Synthesis of Novel *trans*-4-(Substituted-benzamido)-3,4-dihydro-2*H*-benzo[*b*]-pyran-3-ol Derivatives as Potential Anticonvulsant Agents with a Distinctive Binding Profile†

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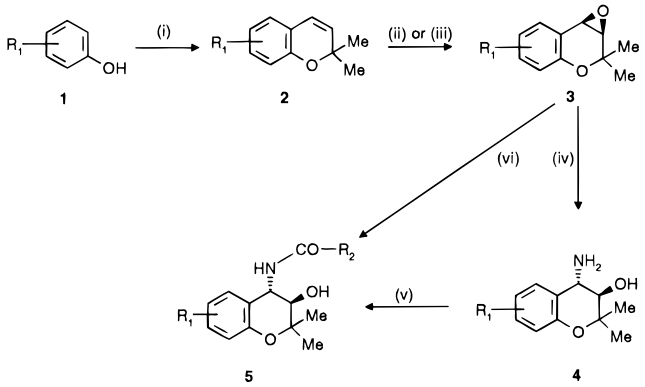
In our earlier communication,[1] we reported that fluorobenzamides such as **5f**, possessing 3*R*,4*S* stereochemistry, showed good anticonvulsant activity in the mouse maximal electroshock seizure threshold (MEST) model, while being devoid of antihypertensive properties. In contrast, the corresponding 3*S*,4*R* enantiomers, like the structurally and stereochemically related activator of ATP-sensitive potassium channels, levcromakalim[2] (**6**), showed potent antihypertensive activity. The 3*S*,4*R* enantiomers additionally showed little anticonvulsant activity. A subsequent communication[3] confirmed that a 4*S* configuration of the fluorobenzamide is crucial in conferring anticonvulsant activity without antihypertensive activity. Although the 3-hydroxyl group appears essential for anticonvulsant activity, its stereochemistry is not important. These early data clearly indicated that the mechanism of the anticonvulsant action of compounds such as **5f** was unlikely to involve the modulation of ATP-sensitive potassium channels. It was clearly important to gain a fuller understanding of structure−activity relationships (SAR) within the series.

This communication describes the discovery of analogues of **5f** such as the 4-fluorobenzamide **5b** (SB 204269) with markedly enhanced anticonvulsant potency *in vivo*. In order to facilitate an understanding of the mechanism of their anticonvulsant action, [^{3}H]-SB 204269 was prepared by [^{3}H] hydrogenolysis of the corresponding 2,5-dibromobenzamide of **5b.** [^{3}H]SB 204269 was then used to identify a novel highly enantioselective binding site in the brain of several species including humans. Extensive pharmacological characterization illustrated the unique nature of the [^{3}H]SB 204269 binding site as a mechanistic target. Having identified this unique binding site, displacement of the radioligand was used to study the SAR directly for this intriguing class of compounds. For the series of benzopyrans described here, affinity for this site was found to correlate with their anticonvulsant activity in rodent models.

Chemistry. Key intermediates for the synthesis (Scheme 1) of the compounds described in the Table 1 are the racemic 4-amino-3,4-dihydro-2,2-dimethyl-2*H*-benzo[*b*]pyran-3-ols **4,** which were synthesized using

Scheme 1.[a] Synthetic Route to 4-Amidobenzo[*b*]pyran-3-ols



[a] Reagents: (*i*) 3-chloro-3-methylbutyne and procedures as in ref 4; (*ii*) for *trans* racemate, *m*-CPBA, and procedures as in ref 4; (*iii*) for 3R,4S enantiomers, epoxidation using procedures in ref 5; (*iv*) NH_3, EtOH; (*v*) Et_3N, CH_2Cl_2, R_2COCl (method A); (*vi*) KOBu^t, Bu^{t}OH, R_2CONH_2 (method B).

