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3-Amino-1,2-benzisoxazoles: A New Family of Potent Inhibitors of LTB_4 Binding to the Human Neutrophils

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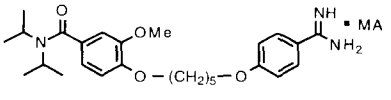
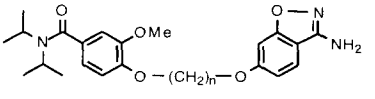
Abstract: A new family of 3-amino-1,2-benzisoxazoles was designed and synthesized to be a potent inhibitors of LTB_4 binding to the human neutrophils. HS-1141 appears to be one of the strongest inhibitors of LTB_4 binding reported so far (IC_{50} = 7nM). © 1997, Elsevier Science Ltd. All rights reserved.

5-(*S*), 12(*R*)-Dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (LTB_4) is one of the family of leukotrienes derived from the release of arachidonic acid from cellular membrane lipids and subsequent metabolism by 5-lipoxygenase. LTB_4 is released from neutrophils, monocytes, mast cells, and alveolar macrophages in response to a wide variety of stimuli. It stimulates aggregation[1] and degranulation[2] of human neutrophils, induces chemotaxis of leukocytes[3] and promotes the generation of superoxide.[4] Enhanced concentrations of this eicosanoid have been observed in tissues of patients with psoriasis,[5] inflammatory bowel disease,[6] rheumatoid arthritis,[7] gout,[8] bronchial asthma,[9] cystic fibrosis,[10] adult respiratory distress syndrome (ARDS),[11] and cerebral hemorrhage.[12] Hence, this product of arachidonic acid metabolism may play an important proinflammatory role in disease. However, in order to clearly define the role of LTB_4 in human inflammatory disease, potent, selective, and bioavailable antagonists are needed.

Several research groups have already reported synthetic LTB_4-receptor antagonists. Structurally, these compounds fall into three principal categories: (1) leukotriene analogs, based on the natural product, such as SM-9064,[13] or U-75302,[14] (2) hydroxyacetophenone derivatives, related to the prototype LTD_4 antagonist FPT55712, such as LY255283,[15a,b] or SC-41930,[16] (3) dicarboxylic acids, such as LY223982,[15c,d] and ONO LB457,[17] and (4) miscellaneous types, such as CP 105696,[18] and CGS-25019C series.[19]



CGS 25019C



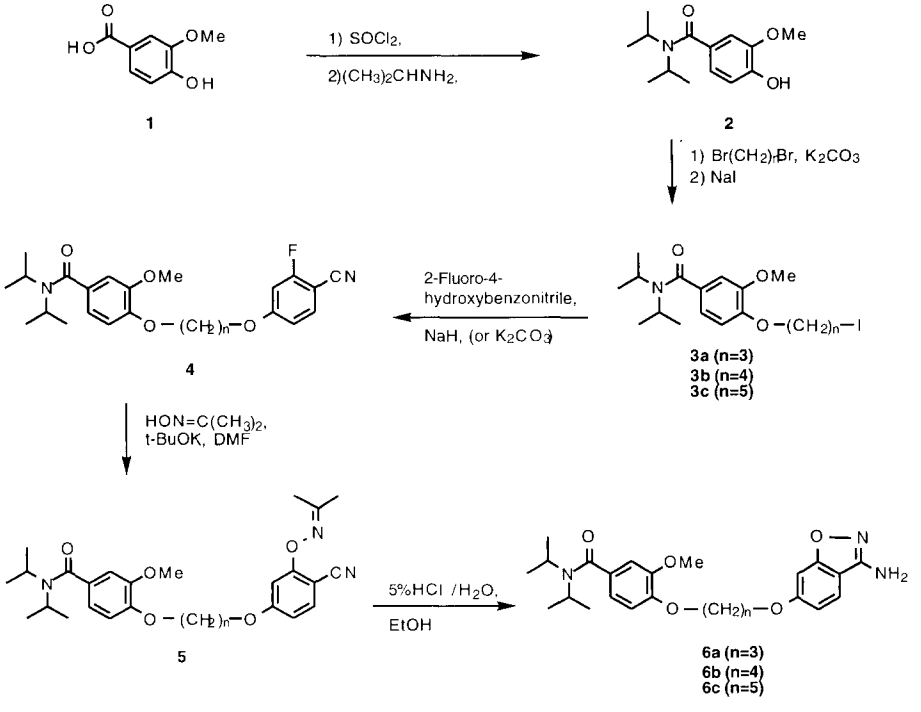
6a (n=3)
6b (n=4)
6c (n=5)

