Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 18, pp. 2101-2104, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00356-8

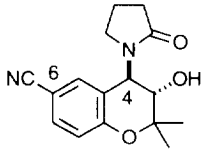
CHIRAL SPIROCYCLIC BENZOPYRAN POTASSIUM CHANNEL OPENERS: EVIDENCE FOR THE ACTIVE CONFORMATION OF LEVCROMAKALIM

R. C. Gadwood,* L. M. Thomasco, V. E. Groppi, B.-A. Burnett, S. J. Humphrey, M. P. Smith, and W. Watt

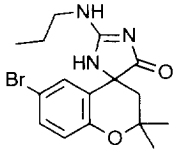
Departments of Medicinal Chemistry Research, Cell Biology and Inflammation Research, Cardiovascular Pharmacology, and Physical and Analytical Chemistry, Upjohn Laboratories, Kalamazoo MI 49001

Abstract. Both enantiomers of a spirocyclic benzopyran imidazolone were prepared in high enantiomeric purity. The *S*-isomer was found to be a potent potassium channel opener while the *R*-isomer was completely inactive. Comparison of this structurally rigid compound with levcromakalim suggests that the biologically active conformation of levcromakalim is the same as its preferred conformation in solution and solid state.

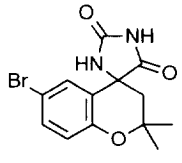
Levcromakalim (**1**) is a potent hypotensive agent thought to exert its vasorelaxant effect via opening of cell membrane ATP-sensitive potassium channels in smooth muscle. Its discovery has given rise to a rich and varied literature on the SAR of benzopyran potassium channel openers.[1,2] The key functional groups required for optimum activity in these compounds are well recognized and include an electron-withdrawing substituent at C6 and a hydrogen-bond accepting functional group at C4. However, little is known about the required three dimensional relationships among these structural features. Solution phase and solid state data suggest that the lactam and benzopyran rings of **1** prefer to be orthogonal to one another with the carbonyl parallel to the C4-H bond.[3-5] However, this low energy arrangement is not necessarily the active conformation since bond rotation could occur prior to binding.



1



2

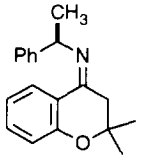


3

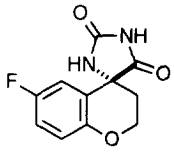
Information on the biologically active conformation of levcromakalim could lead ultimately to more potent or selective potassium channel openers. Evidence for the active conformation of levcromakalim requires a rigid system having both potent biological activity and clearly defined spatial relationships. The recently reported[6] spirocyclic benzopyran imidazolones (SBI's, **2**) meet these criteria and, in chiral form, present a unique tool to determine the preferred active conformation of levcromakalim.

The strategy for synthesis of the enantiomers of **2** relies on the preparation of **3** in optically pure form. Since the chiral center of **3** is quaternary, it was expected that homochiral **3** would ultimately lead to optically pure **2** without racemization. Although both asymmetric syntheses and resolution methods have been described

for preparation of spirohydantoins in chiral form, none were successful in the case of **3**.[7-10] Most notable was our inability to add HCN to the chiral imine **4** using either HCN in ethanol or $TMSCN/TiCl_4$ in methylene chloride.[11] This method had been previously used for the successful asymmetric synthesis of the closely related spirohydantoin, sorbinil (**5**).[10] The failure of this reaction in the case of **3** is presumably due to the additional steric hindrance introduced by the *gem*-dimethyl groups.



4



5

Resolution of **3** was ultimately accomplished by reaction with *R*-α-methylbenzylisocyanate[12] and separation of the resulting diastereomeric ureas (**6** and **7**) using medium pressure silica gel chromatography (Scheme I).[13] The less polar urea diastereomer (**6**) was isolated in pure form (>99%) while the more polar diastereomer (**7**) was obtained as an approximately 95:5 mixture with **6** (based on NMR integration). Hydrolysis of **6** and **7** with methanolic sodium methoxide gave the two enantiomers of **3**. As expected, the enantiomer produced from **6** was isolated in optically pure form (> 99.4% ee), while that isolated from **7** was only 95% optically pure (90% ee) as determined by HPLC analysis.[14] The latter enantiomer was obtained optically pure (> 99.4% ee) after three recrystallizations. Since **3** was a key synthetic intermediate from which the chirality of all subsequent intermediates was derived, an absolute assignment of configuration was made at this point in the synthesis. The enantiomer isolated from **7** was shown by X-ray crystallography[15] to be *R*-**3** (Figure 1) and the enantiomer derived from **6** was therefore assigned as *S*-**3**. The structures of **6** and **7** were correspondingly assigned as those shown in Scheme I.[16]