procedures previously described.[4] The exceptions are the chiral amino alcohols[5c] **4b**, **4c**, **4f**, and **4i** (precursors to **5b**, **5c**, **5f**, and **5i),** which were prepared by enantioselective epoxidation of the corresponding chromenes **2** using Mn(III) salen catalysts,[5] followed by treatment with ethanolic ammonia. The amino alcohols **4** were then converted into the amides shown in the Table 1 by reaction with the appropriate acid chloride (method A). Alternatively, the benzopyran epoxides **3** were converted directly into the benzamides **5a** and **5e** on treatment with the anion of 4-fluorobenzamide in *tert*-butyl alcohol at 40 °C (method B). Optical purity was determined by HPLC on a Chiracel OD, P45SC (Daicel) column using phosphate buffer (pH 3)/methanol/acetonitrile as eluent.[3] (X-ray analysis of **5b** also confirmed the absolute stereochemistry; data not presented.)

Characterization of [^{3}H]SB 204269 Binding Site.[6] [^{3}H]SB 204269 binding to rat forebrain membranes is of moderate affinity (K_D 40 nM) and fairly high density (B_{max} 220 fmol/mg of protein). The binding is highly enantioselective, since in competition studies the enantiomers (+)-**5b** (3*R*,4*S*) and (−)-**5c** (SB 204268, 3*S*,4*R*) had pK_i values of 7.3 and <4.5, respectively (Figure 1).

In addition to rat forebrain, [^{3}H]SB 204269 binding has been observed in mouse, cat, dog, marmoset, and human brains. In all species, affinities were similar. Further details will be published elsewhere.[6]

None of the standard anticonvulsant agents (diazepam, phenytoin, valproate, and phenobarbitone) or the newer compounds in clinical development (gabapentin, lamotrigine, and levetiracetam) have shown any activity (at up to 100 μM) in the [^{3}H]SB 204269 binding assay.[6] In addition, although the benzamides **5** are structurally, but not stereochemically, related to the benzopyran class of ATP-sensitive K^+ channel activators, none of the activators of this or other classes (levcromakalim, aprikalim, or pinacidil) showed significant affinity for the binding site.[6] These studies demonstrate the unique mechanistic nature of this class of anticonvulsant 4(*S*)-amidobenzopyran-3(*R*)-ols.

Results and Discussion. *In vitro* potency (Table 1) was measured using the radioligand binding procedure.[6] Compounds were evaluated for oral anticonvulsant activity, in groups of 10−20 naive mice (male CD1-

† This work was conducted in compliance with the Home Office (U.K.) Guidance on the operation of the Animals (Specific Procedures) Act 1986, and was reviewed and approved by the SB Procedures Review Panel.

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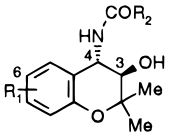
Table 1. Chemical and Biological Data for *trans* 4-(Substituted-amido)-2*H*-benzo[*b*]pyran-3-ols



