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A Congener Study of Zileuton Reveals Interesting Effects on Glucuronidation Rates§

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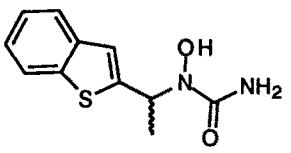
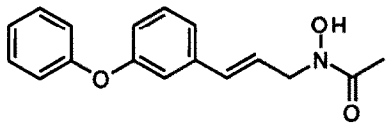
Abstract: Heteroatom substitution within the zileuton nucleus led to a congener series which was evaluated for potency and rate of glucuronidation, a major route for elimination of zileuton. Subtle changes in structure were found to significantly effect glucuronidation rate.

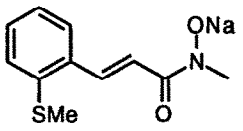
For the past several years, the focus of attention on the arachidonate 5-lipoxygenase (5-LO) pathway has given rise to a broad range of structures for inhibiting the enzyme, its products or its activation.[1] Hydroxamates were demonstrated as 5-LO inhibitors some time ago by Corey[2] and refinement of this basic approach has led to a number of interesting compounds. Zileuton[3a] (A-64077, 1), BWA4C[3b] and Ly-233569[3c] are representative structures. While zileuton displayed good efficacy in several models, its duration of action was limited primarily by rapid glucuronidation[4] and elimination. Notable work by the Abbott group has proceeded ultimately from zileuton to A-78773[3d], a more potent and metabolically more durable compound. Promising clinical results have recently been reported for the Abbott compounds.[4a,5] With knowledge of the glucuronidation problem and its



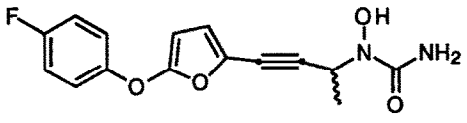
Zilueton (1)



BWA4C



Ly-233569

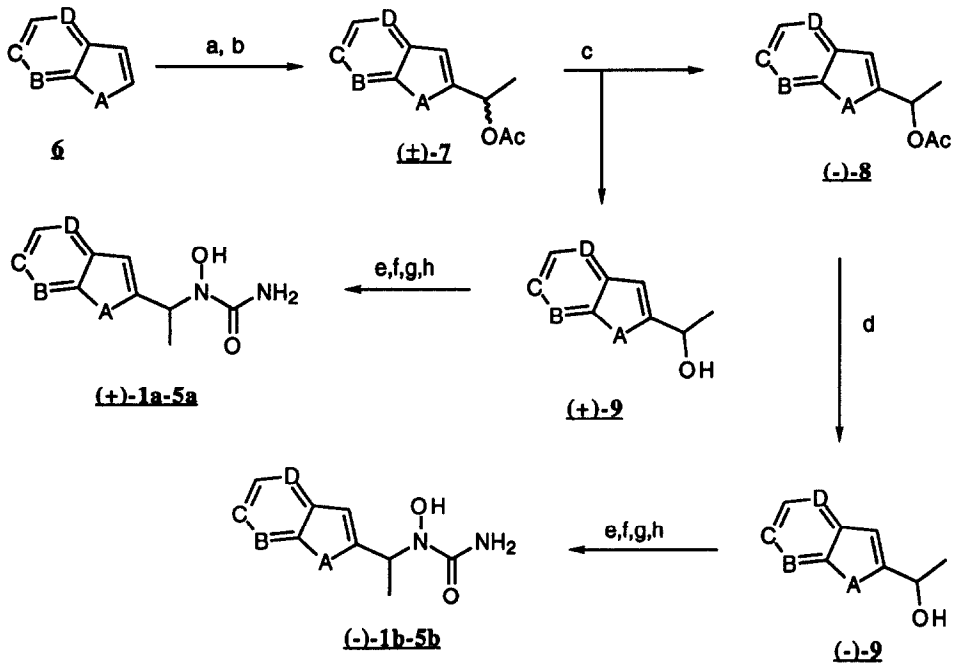


A-78773

effect on the duration of action of hydroxyurea 5-LO inhibitors, we examined a series of heteroaromatic congeners of zileuton (**1** - **5**) as individual enantiomers for potency and resistance to glucuronidation. We specifically were interested in the effect of placement of different heteroatoms in the intact benzothiophene nucleus.

