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IDENTIFICATION OF (-)-*cis*-6-ACETYL-4*S*-(3-CHLORO-4-FLUORO-BENZOYLAMINO)-3,4-DIHYDRO-2,2-DIMETHYL-2*H*-BENZO[b]PYRAN-3*S*-OL AS A POTENTIAL ANTIMIGRAINE AGENT

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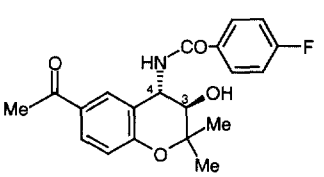
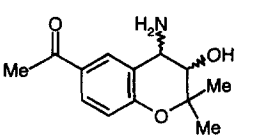
Abstract: Optimisation of novel *cis*- and *trans*-4-(substituted-amido)benzopyran-3-ol derivatives has led to the identification of SB-220453 **20** with an *in vivo* pre-clinical CNS profile predictive of potential antimigraine activity.

Keywords: Chromans, migraine, polycyclic heterocyclic compounds, SB-204269 receptor.

In this paper, a new class of potential antimigraine agents acting via a novel mechanism completely distinct to that of sumatriptan (5-$HT_{1D/1B}$ agonist) is described and SB-220453 is identified as a pioneer agent for further evaluation. Migraine is characterised by a complex cascade of physiological and molecular events resulting in activation of pain pathways and headache and there is growing evidence that increased cortical excitability underlies these changes.[1,2] In an earlier communication,[3] a series of 3*R*,4*S* benzopyrans was reported which were particularly efficaceous in increasing the seizure threshold in rodent maximal electroshock models. Such models of excessive neuronal excitability can also be used to predict anticonvulsant activity and indeed, the fluorobenzamide **1** (SB-204269) was identified as a candidate for clinical evaluation for the treatment of epilepsy.[4,5] Compounds of this class have been shown to interact at a unique binding site in the brain of several species including man which was revealed by high affinity for [^{3}H] SB-204269.[6]



1 SB-204269



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It was reported earlier that 4*S* stereochemistry at the point of attachment of the benzamide moiety was essential for high affinity for this binding site and that a 6-acetyl group was the optimal substituent. Further analogues of SB-204269 have now been prepared to explore fully the stereochemical preferences about the benzopyran nucleus at C(3) and C(4) and to optimise the substitution pattern in the benzamide moiety. In earlier studies, the synthesis of *trans* benzamides from the individual enantiomers of amino alcohol **23** and subsequent conversion into their *cis* counterparts with diethylaminosulfur trifluoride were reported.[4,7]

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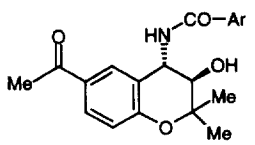
Application of these published procedures furnished the desired compounds (see Table 1) in good yield and high (>99.8% ee) enantiomeric purity.



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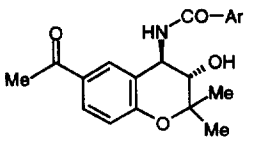
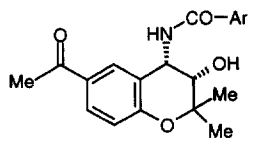
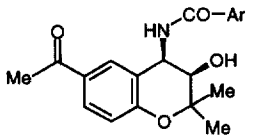


13-14 (3*S*, 4*R*)



15-21 (3*S*, 4*S*)



22 (3*R*, 4*R*)

Table 1: Chemical and Biological Data for 4-Amido-2*H*-benzo[b]pyran-3-ols

Cpd[a,b]	mpt °C	Ar	[^{3}H] SB-204269 Binding[c] pKi	rodent MEST,[d] % increase in seizure threshold at 10 mg/kg p.o.	
				Mouse at 1h post dose	Rat at 4h post dose
1	164	4-FPh	7.3	102*	600*
2	195	2-ClPh	7.7	140*	478*
3	172	3-ClPh	8.0	90*	945*
4	204	2-Cl, 4-FPh	7.5	162*	1081*
5	161	3-Cl, 4-FPh	7.9	118*	849*
6	204	2,3-diClPh	8.0	146*	1070*
7	188	2,6-diClPh	5.3	10ns	Nd
8	223	2,3,5-triClPh	7.5	33*	Nd
9	228	3-pyridyl	6.5	30*	Nd
10	267	2-pyrazinyl	4.8	-15ns	Nd
11	213	3-thienyl	6.8	50*	Nd
12	172	2-Cl(3-thienyl)	7.8	95*	519*
13	161	4-FPh	<4.5	8ns	Nd
14	116	3-ClPh	<5.2	Nd	Nd
15	161	4-FPh	7.1	70*	Nd
16	110	3-ClPh	7.9	93*	604*
17	176	2-ClPh	7.5	84*	424*
18	106	2,3-diClPh	7.8	116*	768*
19	189	2-Cl, 4-FPh	7.2	153*	269*
20	152	3-Cl, 4-FPh	7.9	143*	1192*
21	146	2-Cl(3-thienyl)	7.5	47*	383*
22	155	3-ClPh	<5.4	Nd	Nd

