 1H), 4.70 (m, 1H), 4.96 (m, 2H), 7.43 (dd, 1H), 7.60 (m, 2H). HPLC–MS (parameters: column symmetry, 50 × 2.1 mm, C_{18}, 3.5 μm; eluent A: MeCN + 0.1% HCOOH; B: 0.3 g 30% aq HCl in 1 L H_2O; 0–4 min: from 10% to 90% A; 4–6 min: 90% A, 0.5 mL/min, 40 °C): R_f = 3.34 min, peak area (UV detection at 210 nm) = 100%, [M+H] = 387. Elemental anal. for $C_{18}H_{14}ClF_3O_4$ (386.75). Calcd C, 55.90; H, 3.65; Cl, 9.17; O, 16.55. Found C, 55.82; H, 3.50; Cl, 9.23; O, 16.82. Solubilities of *rac*-**16**: (solvent) 14 mg/L (buffer, pH 6.5), 12 mg/L (3 mM Taurocholat), and 70 mg/L (15 mM Taurocholat).

7. Neuroprotection model: (a) Mauler, F.; Mittendorf, J.; Horváth, E.; De Vry, J. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 359; Brain edema model: (b) Mauler, F.; Hinz, V.; Augstein, K.-H.; Faßbender, M.; Horváth, E. *Brain Res.* **2003**, *989*, 99.

8. *rac*-**16** has been separated into its enantiomers (Chiralpak AD-H; 250 × 20 mm; 5 μm; UV-detection (254 nm); Eluent: *i*-hexane/*i*-propanol 10:1 (v/v); 15 mL/min). The enantiomers were tested in the primary assay and the eudismic ratio is 10. The absolute configuration of the eutomer has not been determined.