compd[a,b]	mp, °C	method	R_1	R_2	stereochem	[^{3}H]SB 204269 binding pK_i	mouse MEST po % increase in seizure threshold at dose[c] 10 mg/kg	3 mg/kg
5a	175	B	5-COMe	4-FPh	racemic	5.09 ± 0.07	NT	NT
5b	163−4	A	6-COMe	4-FPh	3*R*,4*S*	7.32 ± 0.03	102*	89*
5c	161	A	6-COMe	4-FPh	3*S*,4*R*	<4.5	8	NT
5d	190−3	A	7-COMe	4-FPh	racemic	5.51 ± 0.03	NT	NT
5e	262−4	B	8-COMe	4-FPh	racemic	5.00 ± 0.02	5	NT
5f	224−5	A	6-CN	4-FPh	3*R*,4*S*	6.00 ± 0.11	41*	15*
5g	197−8	A	6-NO_2	4-FPh	racemic	6.20 ± 0.07	45*	2
5h	239−40	A	6-SO_2Ph	4-FPh	racemic	5.84 ± 0.13	−7	NT
5i	172	A	6-C_2F5	4-FPh	3*R*,4*S*	6.35 ± 0.05	−8	NT
5j	164−5	A	6-F	4-FPh	racemic	5.38 ± 0.15	10	NT
5k	185−6	A	6-Me	4-FPh	racemic	5.30 ± 0.02	5	NT
5l	205−7	A	6-COEt	4-FPh	racemic	6.80 ± 0.01	35*	NT
5m	217−20	A	6-COPh	4-FPh	racemic	6.12 ± 0.06	NT	
5n	198	A	6-CO_2Me	4-FPh	racemic	6.69 ± 0.04	59*	NT
5o	>296	A	6-$CONH_2$	4-FPh	racemic	6.06 ± 0.02	6	NT
5p	188−91	A	6-COMe	Ph	3*R*,4*S*	7.30 ± 0.05	110*	65*
5q	215	A	6-COMe	4-ClPh	racemic	7.16 ± 0.01	55*	NT
5r	193−5	A	6-COMe	2-ClPh	3*R*,4*S*	7.65 ± 0.06	140*	94*
5s	170−2	A	6-COMe	3-ClPh	3*R*,4*S*	8.04 ± 0.05	90*	78*
5t	171−3	A	6-COMe	3-IPh	3*R*, 4*S*	8.28 ± 0.03	51*[d]	NT
5u	209−10	A	6-COMe	cyclohexyl	racemic	5.90 ± 0.04	NT	NT

[a] ^{1}H NMR spectra were consistent with assigned structures. [b] All compounds gave satisfactory C, H, N analyses (±0.4%). [c] *$p < 0.05$ compared to vehicle control animals see ref 12. In all experiments, the CC_{50}'s for vehicle-treated controls fell within the range of 12−14 mA. Percent changes for drug-treated groups are derived from studies where standard errors were less than 10% of the CC_{50} values. [d] Data for the racemate; NT = not tested.

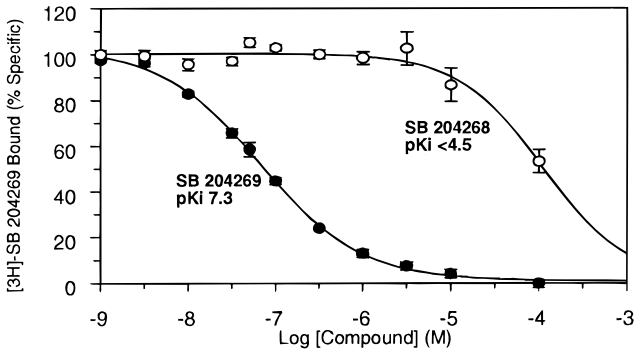
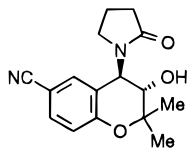


Figure 1. Competition for [^{3}H]SB 204269 binding to rat forebrain.

Charles River, 25−30g), in the mouse MEST threshold test using an "up and down" method of shock titration.[7]

The enantioselectivity of the [^{3}H]SB 204269 binding discussed above is entirely consistent with previous reports[1,3] on 6-cyano-4-(fluorobenzamido)benzopyrans, where good oral anticonvulsant activity was only observed for compounds such as **5f** with 4*S* stereochemistry. In contrast to the high affinity and *in vivo* potency of the 3*R*,4*S* enantiomer **5b**, the 3*S*,4*R* enantiomer **5c** showed only very weak binding affinity (pK_i <4.5) and was also inactive in the mouse MEST test at a dose of 10 mg/kg po. Indeed, for a range of compounds, a qualitative correlation was found between *in vitro* binding affinity and *in vivo* activity, supporting the relevance of this novel site to anticonvulsant activity (Table 1). As it became established that potent anticonvulsant activity of the *trans* compounds was associated only with the 4*S* enantiomer, many compounds were tested as *trans* racemates and generally only compounds of particular interest were tested as 3*R*,4*S* enantiomers. Thus, in interpreting the data in Table 1, it should be borne in mind that the racemate typically has half the potency of the 3*R*,4*S* enantiomer; this is further exemplified with data (not in Table 1) on the racemate of **5b**, both *in vitro* (pK_i 6.91 ± 0.05) and *in vivo* (57% increase in seizure threshold at 10 mg/kg po).