[a] ^{1}H NMR and m/z spectra were consistent with assigned structures; for **20** see ref [10];

[b] All compounds gave satisfactory C, H, N analyses (± 0.4%);

[c] Procedure as detailed in ref [5];

[d] Procedure as detailed in ref [8, 9]; * P<0.05, ns = non-significant; Nd = not determined.

Discussion

Compounds were screened for their ability to displace [^{3}H] SB-204269 in the rat forebrain and potent compounds were examined *in vivo,* initially in the mouse MEST model at a dose of 10 mg/kg p.o. Selected compounds were examined further in the same model in the rat and those of particular interest taken forward to a trigeminal nerve extravasation model of migraine.

Although replacement of the 4-fluoro in **1** (SB-204269) by 4-chloro led to a reduction in affinity, the 2-chloro (**2**) and 3-chloro (**3**) analogues had enhanced potency.[4] The effects of combinations of halogen substituents were then explored (Table 1). The 2-chloro-4-fluoro (**4**) and 3-chloro-4-fluoro (**5**) analogues retained the affinities of the respective des-fluoro compounds **2** and **3**. The 2,3 dichloro **6** retained the high affinity of the 3-chloro **3** but addition of a further 5-chloro substituent (**8**) resulted in a three-fold reduction in affinity and a marked reduction in *in vivo* activity. Interestingly, in the case of the 2,6-dichloro substituted compound **7,** there was a more dramatic reduction in both *in vitro* and *in vivo* potency.

Replacement of the benzamide group by a heteroaryl amide provided some interesting results. For nitrogen containing heterocycles, 3-pyridyl **9** (pKi 6.5) had thirty-fold lower affinity than 3-chloro **3** and 2-pyrazinyl **10** (pKi 4.8) had very low affinity. The unsubstituted 3-thienyl **11** showed modest affinity (pKi 6.8) but the introduction of a 2-chloro substituent (**12**) resulted in a ten-fold increase in affinity up to the same level as seen with 2-chloro benzamide **2**. Clearly, there appears to be an appropriately positioned hydrophobic pocket in the binding site.

It has been previously reported,[4] that 4*S* stereochemistry was crucial for high affinity (e.g. compare **1** and **13**), whereas the stereochemistry of the 3-hydroxyl group had little affect on the activity of a related series of 6-cyano substituted benzopyrans.[7] Therefore, in the current higher affinity 6-acetyl series, the *cis* (3*S*, 4*S*) analogues (**15** to **21**) of the more potent *trans* (3*R*, 4*S*) compounds were prepared for comparison. In general, this inversion of stereochemistry of the hydroxyl at C(3) led to similar or a marginal reduction in affinity. To define fully the effect of stereochemistry at C(3) and C(4), all isomers of the highly potent 3-chlorobenzamide **3** (pKi 8.0) were prepared. The data confirmed that 4*R* compounds (**14, 22**) have very low affinity for the SB-204269 binding site. Indeed the eudismic ratios for *trans* 4*S* **3** and *cis* 4*S* **16** enantiomers are in excess of six hundred and three hundred-fold respectively.

Compounds **3** to **6**, **12**, and **16** to **20** exhibited a similar level of activity to SB-204269 in the mouse MEST test. Further evaluation of these in rat MEST at 4h post-dose confirmed high activity and good duration of action. From Table 1, it can be seen that a number of both *cis* and *trans* benzamides had an overall profile in the rat MEST model comparable to or better than that of SB-204269. Of these, benzamides **3, 4, 6** and **20,** which showed increased efficacy, were selected for a full examination of their duration of action in the rat (Table 2). All four compounds showed a better profile *in vivo* in terms of level of effect to SB-204269. All compounds showed a rapid onset of pharmacological effect (15 minutes post-dose) with a good duration of action (> 6 hours post-dose). However, both **4** and **20** were clearly superior with high efficacy at the lower dose of 3mg/kg p.o. and, based on its overall profile, *cis* **20** was selected for further evaluation.

