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STEREOCHEMICAL DIFFERENTIATION OF ANTICONVULSANT AND ANTIHYPERTENSIVE EFFECTS IN 4-(FLUOROBENZOYLAMINO)-BENZOPYRANS

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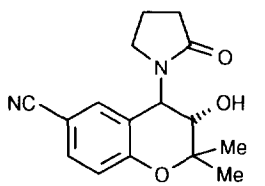
Abstract. Replacement of the 2-pyrrolidinone group of cromakalim **1** by the fluorobenzoylamino group has introduced anticonvulsant activity. It is particularly noteworthy that this activity is found predominantly in the 3*R*,4*S* enantiomeric series. In contrast, antihypertensive activity is restricted to the 3*S*,4*R* enantiomeric series.

Cromakalim **1** is the pioneer compound of a new class of antihypertensive agents which act *via* the relaxation of vascular smooth muscle caused by the opening of ATP-sensitive potassium channels. Its blood pressure lowering activity[1] has been shown to reside virtually exclusively in the 3*S*,4*R* enantiomer, levcromakalim **2**. Subsequently, mechanistic studies have demonstrated that only this enantiomer modulates the ATP-sensitive potassium channel in smooth muscle.[2]



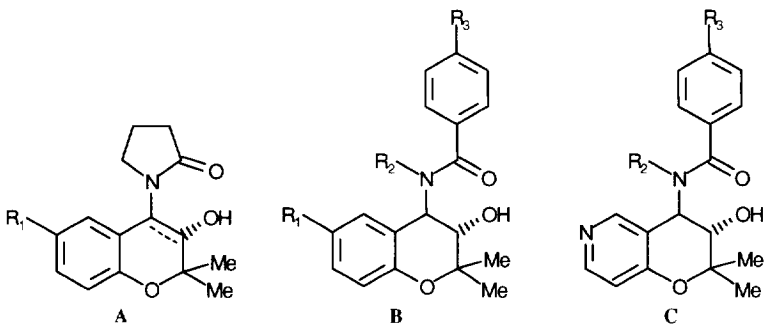
1 cromakalim (±)-*trans*

2 levcromakalim (-)-3*S*,4*R*

The possible use of potassium channel activators in the treatment of epilepsy has been considered following reports that cromakalim **1** or levcromakalim **2**, when administered intracerebroventricularly, inhibit both chemically-induced seizures in rodents[3,4] and spontaneous seizures in genetically epileptic rats.[5] The effect is stereospecific, the (+)-3*R*,4*S* enantiomer of **1** being ineffective at doses at which **2** was effective.[3] However, we have found that cromakalim when administered systemically (orally or intraperitoneally) does not inhibit seizure activity in a mouse maximal electroshock threshold model (MEST), even at a dose of 10 mg/kg which is much greater than that required for antihypertensive activity.[1] Although the models are different, a possible explanation for the present lack of activity of cromakalim is its poor ability to penetrate the CNS. Thus, potassium channel activators which more readily penetrate the CNS may have therapeutic potential in the treatment of epilepsy.

In a search for compounds that are potentially more brain penetrant than cromakalim, a series of more lipophilic analogues was tested in the mouse MEST model[6] (Table 1). In this model, the threshold voltage (applied *via* a subcutaneous and a buccal electrode) for inducing tonic hindlimb extension seizures in 50% (CV_{50}) of the animals was determined using groups of 10-20 mice (male, 25-30g, CD1-Charles River). The effects of drug treatments were expressed as a percentage change from vehicle control values and statistical comparisons between treatment groups were made according to the method of Litchfield and Wilcoxon.[7] The compounds were administered orally as a fine suspension in 1% methylcellulose one hour before electroshock application.

Table 1. Effect of standards, cromakalim (1) and analogues in the mouse MEST model



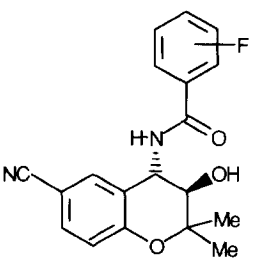
Cpd. No.[a]	A/B/C (racemic)	R_1	R_2	R_3	Dose (mg/kg po)	% Change in Seizure Threshold
1	A	CN	-	-	10	+4
					10[b]	-16
3[c]	A	*t*-Bu	-	-	10	+12
4[d]	A	CN	-	-	30	+4
5	B	CN	H	H	10	+29*
6	B	CN	H	F	10	+49*
7	B	CN	H	CN	30	+7
8	B	CN	H	OH	30	-2
9	B	H	H	F	30	+13*
10	B	CF_3	H	F	10	+33*
11	C	-	H	F	10	+5
12	B	OMe	H	F	30	+28*
13	B	*i*-Pr	H	F	30	+19*
14	B	Ph	H	F	30	+20*
15	B	CN	Me	F	30	+26*
		Carbamazepine		ED_{50}[e] 12.7 mg/kg p.o.		
		Phenobarbitone		ED_{50}[e] 10.6 mg/kg p.o.		