CGS-25019C has a unique structure with a basic aryl amidine group instead of the usual acidic functionality. In phase I clinical trials, CGS-25019C provided maximal inhibition of *ex vivo* LTB_4-induced CD11b upregulation 3-4 h after oral dosing in healthy volunteers, and 100% inhibition was observed at doses of

300 mg/day and above.[19d] Gastrointestinal side effects were observed at doses above 500 mg, potentially limiting the role of this compound in further elucidating the clinical potential of CGS-25019C. Since it could be speculated that this gastrointestinal side effects are originating from the basic aryl amidine group, we synthesized 3-amino-1,2-benzisoxazoles series.

The synthetic route of **6a-c** began with diisopropyl amide formation with 4-hydroxy-3-methoxy benzoic acid with thionyl chloride and diisopropyl amine in 70% yield. The resulting alcohol **2** was alkylated with dibromobutane and potassium carbonate in acetonitrile in 62% yield followed by the iodide formation with sodium iodide in acetone to generate iodide **3b** in 66% yield. Iodide **3b**, which were then coupled with 2-fluoro-4-hydroxybenzonitrile using sodium hydride in DMF, gave ether **4b** in 56% yield. Utilizing the known methodology of 3-amino-1,2-benzisoxazole formation from *o*-fluorobenzonitrile,[20] the reaction of intermediate **4b** with acetone oxime and potassium *t*-butoxide in DMF gave the *O*-(*o*-cyano)-arylacetone oxime **5b** in 96% yield. *O*-(*o*-Cyano)-arylacetone oxime **5b** was refluxed in 1:1 mixture of ethanol and 5% aqueous hydrochloric acid and neutralized to give **6b**[21a] in 64% yield. Compounds **6a**[21b] and **6c**[21c] were prepared using analogous procedure with comparable yields.

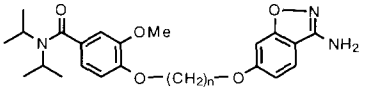
SCHEME 1



Compounds **6a-c**, which are all new compounds, were assayed for their ability to inhibit the binding of [^{3}H]-LTB$_4$ to receptors on intact human PMNs (table 1).[22, 23] Compound **6b**, with C$_4$ chain and 3-amino-1,2-benzisoxazole substituting aryl amidine, appears to be one of the strongest inhibitors of LTB$_4$ receptor binding

reported so far (IC_{50} = 7nM). When the ether connecting two aromatic portions was changed from C_4 to C_3 and C_5, the inhibition activity was reduced. The inhibition of these compounds against LTB_4 binding to human neutrophil may come from the LTB_4 receptor antagonistic activity like CGS-25019C.

Table 1. Inhibition of LTB_4 Receptor Binding to the Human Neutrophils



Cmpd. No.	HS #	n	Inhibition(%)*			IC_{50}
			10 nM	100 nM	1 μM	
6a	HS-1151	3	<10	37	92	
6b	HS-1141	4	64	95	99	7 nM
6c	HS-1132	5	<10	10	90	

* Inhibition (%) values are stated as the mean of at least three determinations.