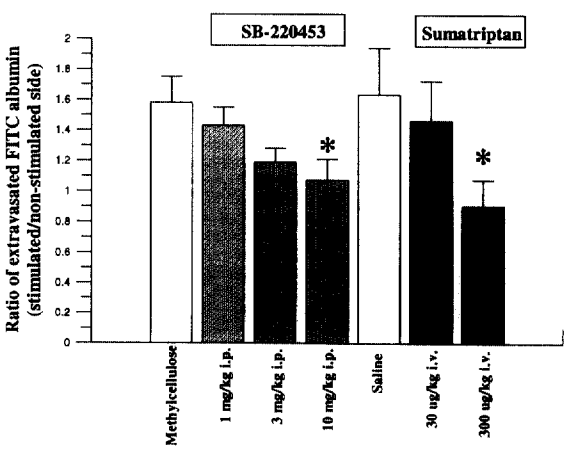
Table 2: Duration of Action of Benzamides in the Rat MEST Test.

Compound.	Dose mg/kg p.o.	% Increase in Seizure Threshold in Rat MEST[a] Time post-dose (hours) 0.25	0.5	1	2	4	6
1	10	48	90	180	420	570	390
3	10	157	273	507	796	945	983
4	3	90	208	300	660	900	1060
6	10	104	286	880	1124	1070	1076
20	3	76	202	369	576	533	638

[a] Procedure as detailed in ref [8, 9]; all figures $P<0.05$.

The impressive activity of **20** (SB-220453) in animal seizure models indicates that this compound is very effective at moderating abnormally high levels of neuronal excitability. This suggests that it may have potential utility in treating migraine. For this condition, it has been postulated that neurogenic inflammation (vasodilatation and plasma protein extravasation) may be important to the pathophysiology of migraine headaches and to the action of antimigraine drugs.[11] Neurogenic plasma extravasation develops in dura mater following trigeminal nerve stimulation and this can be detected by leakage of an albumin tracer.[12] It has been shown that SB-220453 inhibits neurogenic plasma protein extravasation in the meninges to a similar degree to sumatriptan (5-$HT_{1D/1B}$ agonist) (see Fig. 1). Thus, at the highest dose of SB-220453 (10 mg/kg i.p.), there was a complete block of neurogenic extravasation (stimulated/non-stimulated ratio of 1). Also, in contrast to sumatriptan, SB-220453 has been shown to antagonise cortical spreading depression (data not shown[13]), a phenomenon that has been linked to activation of trigeminal pain fibres[1] and initiation and maintenance of the migraine attack.[14] Hence these preliminary studies in animal models of migraine suggest that SB-220453 may be a useful treatment of this condition.

Figure 1: Comparison of SB-220453 and Sumatriptan in the Rat Extravasation Model[a].



a. Extravasation ratios (stimulated/non-stimulated sides) of FITC-albumin following electrical trigeminal nerve stimulation in rats. Animals were pretreated with methylcellulose (1% methylcellulose in 0.9% saline), saline, SB-220453 or sumatriptan. Data are represented as means±standard error of mean and significant differences between populations were assessed using Mann-Whitney U-test ($p<0.05$) (drug versus respective vehicle groups;n=6-8).

SB-220453 appears to interact solely with the SB-204269 binding site. It has shown no significant activity (selectivity > 100-fold) in over 70 receptor, ion channel and enzyme assays, including 5-$HT_{1D/1B}$ receptors. The lack of affinity for 5-$HT_{1D/1B}$ receptors confirms that SB-220453 acts by a different mechanism to sumatriptan. As would be expected for a structure of this type with 4*S* stereochemistry, it has no liability to produce cardiovascular side-effects (no significant effect on either heart rate or blood pressure in the conscious normotensive rat at 50 and 100 mg/kg p.o.; data not presented). In addition, the compound had no overt behavioural depressant effects at doses between 50 – 200 mg/kg p.o. when examined in the mouse Irwin profile test and there was no impairment of performance in the rat rotarod test (which is a sensitive paradigm for the detection of neurological deficits such as sedation and motor incoordination[15]) at doses up to 120 mg/kg p.o.

Summary

The *cis* benzopyran SB-220453 has high affinity (pKi 7.9) for the novel [^{3}H] SB-204269 binding site within the brain. It has high efficacy in animal seizure models indicating that it is very effective at moderating abnormally high levels of neuronal excitability. This suggests it may have potential in the treatment of migraine. Indeed, its excellent overall profile in the antimigraine animal models reported here, and others which will be reported in full elsewhere, has led to its selection as a pioneer agent for the treatment of migraine.

Acknowledgement

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References and Notes

[1] Moskowitz, M., Nozaki, K. and Kraig, R. *J. Neuroscience*, **1993**, *13*, 1167-1177.

[2] Parsons, A.A. *Current Opinion Neurol.*, **1998**, *11*, 227-231.

[3] Blackburn, T.P., Buckingham, R.E., Chan, W.N., Evans, J.M., Hadley, M.S., Thompson, M; Upton, N., Stemp, G. and Vong, A.K. *Bioorg. Med. Chem. Lett.*, **1995**, *5* 1163 - 1166.