[a]benzamides prepared as in ref 8, had satisfactory elemental analyses and spectroscopic data (see EP126 311 for compounds **5**, **7** and **8**)
[b]administered intraperitoneally, 30 min pretest
[c]ref 9 : [d]ref 1, compound formed by water elimination across 3,4-bond
[e]dose producing a 50% elevation in seizure threshold
*p <0.05 compared to vehicle controls

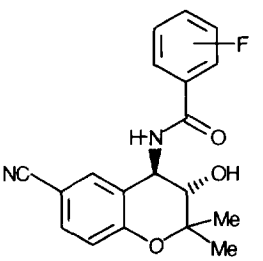
Simple modification of cromakalim **1** to give a range of more lipophilic compounds such as the 6-*t*-butyl- **3** and chromene **4** analogues did not introduce anticonvulsant activity. Surprisingly, replacement of the 2-pyrrolidinone moiety in cromakalim by benzoylamino (**5**) introduced anticonvulsant activity which was further enhanced by substitution of a fluorine atom at the *para* position (**6**). Other substituents such as cyano (**7**) and hydroxyl (**8**) at this position attenuated activity. Removal of the 6-cyano group (**9**) from **6** resulted in a reduction in activity which could be restored by the insertion of the electron-withdrawing trifluoromethyl group (**10**). However, changing the ring to pyranopyridine **11** resulted in loss of activity, whereas insertion of electron donating substituents (**12**, **13**, **14**) gave compounds with modest anticonvulsant activity. Methylation of the amidic nitrogen atom (**15**) of compound **6** led to reduced activity. Thus a series of fluorobenzoylamino benzopyrans has been shown to produce oral anticonvulsant activity in the mouse MEST model and the potency of **6** compares favourably with that of clinically effective anticonvulsants such as carbamazepine and phenobarbitone.

All the above data refer to racemic compounds and it was clearly important to establish which enantiomer was responsible for the anticonvulsant activity. The individual **16**, **17** (3*R*,4*S*) and **18**, **19** (3*S*,4*R*) enantiomers of **6** and the closely related *meta*-fluoro analogue were prepared[10] and tested in the mouse MEST test. Most significantly, it was found that anticonvulsant activity resided predominantly in the 3*R*,4*S* enantiomers **16**, **17** (Table 2). These have the opposite stereochemistry to that of levcromakalim **2**, the active antihypertensive enantiomer of cromakalim. Indeed, the 3*R*,4*S* enantiomer **17** had no effect on blood pressure (Fig 1) in the spontaneously hypertensive rat (SHR) at a dose of 10 mg/kg p.o. In contrast, and as expected from its equivalent stereochemistry to that of levcromakalim, the 3*S*,4*R* enantiomer **19** showed marked antihypertensive activity. Thus it seems very unlikely that the anticonvulsant activity of **16** and **17** is a result of an interaction with the type of ATP-sensitive potassium channel that modulates the cardiovascular actions of **2**.[2] The mechanism of action of the anticonvulsant activity of **16** and **17** and further examples of the 3*R*,4*S* series is currently being explored. Further structure-activity relationships will be reported at a later date.

Table 2. Effect of compounds 16 - 19 in the mouse MEST model (10 mg/kg po)



(3*R*,4*S*) **16**, *p*-F; **17**, *m*-F

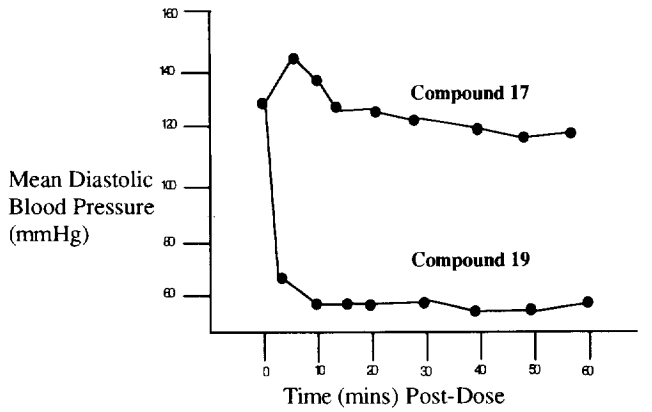


(3*S*,4*R*) **18**, *p*-F; **19**, *m*-F

Cpd. No.	% Increase in Seizure Threshold
16	41*
17	50*
18	17
19	12*

*: $p<0.05$ compared to vehicle controls

Figure 1. Effect of enantiomers 17 and 19 in SHRs (10 mg/kg po)



Diastolic blood pressure was monitored using a tail cuff at 5-10 min intervals for a period of 1h following oral administration of test compounds in suspension in 1% w/v methylcellulose to groups of 3 SHRs. Standard errors were determined and fell within the limits of the data points.

In conclusion, the initial premise that a more brain penetrant analogue of levcromakalim would show anticonvulsant activity *via* an action at classical ATP-sensitive potassium channels after systemic administration has not been proven. However, this paper reports the more important and very surprising finding that fluorobenzamides in the opposite enantiomeric *trans* 3*R*,4*S* series possess potent anticonvulsant properties with minimal antihypertensive effects.

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