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Synthesis and anticonvulsant activity of new 4-aminopyrazoles and 5-aminopyrazol-3-ones

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In the course of our investigations of antiepileptic drugs new 4-aminopyrazoles **1** and 5-aminopyrazole-3-ones **2**, **3** were synthesised and tested for anticonvulsant activity.

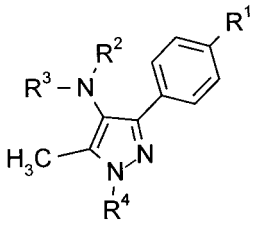
4-Aminopyrazoles **1** are obtained starting with substituted benzoyl acetones, which react with hydrazine via the hydroxyimino compounds to 4-nitrosopyrazoles [1]. Nitrosopyrazoles were reduced with sodium dithionite to yield 4-aminopyrazoles [2]. These compounds can be isolated as hydrochlorides and give crystalline acetyl derivatives [3]. 4-Amino-5-methyl-3-phenylpyrazole and the acetylated compound are known from the literature [4].

The synthesis of 5-aminopyrazole-3-ones **2** starts with substituted benzyl cyanides. These compounds are reacted with diethyl carbonate and are finally cyclisised with hydrazine and sodium methylate to yield 5-amino-pyrazole-3-ones [5]. Known compounds in this series are 5-amino-4-(4-chlorophenyl)-pyrazole-3-one and 5-amino-4-phenyl-pyrazole-3-one [5, 6].

5-Anilinopyrazole-3-one **3** can be synthesised in a one pot procedure starting with methyl acetoaceticacid ester, sodium methylate, substituted aryl isocyanates and hydrazine hydrate [7]. Of this series of compounds, only 5-anilino-pyrazole-3-one was described in the literature.

All new compounds are listed in the Table. Structural assignments were made by elemental analysis, IR and $^{1}H/^{13}C$-NMR-spectroscopy.

The anticonvulsant activity and pharmacological profile of these pyrazoles were investigated using standard methods [8, 9]. Compounds **1a** and **1b** potently protect rats in the maximal electroshock seizure (MES) test, in particular



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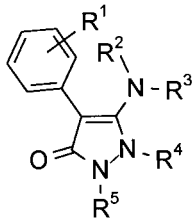

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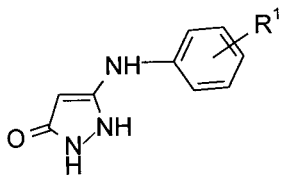

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compound **1b** with an $ED_{50} = 24$ mg/kg (rats, p.o.). The interaction with neuronal sodium channels of the cell line B50 was investigated for compound **1b** using phenytoin as standard. At a concentration of 100 μmol the sodium channel blocking effect of compound **1b** was as efficacious as phenytoin. At a concentration of 10 μmol this blocking effect was smaller in comparison with phenytoin. (To evaluate these effects the blocking of the static current in the course of membrane depolarisation from −80 to 0 mV and the frequency potentiation with a stimulus of 10 Hz compared to a stimulus of 1 Hz were used).

Compound **1b** corresponds with the pharmacophore model for sodium channel blocking agents recently proposed by our group [10]. The pharmacological profile corresponds with phenytoin and carbamazepine as well which means that pentetrazole-induced seizures (PTZ) are not suppressed by the active pyrazoles. Compounds **1a** and **1b** were not neurotoxic (rotorod).

Table: Newly synthesised compounds

Compd.	R^1	R^2	R^3	R^4	R^5	Yield (%)	mp. (°C)
1a	Cl	H	H	H		23	133–135[a]
1b	Br	H	H	H		28	127–129[a]
1c	H	$COCH_3$	H	H		94	117–120[b]
1d	Cl	$COCH_3$	$COCH_3$	$COCH_3$		77	143–146
1e	Br	$COCH_3$	H	$COCH_3$		43	212–215
1f	Br	$COCH_3$	$COCH_3$	$COCH_3$		46	144–145
2a	3,4-Cl_2	H	H	H	H	13	234–236[b]
2b	2,4-Cl_2	H	H	H	H	39	219–221[b]
2c	2,6-Cl_2	H	H	H	H	36	244–246
2d	4-Cl	$COCH_3$	H	H	H	48	208–210
2e	4-Cl	$COCH_3$	$COCH_3$	$COCH_3$	$COCH_3$	93	91–94
2f	4-Cl	COC_6H_5	H	H	H	16	268–270
2g	4-Cl	H	H	COC_6H_5	H	18	177–180
2h	2,4-Cl_2	H	H	C_6H_5	H	19	265–266
3a	2-methyl					58	195–197
3b	2,3-dimethyl					22	227–229
3c	2-Cl					32	178–180
3d	4-Cl					24	173–177
3e	2-F					39	269–271
3f	2,4-Cl_2					56	238–240