The synthesis of these compounds is outlined in Scheme 1. For compounds **1, 3, 4** and **5** the parent heterocycles **6**[6] were lithiated and trapped with acetaldehyde to afford the alcohols in good yield after purification by chromatography. Although the starting alcohol for **2** could also be prepared in this manner, we found it more convenient to prepare as shown below in Scheme 2. Acetylation under standard conditions led to the racemic acetates **7**. Lipase catalyzed hydrolysis[7] to the point of consumption of one acetate enantiomer led to enantiomerically pure acetates (-)-**8** in 30 - 35% yield. These were subsequently converted to alcohols (-)-**9** in quantitative yield . The recovered alcohols were reacetylated and submitted to limited hydrolysis using the lipase to obtain the alcohols (+)-**9**. After this recycle, the (+)-alcohols were obtained in 15 - 30% yield. Chiral chemical shift reagents or chiral HPLC could be used to conveniently monitor the progress of the hydrolyses. Displacement

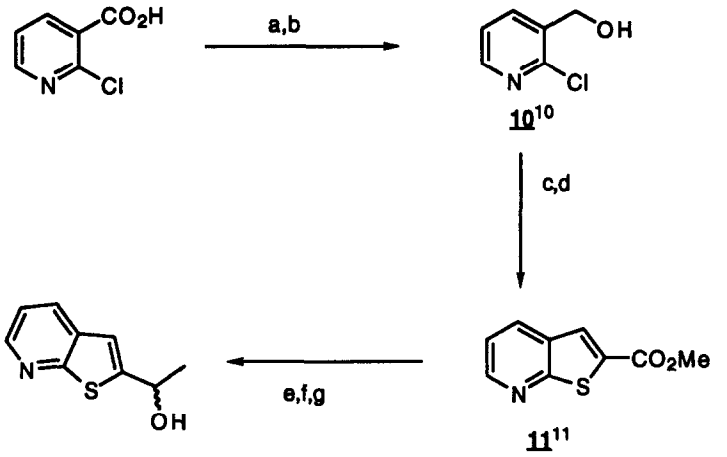
Scheme 1 - Synthesis of N-Heteroarylethyl Hydroxy Ureas



(a) n-BuLi, THF, -78°C then CH_3CHO; (b) Ac_2O, pyr, rt; (c) C. Cylindracea lipase, pH = 6.9 phosphate buffer, 40°C, 3 - 48 h; (d) K_2CO_3, MeOH, H_2O; (e) PPh_3, diethyl azodicarboxylate, AcONHAc, C_6H_6, THF, 10°C, 30 min; (f) K_2CO_3, MeOH, H_2O, rt, 1 h; g - 6N aqueous HCl, MeOH, rt, 24 h; (h) NaOCN, CF_3CO_2H, MeOH, $C_6H_5CH_3$, 10°C

of the alcohols under controlled Mitsunobu conditions similar to those reported[8] and careful hydrolysis gave the hydroxyureas in 15 - 20% yield. Use of 5 - 20% THF in benzene as solvent was critical. More polar solvent mixtures led to significant racemization. Under our conditions a small amount of racemization invariably occurred at this step. Generally, the enantiomeric purity could be upgraded by simple recrystallization. After recrystallization to constant enantiomeric purity, the hydroxamates were hydrolyzed to the hydroxylamines. Conversion to the hydroxyureas[9] was accomplished in a straightforward manner. Overall yield for these last two steps was 55 - 75%.