[4] Chan, W.N., Evans, J.M., Hadley, M.S., Herdon, H.J., Jerman, J.C., Morgan, H.K.A., Stean, T.O., Thompson, M., Upton, N., and Vong, A.K. *J. Med. Chem.*, **1996**, *39*, 4537 - 4359.

[5] Herdon, H.J., Jerman, J.C., Stean, T.O., Middlemiss, D.N., Chan, W.N., Vong, A.K., Evans, J.M., Thompson, M. and Upton, N. *Brit. J. Pharmacol.*, **1997**, *121*, 1687 - 1691.

[6] Herdon, H., Jerman, J., Stean, T.O., Chan, W., Middlemiss, D.N. and Upton, N. *Eur. J. Pharmacol.*, **1996**, 314, R7 – R8.

[7] Brown, T.H., Campbell, C.A., Chan, W.N., Evans, J.M., Martin, R.T., Stean, T.O., Stemp, G., Stevens, N.C., Thompson, M., Upton, N. and Vong, A.K. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2563 - 2566.

[8] Upton, N., Blackburn, T.P., Campbell, C.A., Cooper, D., Evans, M.L., Herdon, H.J., King, P.D., Ray, A.M., Stean, T.O., Chan, W.N., Evans, J.M and Thompson, M. *Brit. J. Pharmacol.*, **1997**, *121*, 1679 - 1686.

[9] Compounds were evaluated for oral anticonvulsant activity in groups of 12 naive mice (male CD1-Charles River, 25-30g) in the mouse MEST test using an "up and down" method of shock titration as described in Upton, N. *Trends Pharmacol. Sci.* **1994**, *15*, 456 - 463. Percentage increases for drug-treated groups are devised from studies where standard errors were less than 10% of the CC_{50} values and with $p<0.05$ compared to vehicle control animals. In all experiments, the CC_{50} values for vehicle-treated controls fell within the range of 12-14mA.

[10] Physical and spectroscopic data for **20**.

Found: C, 61.16; H, 4.93; N, 3.63%, $C_{20}H_{19}ClFNO_4$ requires: C, 61.31; N, 4.89; N, 3.57%; $[\alpha_D]^{20}$ - 102° (c.1.1% in MeOH); ν_{max}(KBr): 3360, 3310, 2950, 1680, 1640, 1260, 840 cm^{-1}; ^{1}HNMR (270MHz, $CDCl_3$) δ: 1.40 (3H, s, CMe_2), 1.56 (3H, s, CMe_2), 2.12 (1H, d, J=8Hz, *ex* D_2O), 2.50 (3H, s, COMe), 3.84 (1H, dd, J=9,3Hz, CHOH), 5.62 (1H, dd, J=9,3Hz, CHNH), 6.91 (1H, d, J=9Hz), 6.95 (1H, brd, J=9Hz), 7.23 (1H, t, J=7Hz), 7.75 (1H, m), 7.83 (1H, dd, J-9,2Hz), 7.92 (1H, d, J=2Hz), 7.96 (1H, br, CONH); m/z: 391 (M+,1%), 373 (4), 358 (88), 203 (50), 157 (100).

[11] Young, W.B., Silberstein, S.D. and Dayno, J.M. *Seminars in Neurology*, **1997**, *17*, 325 - 333.

[12] Methods were developed from Spokes, R.A. and Middlefell, V.C. *Eur. J.Pharmacol.* **1995**, *281*, 75-79. In brief, SB-220453 (1, 3 or 10mg/kg i.p.) or vehicle was administered to male rats (190-350g). Between 20-25 min later they were anaesthetised (pentobarbitone 60mg/kg i.p.) and administered fluorescein isothiocyanate (FITC)-albumin (rat) (50mg/kg i.v.). After a further 10 min the trigeminal ganglion was stimulated for 5 min (1.2mA, 5Hz, 5ms), followed 30 min later by terminal transcardiac perfusion with heparinised saline. Only animals with complete perfusion (post-perfusion FITC-albumin < 4% of pre-perfusion levels) were included for analysis. In separate experiments, sumatriptan (30 or 300ug/kg i.v.) or saline was administered 5 min post FITC-albumin. FITC-albumin was extracted in phosphate buffered saline and determined by Cytofluor plate reader.

[13] *SCRIP* **1998**, *2329*, 19.

[14] Moskowitz, M. and Macfarlane, R. *Cerebrovascular and Brain Metabolism Reviews*, **1993**, *5*, 159 - 177.

[15] For methods see: *"Animal Models in Psychiatry and Neurology"*. Hannin, I. and Usdin, E. Eds; Pergamon Press, Oxford, **1977**.