[a] hydrochloride × butanol, [b] hydrate

IR (cm^{-1}): 3400, 3343 (NH; **1a**, **b**), 1646 (C=O; **1c**), 1747, 1723 (C=O; **1d**, **1f**), 3250 (NH; **1e**), 1600–1650 (C=O; **2a–c**, **h**), 1680–1785 (C=O; **2d–g**), 1676–1703 (C=O; **3a–f**)

^{13}C-NMR (δ): 9.43–11.76 (CH_3, **1a–f**), 168.94–172.63 (C=O; **1c–f**), 161.10–167.63 (**C_3**=O; **2a–h**), 171.59–173.57 (**C_5**=O; **3a–f**)

The acetylated compound 1c shows no anticonvulsant activity at all. This result also corresponds with the proposed pharmacophore although a direct comparison of compound 1c with 1b is limited because of the difference in the experimental partition coefficient octanol/water (log P of 1b = 1.39; log P of 1c = 0.08).
A correct distance range between the exocyclic NH_2-group and the endocyclic NH-group and also the free access to these functionalities are essential for anticonvulsant activity. Deviations of this core structure this can also mean more polar groups in the heterocyclic system result in inactive compounds like 2a. Therefore all 5-amino-pyrazole-3-one 2, 3 show no anticonvulsant activity.

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Reverse-phase liquid chromatographic UV method for analysis of hexoprenaline sulphate and selection of chromatographic conditions suitable for multi-β-agonist analysis

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Hexoprenaline is a widely prescribed potent β_2-agonist [1]. It appears to be mostly used as a stimulant in obstetric practice [2]. Besides this use as a tocolytic it also relieves reversible airway obstruction, including status asthmaticus [1]. Although, this drug is widely used, drug standards for hexoprenaline have not been published in major compendia. This has necessitated the need to develop specific assay procedures for hexoprenaline in the presence of related compounds and potential degradation products.
A number of single and multi-component high performance liquid chromatographic (HPLC) methods have been reported for the analysis of β_2-agonists similar in structure to hexoprenaline [3–5]. The application of HPLC is limited when determining β_2-agonists in bio-samples because of insufficient selectivity and lack of sensitivity of common HPLC detectors [4]. Consequently HPLC is rarely used to determine the illicit use of the compounds in zootechnics and in sports [4]. GC separations of these compounds after purification of analytes by means of either liquid-liquid partition or solid-phase extraction are used to overcome these problems [6]. However, HPLC has significant advantages over GC for the analysis of these compounds in pharmaceutical dosage forms [4, 5, 7]. Unfortunately no reported method specific for hexoprenaline, either as the sulphate or hydrochloride salts, could be found. In this study the applicability of HPLC for assaying hexoprenaline sulphate in pharmaceutical dosage forms was investigated.
Isocratic elution (Fig. 1) was performed using a mobile phase of methanol and water (45 : 55 v/v) containing 0.1 M ammonium acetate and 0.1 M triethylamine with 0.1 M formic acid at a pH of 3.5 [4]. Fig. 1 shows the chromatogram obtained for the direct injection of a sample containing hexoprenaline · H_2SO_4 75 μg/ml and spiked with clenbuterol 50 μg/ml. The capacity factor, k′, for hexoprenaline was 2.96 and for clenbuterol 1.20 showing satisfac-

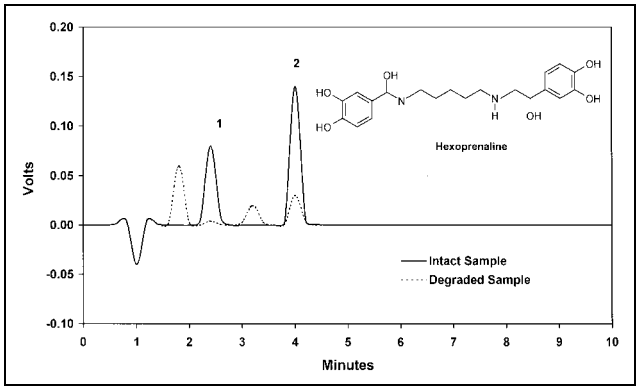

Fig. 1: HPLC chromatogram (285 nm) produced after injection 50 μl of a sample containing 75 μg/ml hexoprenaline sulphate (2) and spiked with 50 μg/ml clenbuterol (1). Mobile phase 45% methanol in buffer with pH about 3.8