Scheme 2



(a) KOH, H_2O, DMSO then MeI, 1h, rt (68%); (b) $LiAlH_4$, THF, -10 - 0°C (92%); (c) PCC/Al_2O_3, CH_2Cl_2, rt, 20h; (d) DBU, $HSCH_2CO_2Me$, $PhCH_3$, 45 min, rt (80%); (e) $LiAlH_4$, THF, -10°C (65%); (f) PCC/Al_2O_3, $ClCH_2CH_2Cl$, rt, 6h (74%); (g) MeMgBr, Et_2O, THF, -20°C, 45 min (100%)

Compounds were screened for potency in human whole blood using several donors by multiple radioimmunoassay determination of LTB_4 production 30 minutes after stimulation with A23187 ionophore.[12] Susceptibility to glucuronidation was determined by HPLC determination of glucuronidation rates over a 90 minute incubation period using human microsomal protein obtained as a pool from five donors.[13] These glucuronidation rates agreed with those observed for individual donors. The results of these assays are outlined in Table 1.

Although significant gains in potency were not observed, potency itself is not sufficient for successful *in vivo* showing with hydroxyureas given the problems associated with rapid elimination of the derived glucuronides. With respect to the stereochemistry at the hydroxyurea bearing carbon, the dextrorotatory isomers were generally the more potent. This contrasts with earlier reports from the Abbott group. Within their series the stereochemistry at the hydroxyurea bearing carbon has little effect on potency. Effects on glucuronidation rates

within the series were more pronounced. When compared with the parent compound **1**, minor changes in the heterocycle resulted in significant changes in the observed glucuronidation rates. As expected[4a], one hydroxyurea enantiomer was glucuronidated more rapidly. In general, the addition of a heteroatom to the six-membered ring of the parent system (**1**) produced compounds more resistant to glucuronidation than **1**. Compounds **3** as the exceptions demonstrate the effect of subtle structural changes on glucuronidation rates.

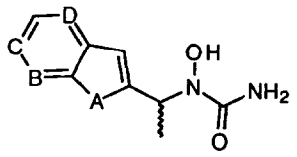


Table 1 - IC_{50} and Glucuronidation of N-Heteroarylethyl Hydroxy Ureas

Cmpd	A	B	C	D	IC_{50} Human Whole Blood† (LTB_4, μM (95% CL))	Glucuronidation Rate¶ (nmole/min/mg protein (95% CL))
(-)-1a	S	CH	CH	CH	1.88 (1.13 - 2.62)	0.155 (0.085 - 0.226)
(+)-1b	S	CH	CH	CH	0.64 (0.39 - 0.89)	0.025 (0.005 - 0.045)
(-)-2a	S	N	CH	CH	3.70 (3.67 - 3.92)	0.058 (0.047 - 0.069)
(+)-2b	S	N	CH	CH	0.95 (0.92 - 0.98)	0.012 (0.012 - 0.013)
(-)-3a	S	CH	N	CH	2.00 (1.82 - 2.19)	0.241 (0.185 - 0.296)
(+)-3b	S	CH	N	CH	1.50 (1.45 - 1.55)	0.053 (0.047 - 0.059)
(-)-4a	S	CH	CH	N	6.62 (6.54 - 6.70)	0.037 (0.031 - 0.043)
(+)-4b	S	CH	CH	N	3.12 (3.00 - 3.24)	0.0060 (0.003 - 0.009)
(-)-5a	O	N	CH	CH	11.3 (10.4 - 12.1)	not detected¥
(+)-5b	O	N	CH	CH	2.78 (2.54 - 3.02)	not detected¥

† - n = 7 for **1a** and **1b**; n = 4 for **2 - 7**.
¶ - Results shown represent data obtained from a single experiment using microsomes pooled from five donors. Results are representative of multiple determinations (n = 3) using microsomes from individual donors.
¥ - Niether disappearance of the parent compound with time nor UDGPA dependent appearance of the product was observed (limit of detection 0.001 nmol/min/mg).