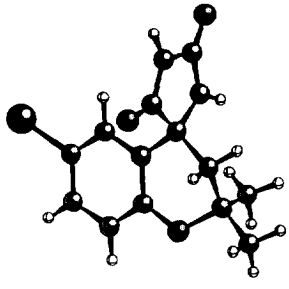
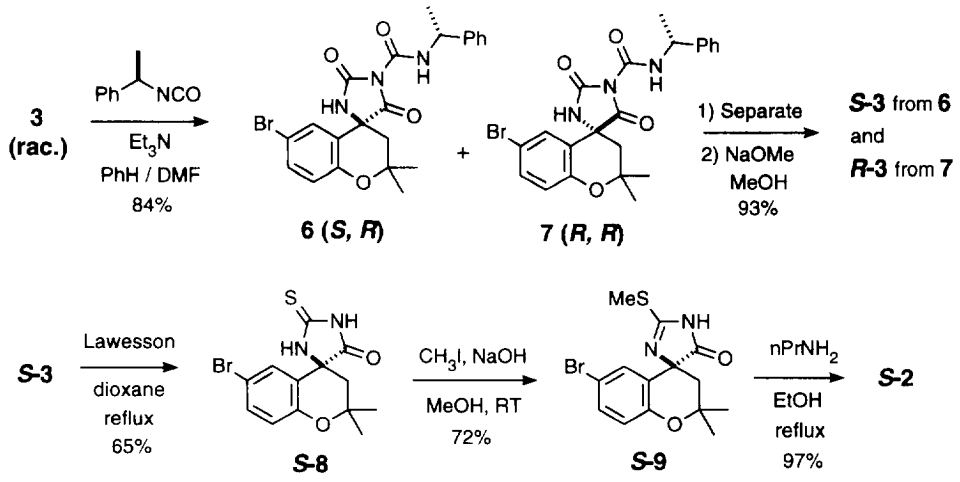


Figure 1. X-ray structure of *R*-**3**.

The individual enantiomers of **3** were converted to *R*-**2** (U-99,752) and *S*-**2** (U-99,751) by conversion to the thiohydantoins (**8**) with Lawesson's reagent, methylation to afford **9**, and displacement with propylamine.[16] No racemization of the chiral center was observed in any of these steps. Analysis of *R*-**2** and *S*-**2** by HPLC showed both enantiomers to be > 99.4% ee.[17]

Scheme I



(*R*-**2** was prepared from *R*-**3** in identical fashion.)

Both enantiomers of **2** were tested in vitro for their ability to hyperpolarize A10 (smooth muscle) cells via opening of ATP-sensitive potassium channels.[18] The *S*-enantiomer of **2** was found to be exceedingly active, with an EC_{50} of 4 nM.[19] In contrast, *R*-**2** was completely inactive even at concentrations up to 30 μM. In vivo, *S*-**2** was remarkably potent at lowering blood pressure (ED_{30} = 2.5 μg/kg) when administered i.v. to CUP-anesthetized rats. As is commonly observed with PCO's, tachycardia accompanied the decrease in mean arterial pressure. Again, *R*-**2** was inactive relative to the vehicle control even at cumulative doses up to 10 mg/kg. Although it is commonly observed that potassium channel activity resides primarily in only one enantiomer of chiral benzopyran PCO's, the degree of discrimination between the SBI enantiomers is unprecedented. In more conformationally flexible benzopyran PCO's, the activity of the distomer presumably stems from its ability to adopt a conformation minimally acceptable to the target protein. In contrast, the rigidity of **2** makes this impossible and as a result the distomer is completely inactive.

The identification of the eutomer of **2** as the 4*S*-isomer can be used to deduce the active conformation of **1**. Figure 2 illustrates the three-dimensional relationships between the structures of **1** and *S*-**2**.[20] Based on SAR data previously published, it has been suggested that the imidazolone N1 nitrogen is a bioisostere for the important carbonyl oxygen of the lactam in **1**.[6] Granting this assumption, the only conformation of **1** which places the lactam carbonyl in the same location as the N1 nitrogen of *S*-**2** is that shown in Figure 2. This is coincident with the preferred solution and solid state conformation of **1**. Based on Figure 2, the optimum arrangement appears to be a hydrogen-bond accepting group positioned 15° below the plane of the benzopyran ring system and 2.3 - 2.9 Å from the C4 carbon. The synthesis and testing of other rigid analogues of the benzopyran PCO's should provide further insight into the SAR of this important class of compounds.