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21. (a) **6a**: a yellow solid: mp 166-168 °C; R_f 0.25 (SiO_2, 75% EtOAc-Hexane); IR (KBr) 3449, 3331, 2961, 2936, 1614, 1447, 1381, 1371, 1258, 1169, 1020 cm^{-1}; ^1H NMR (200MHz, $CDCl_3$) δ 1.25-1.91(m, 12H) , 2.35(t, 2H, J = 6.2Hz), 3.72(br, 2H), 3.86(s, 3H), 4.24(t, 4H, J=5.8Hz), 6.82-6.91(m,5H), 7.35(d, 1H, J = 8.2Hz); ^{13}C NMR (50MHz, $CDCl_3$) δ 20.7, 28.9, 48.3, 55.8, 64.8, 65.4, 93.6, 109.4, 109.9, 112.6, 112.9, 118.1, 120.6, 131.7, 148.6, 149.2, 157.8, 161.4, 164.6, 170.8. (b) **6b**: a white solid: mp 153-154 °C; R_f 0.1 (SiO_2, 50% EtOAc-Hexane); IR (KBr) 3448, 3186, 2957, 2934, 1605, 1454, 1379, 1368, 1267, 1169, 1024 cm^{-1}; ^1H NMR (200MHz, $CDCl_3$) δ 1.20-1.50(m, 12H) , 2.05(m, 4H) 3.75(br, 2H), 3.85(s, 3H), 4.13(m, 4H), 6.86-6.79(m, 5H), 7.35(d, 1H, J = 8.4Hz); ^{13}C NMR (50MHz, $CDCl_3$) δ 20.7, 25.8, 48.0, 55.8, 67.9, 68.4, 93.3, 109.2, 109.8, 112.1, 118.1, 120.6, 131.4, 148.6, 149.1, 157.8, 161.4, 164.7, 170.8 Anal. Calcd. for $C_{25}H_{35}N_3O_5$: C, 65.62; H, 7.71; N, 9.18. Found: C, 65.70; H, 7.49; N, 8.87. (c) **6c**: a white solid: mp 87-88 °C; R_f 0.1 (SiO_2, 50% EtOAc-Hexane); IR (KBr) 3414, 3339, 2959, 2932, 1620, 1449, 1396, 1371, 1258, 1171, 1032 cm^{-1}; ^1H NMR (200MHz, $CDCl_3$) δ 1.34(m, 12H) , 1.68(m, 2H), 1.90(m, 4H), 3.72(br, 2H), 3.84(s, 3H), 4.02(q, 4H, J = 6.2Hz), 6.86-6.74(m, 5H), 7.34(d, 1H, J = 8.4Hz); ^{13}C NMR (50MHz, $CDCl_3$) δ 20.6, 22.4, 28.5, 28.6, 49.1, 55.7, 68.0, 68.5, 93.2, 109.3, 109.8, 112.2, 112.7, 118.1, 120.7, 131.2, 148.7, 149.0, 157.9, 161.4, 164.5, 170.7 Anal. Calcd for $C_{26}H_{37}N_3O_5$: C, 66.50; H, 7.51; N, 8.95. Found: C, 66.74; H, 7.85; N, 8.69.
22. **PMN Isolation**: Neutrophils (PMN) were purified from the freshly drawn human blood by standard techniques of dextran T-500 sedimentation and centrifugation on Ficoll/Paque (Pharmacia) followed by hypotonic lysis of erythrocyte.[24] The purified PMN were resuspended to a final concentration of 3 x 10^7 cells/ml in HBSS (Hank's balanced salt solution, Gibco).
23. **LTB_4 Receptor Binding Assay**: The assay was performed following the method described elsewhere.[25] LTB_4 receptor binding assay were performed in 12 x 75 mm polypropylene tubes containing 0.5 nM of [^{3}H]-LTB_4 (200Ci/mmol), competitive compound, and cells suspended in HBSS (3 x 10^6 cells) (final volume : 200µl). The tubes were incubated on ice for 45 min. Free and PMN bound [^{3}H]-LTB_4 were separated by the filtration through Whatman GF/C filter. The filter were then washed three times with 5 ml of ice cold Tris buffer (pH 7.4). The filter were air-dried and placed into scintillation vials. The radioactivity was measured by liquid scintillation spectrometry. The specific binding was determined as the count difference between total binding and binding in the presence of 1000-fold excess of unlabeled LTB_4. The LTB_4 binding activity was calculated from the percent inhibition of specific [^{3}H]-LTB_4 binding at various concentrations.
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