Although the role of glucuronidation in effecting the *in vivo* efficacy of many specific therapeutic agents is known, general SAR studies of glucuronidation within series of compounds have not been widely published.[14] In refining series for *in vivo* potency, one is often faced with the need to minimize this mode of metabolism while retaining potency. Alteration of the steric environment at the glucuronidation site, more fundamental structural changes or substitution of the offending functional group with a bioequivalent surrogate can sometimes offer a solution. The Abbott group has reported a number of fascinating examples of the first two approaches within their series of 5-lipoxygenase inhibitors.[4a] In our particular study, we have demonstrated that it is possible to significantly alter glucuronidation rates with small effects on potency by using simple heteroatom substitution in an

existing aromatic ring. While it is impossible to know if such an approach will be useful to minimize glucuronidation in other classes of molecules, we hope that these examples will stimulate further work to determine what *subtle* structural changes can be useful in this context.

References and Notes:

§ - Contribution #896 from the Institute of Organic Chemistry.

1. Musser, J.H.; Kreft, A.F. *J. Med. Chem.* **1992**, *35*, 2501.

2. Corey, E.J.; Cashman, J.R.; Kantner, S.S; Corey, D.R. *J. Am. Chem. Soc.* **1984**, *106*, 1503.

3. a) Carter, G.W.; Young, P.R.; Albert, D.H.; Bouska, J.; Dyer, R.; Bell, R.L.; Summers, J.R.; Brooks, D.W. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 929. b) Jackson, W.P; Islip, P.J.; Kneen, G.; Pugh, A.; Wates, P.J. *J. Med. Chem.* **1988**, *31*, 499. c) Hart, R.A.; MacDonald, B.R.; Simpson, P.J.; Wang, L.; Towner, R.D.; Ho, P.P.K.; Goodwin, R.M.; Breau, A.P.; Suarez, T.; Mihelich, E.D. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 94.

4. a) Brooks, D.W. 210th National Meeting, Chicago, 1993, Poster #41 MEDI. b) Braeckman, R.A.; Granneman, G.R.; Rubin, P.; Kesterson, J.W. *J. Clin. Pharmacol.* **1989**, *29*, 22 (abstract). c) Sweeny, D.J.; Bouska, J.; Machinist, J.; Bell, R.L.; Carter, G.; Nellans, N.H. *Drug Metab. Dispos.* **1992**, *20*, 328. d) Bell, R. L.; Brooks, D. W.; Young, P. R.; Lanni, C.; Stewart, A. O.; Bouska, J.; Malo, P. E.; Carter, G. W. *J. Lipid Mediators*, **1993**, *6*, 259.

5. Brooks, D.W.; Summers, J.B.; Stewart, A.O.; Bell, R.L.; Bouska, J.; Lanni, C.; Young, P.R.; Rubin, P.; Carter, G.W. in *Perspectives in Medicinal Chemistry*, Testa, B.; Kyburz, E.; Fuhrer, W.; Giger, R. eds. Verlag Helvetica Chimica Acta, Basel **1993**, 119.

6. Thieno[2,3-b]pyridine: Klemm, L. H.; Klopfenstein, C. E.; Zell, R.; McCoy, D. R.; Klemm, R. A. *J. Org. Chem.* **1969**, *34*, 347. Thieno[2,3-c]pyridine: Dressler, M. L.; Joullie, M. M. *J. Het. Chem.* **1970**, *7*, 1257. Thieno[3,2-b]pyridine: Klemm, L. H.; Louris, John N. *J. Het. Chem.* **1984**, *21*, 785. Furo[2,3-b]pyridine: Hickson, C. L.; McNab, H. *Synthesis*, **1981**, 464.

7. For an example see: Xie, Z.-H.; Suemune, H.; Sakai, K *J. Chem. Soc. Chem. Comm.*, **1987**, 838. We substituted *C. cylindracea* lipase (Sigma) for the *P. fluorescens* lipase described.

8. Stewart, A.O.; Brooks, D.W. *J. Org. Chem.* **1992**, *57*, 5020.

9. All new compounds were fully characterized (IR, NMR, mass spec., elem. anal.). Enantiomeric purity was determined by chiral HPLC (AGP, 100 x 4.6 mm). Physical data for typical samples of the hydroxyureas used in this study follow: **1a** - mp = 142-143°, $[\alpha]_D$ = -31.68 (c = 0.142, MeOH), ee = 67%. **1b** - mp = 141-142°.