The markedly enhanced potency of **5b** compared to that of the earlier lead compound **5f** reflects the change in the 6-substituent from cyano to acetyl. The 6-cyano group is a key feature of potassium channel activators with 3*S*,4*R* stereochemistry like levcromakalim (**6**).



6 Levcromakalim

Other electron-withdrawing 6-substituents known[8] to be beneficial in potassium channel activators such as nitro (**5g**), phenylsulfonyl (**5h**), and pentafluoroethyl (**5i**) gave compounds with no improvement over the cyano compound. The 6-fluoro (**5j**) and 6-methyl (**5k**) compounds also showed only weak activity. Thus, not only is there stereochemical differentiation from potassium channel activators SAR but also there are clear differences in the optimal nature of the 6-substituent, 6-acetyl being particularly beneficial. The data suggest that the carbonyl of the 6-acetyl group is likely to be involved in a key interaction with the binding site. Homologation to 6-propionyl (**5l**) resulted in little loss in affinity,

whereas replacement by 6-benzoyl (**5m**) was clearly detrimental. Replacement by 6-methoxycarbonyl (**5n)** gave a compound, isosteric with **5l**, with similar affinity, but replacement by 6-aminocarbonyl (**5o**) resulted in markedly reduced affinity.

As the 6-acetyl moiety appeared to be crucial in conferring high affinity, its position in the benzopyran ring was investigated. Affinity was reduced by around 100-fold when it was moved to any of the alternative aromatic ring positions (**5a**, **5d**, **5e**).

Looking at the benzamide substitution pattern, removal of the 4-fluoro atom from **5b** resulted in no loss of affinity (**5p**), nor did replacement by 4-chloro (**5q**). However, the 2-chloro (**5r**) and 3-chloro (**5s**) compounds showed higher affinity, although the latter compound did not show the anticipated higher oral potency. This discovery of higher affinity for the 3-chloro compound led to the synthesis of the 3-iodo compound (**5t**) which showed one of the highest affinities in this series, although this was not reflected in its *in vivo* activity when examined as the corresponding racemate. Replacement of the benzamide by cyclohexylamide (**5u)** caused a reduction in potency, suggesting a role for the phenyl ring other than that of a lipophilic binding interaction.

Compound **5b** (SB 204269) was selected for further evaluation and studies detailing the preclinical profile, which suggest potential utility in the treatment of epilepsy disorders, will be published elsewhere.[9,10] The compound is very effective at impairing pathologically high levels of neuronal excitability while having minimal effects on normal physiological processes. The absence of sedative potential has also been confirmed in a range of studies.[11]

In conclusion, a novel enantioselective binding site for *trans* 3*R*,4*S* benzamidobenzopyrans which appears to mediate their anticonvulsant activity has been identified in mammalian brain. This has been utilized to study SAR in this mechanistically unique series of anticonvulsants. No other known anticonvusants have been found to have significant affinity for this binding site, and compound **5b** (SB 204269) has been selected for clinical evaluation as a pioneer treatment for epilepsy disorders. Further studies to elucidate the mechanism of action of this novel series of 4-amido benzopyrans are underway.

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Supporting Information Available: Experimental procedures, including analytical and spectroscopic data, for the preparation of all compounds **5** and biological protocol details (6 pages). Ordering information is given on any current masthead page.

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(9) Stean, T. O.; Upton, N. Unpublished results. For example, in the more stringent rat supramaximal electroshock test **5b** shows excellent potency (ED_{50} 6.3 mg/kg po) relative to standards (ED_{50} values of 12.0, ≥ 40, and 60.3 mg/kg po for carbamazepine, gabapentin, and diazepam, respectively) and also produces a high level of efficacy. For methods and rationale, see refs 7 and 10.

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