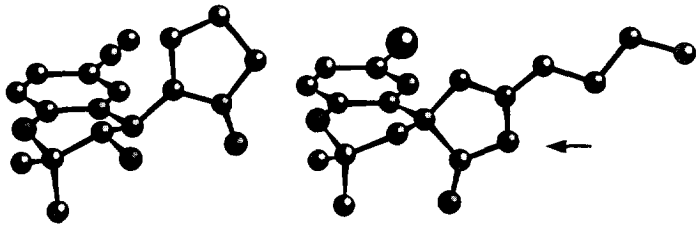


Figure 2. Structures of **1** (left) and *S*-**2**. All hydrogens have been removed for clarity. The arrow points to the N1 nitrogen of **2**.

References and Notes:

1. Longman, S. D.; Hamilton, T. C. *Med. Res. Rev.* **1992**, *12*, 73.
2. Edwards, G.; Weston, A H. *Annu. Rev. Pharmacol.Toxicol.* **1993**, *33*, 597.
3. Thomas, W. A.; Whitcombe, I. W. A. *J. Chem. Soc. Chem. Commun.* **1990**, 528.
4. Gericke, R.; Harting, J.; Lues, I.; Schittenhelm, C. *J. Med. Chem.* **1991**, *34*, 3074.
5. Cassidy, F.; Evans, J. M.; Smith, D. M.; Stemp, G.; Edge, C.; Williams, D. J. *J. Chem. Soc. Chem. Commun.* **1989**, 377.
6. Gadwood, R. C.; Kamdar, B. V.; Cipkus Dubray, L. A.; Wolfe, M. A.; Smith, M. P.; Watt, W.; Miszak, S. A.; Groppi, V. E. *J. Med. Chem.* **1993**, *36*, 1480.
7. Sarges, R.; Goldstein, S. W.; Welch, W. M.; Swindell, A. S.; Siegel, T. W.; Beyer, T. A. *J. Med. Chem.* **1990**, *33*, 1859.
8. Sarges, R.; Bordner, J.; Dominy, B. W.; Peterson, M. J.; Whipple, E. B. *J. Med. Chem.* **1985**, *28*, 1716.
9. Ueda, K.; Tanaka, S.; Kunii, T.; Kagei, K.; Tadashi, S.; Ono, H.; Ohtsuka, I.; Kawase, M.; Ohgoh, T.; Wakabayashi, T. U.S. Patent 4,874,869, October 17, 1989. *CA* **97**: 38936.
10. Sarges, R.; Howard, H. R., Jr.; Kelbaugh, P. R. *J. Org. Chem.* **1982**, *47*, 4081.
11. Imine **4** was prepared from the chromone and α-methyl benzylamine with $TiCl_4$ in toluene.
12. *R*-α-Methylbenzylisocyanate from Fluka (99% ee) was used for this resolution.
13. Pirkle, W. H.; Heire, R.; Ho Hyun, M. *Chirality* **1992**, *4*, 302.
14. Chiracel OD column, 15% IPA/hex. (with 0.1% HOAc), 1 mL/min, UV at 254 nm.
15. X-ray structure determination of *R*-**3**: crystal dimensions: 0.05x0.07x0.18mm^3, colorless, Siemens P1 diffractometer, CuKa radiation, T=293 K, orthorhombic, a=6.368(1), b=14.127(2), c=15.842(1)Å, V=1425.6(2)Å^3, Z=4, d_{cal}=1.599 g cm^3, μ=3.82mm^{-1}, SG:$P2_12_12_1$, 1243 reflections collected, 1152 reflections unique, ($2\theta_{max}$=138°), 901 reflections with $F_o > 3\sigma(F_o)$, R-value=0.045, GOF=2.39, absolute confituration established by anomalous dispersion. Detailed X-ray crystallographic data are available from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2, 1EZ U.K.
16. Specific rotations ($[\alpha]_D^{24}$, CH_3OH): *R*-**3** (+53.0°); *S*-**3** (-52.8°); *R*-**8** (-24.6°); *S*-**8** (+24.9°); *R*-**9** (+101.3°); *S*-**9** (-101.2°); *R*-**2** (+28.0°); *S*-**2** (-28.2°).
17. Chiracel OD column, 30% IPA/Hex., 1 mL/min, UV at 254 nm.
18. Epps D. E.; Wolfe M. L.; Groppi V. E. *Chem. Phys. Lipids* **1994**, *69*, 137.
19. EC_{50} refers to the concentration required to give 50% of the maximum amount of fluorescence change.
20. The structure of **1** is the (inverted) X-ray structure of cromakalim from the Cambridge database. The structure of *S*-**2** is one of two low energy (MM2) conformers which differ by a chair flip.

(*Received in USA* 19 *May* 1995; *accepted* 8 *August* 1995)