$[\alpha]_D$ = +37.04 (c = 0.166, MeOH), ee = 84%. **2a** - mp = 160-162°, $[\alpha]_D$ = -43.32 (c = 0.120, MeOH), ee >98%. **2b** - mp = 160°, $[\alpha]_D$ +43.10 (c = 0.123, MeOH), ee >98%. **3a** - mp = 166-168° $[\alpha]_D$ = -34.84 (c = 0.145, MeOH), ee >98%. **3b** - mp 169-170°, $[\alpha]_D$ = +33.99 (c = 0.145, MeOH), ee >98%. **4a** - mp = 168-168.5°, $[\alpha]_D$ = -45.04 (c = 0.110, MeOH), ee >98%. **4b** - mp = 165-166°, $[\alpha]_D$ = +48.9 (c = 0.126, MeOH), ee >98%. **5a** - mp = 165-167°, $[\alpha]_D$ = -39.6 (c = 0.10, MeOH), ee >97%. **5b** - mp = 172-175°, $[\alpha]_D$ = +41.46 (c = 0.108, MeOH), ee >97%.

10. Murakami, Y.; Sunamoto, J.; Kinuwaki, S.; Hondo, H.; *Bull. Chem. Soc. Japan*, **1973**, *46*, 2187.

11. For the ethyl ester see: Becher, J.; Dreier, C.; Frandsen, E.G.; Wengel, A.S. *Tetrahedron*, **1978**, *34*, 989. and Schneller, S.W.; Clough, F.W.; Hardee, L.E. *J. Het. Chem.* **1976**, *13*, 273.

12. One ml samples of freshly drawn heparinized whole blood were preincubated at 37°C for 15 minutes with the test compounds previously dissolved in 2µl of DMSO. The calcium ionophore A23187 (2µl of a 25 mM solution in DMSO) was added to start the reaction and the incubation was continued for an additional 30 minutes. Plasma was separated by centrifugation in the cold and duplicate aliquots were analyzed for LTB_4 using a commercial radioimmunoassay kit (Amersham). Four concentrations of test compound were tested for each subject. The results were expressed as percent inhibition of LTB_4 production by comparison with solvent controls for each subject. The data for each compound were initially analyzed using a two parameter nonlinear model of the form F(x)=100/(1+(IC_{50}/x)**n) and the slopes, n, ranging from 0.64 to 0.97 with a mean ±SD of 0.80 ± 0.10 were not significantly different from each other. The data were then analyzed with a one parameter model of the form F(x)=100/(1+(IC_{50}/x)**0.8) using the simple algorithm of Nelder and Mead supplied with PCNONLIN, SCI Software, Lexington, KY.

13. Rates of glucuronidation were determined with 0.5 - 2.5 mg/ml of human microsomal protein pooled from five donors (Human Biologics, Inc.). Reaction mixtures contained 84 nM substrate, 5 mM UDGPA, 50 mM sodium phosphate buffer (pH 7.0), 0.03 % Triton X-100 and 5 mM $MgCl_2$. Following incubation at 37°C for 0 (control), 15, 30, 60, and 90 min, 0.250 ml aliquots of the reaction mixture were mixed with 0.050 ml of 40 % trichloroacetic acid and centrifuged to remove precipitated protein. Aliquots of the supernatant were assayed by reverse phase HPLC (Keystone BDS-Hypersil C18, 250 x 4.6 mm, MeCN/ 50 mM aqueous NH_4OAc at pH4.5, 1 ml/min, 254 nm detection. Rates of glucuronidation were determined by linear regression analysis of the time/ concentration curves.

14. For a recent example see: Swart, P.J.; Jansman, F.G.A.; Drenth, B.F.H.; de Zeeuw, R.A.; Dijkstra, D.; Horn, A.S. *Pharm. and Toxicol.* **1991**, *68*, 215. For a QSAR study of phenol glucuronidation see: Kim, K.H. *J. Pharm. Sci.*, **1991**, *80*, 966. For a recent review of glucuronidation in humans see: Miners, J.O.; Mackenzie, P.I. *Pharmac. Ther.* **1991**, *51*, 347.

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