Dual D_2-Receptor and β_2-Adrenoceptor Agonists for the Treatment of Airway Diseases. 1. Discovery and Biological Evaluation of Some 7-(2-Aminoethyl)-4-hydroxybenzothiazol-2(3*H*)-one Analogues

Roger V. Bonnert, Roger C. Brown, David Chapman, David R. Cheshire, John Dixon, Francis Ince,* Elizabeth C. Kinchin, Amanda J. Lyons, Andrew M. Davis, Catherine Hallam, Stephen T. Harper, John F. Unitt, Iain G. Dougall, Dale M. Jackson, Kenneth McKechnie, Alan Young, and Wilfred T. Simpson†

Astra Charnwood, Bakewell Road, Loughborough, Leicestershire LE11 5RH, U.K.

Received July 16, 1998

Introduction. Chronic obstructive pulmonary disease (COPD) and asthma are diseases where there are degrees of hyperreactivity of the airways.[1] This hyperreactivity can lead to an exaggerated reflex response to a variety of stimuli and can give rise to many symptoms of both COPD and asthma (bronchoconstriction, dyspnea, cough, and mucus production).[2] COPD is still a poorly treated disease, even though topically administered β_2-adrenoceptor agonists[3] and topically applied steroids[4] are well-established treatments for asthma. Neither of these classes of compounds was designed to reduce airway hyperreactivity, and relatively little attention has been paid to the provision of compounds that could specifically reduce sensory nerve activity in the lung.

Hyperreactivity of the airways is intimately associated with the neural reflex pathways which contain both afferent and efferent nerves;[5] however, the receptors that modulate the activity of afferent and/or efferent nerves in the lung are not well-characterized. Despite this paucity of information, attempts have been made to antagonize the actions of one of the efferent neurotransmitters, acetylcholine, and this has given rise to antimuscarinic agents such as ipratropium bromide.[6] It has now been recognized that efferent nerve activity is not solely mediated by acetylcholine and that neuropeptides are probably involved in this process.[5] A more efficient approach to the control of reflex nerve activity in the airways would be to modulate the activity of afferent nerves. Our objective has been to discover compounds that are receptor agonists (or antagonists) which would suppress afferent nerve activity in the lung. We also required that these compounds should possess antibronchoconstrictor activity. This dual activity should provide an effective symptomatic treatment for both COPD and asthma and has the added advantage that, if neurogenic inflammation is a significant component of human airway disease, this approach would beneficially affect some aspects of the underlying inflammation in the lung.

Studies of the central and peripheral actions of dopamine have shown that it can act as an inhibitor of a variety of neural systems, e.g., sympathetic[7] and chemoreceptor,[8] via the stimulation of D_2-receptors, although at the time that some of these studies were conducted it would not have been possible to distinguish between D_2/D_3/D_4-receptor agonist effects.[9] Recent work has shown D_2-receptor mRNA to be present in nerves associated with reflex pathways (rat vagal afferent neurons[10] and dorsal root ganglia[11]), however, the presence of D_2-receptors on sensory nerve endings in the lung remains to be demonstrated. Our working hypothesis has been that the stimulation of D_2-receptors on afferent nerves in the lung would lead to the suppression of sensory nerve activity. D_2-Receptor agonist activity should reduce reflex bronchoconstriction, dyspnea, cough, and mucus production; however, it seemed less likely that it would diminish the direct acting bronchoconstrictor activity of locally released mediators of bronchoconstriction. β_2-Adrenoceptor agonists are the most commonly used antibronchoconstrictor agents[3] but have less obvious effects on dyspnea, cough, and mucus production. With this in mind we set out to discover compounds that were dual D_2-receptor and β_2-adrenoceptor agonists, a combination of activities that should provide an effective symptomatic treatment for COPD and asthma. This report describes the discovery and biological evaluation of some 7-(2-aminoethyl)-4-hydroxybenzothiazol-2(3*H*)-one derivatives that possess dual D_2-receptor and β_2-adrenoceptor agonist activity.

Chemistry. The compounds were prepared using the pathways shown in Scheme 1. Reaction of the arylethylamines (**1** or **2**) and the appropriate acid (**3**; see Supporting Information) activated using *N*,*N*′-carbonyldiimidazole (CDI) or dicyclohexylcarbodiimide (DCCI) afforded the amides (**4** or **5**). Subsequent reduction of these amides with diborane and, where necessary, demethylation with aqueous hydrobromic or hydrochloric acid, followed by purification, afforded the secondary amines (**7**) as their salts with hydrochloric, hydrobromic, or trifluoroacetic acid. The reduction of the amides (**4** or **5**) to the amines (**6** or **7**) proved to be difficult reactions, requiring tedious purification procedures; consequently some of the yields are poor. Work is in hand to optimize the yields of the more important compounds, e.g., **7d**, and these studies will be reported in subsequent communications.

Biology. Compounds **7a**−**d** and **8**[12] (see Chart 1) were evaluated in the in vitro systems noted below, and the results are shown in Table 1. D_2-Receptor activity: ligand binding to bovine pituitary membranes[13] (D_2(LB): pK_H, pK_L); field stimulated rabbit ear artery[14] (D_2(REA): $p[A]_{50}$, intrinsic activity (IA) vs 2-amino-6,7-dihydroxy-l,2,3,4-tetrahydronaphthalene = 1.0). β_2-Adrenoceptor activity: guinea pig trachea[15] (β_2(GPT): $p[A]_{50}$, IA vs isoprenaline = 1.0). α_1-Adrenoceptor activity: rabbit ear artery[16] (α_1(REA): $p[A]_{50}$, IA vs phenylephrine = 1.0).

Results and Discussion. Our studies in the D_1-receptor area established that Dopacard (**9**) possesses

* Author for correspondence.
† Deceased.

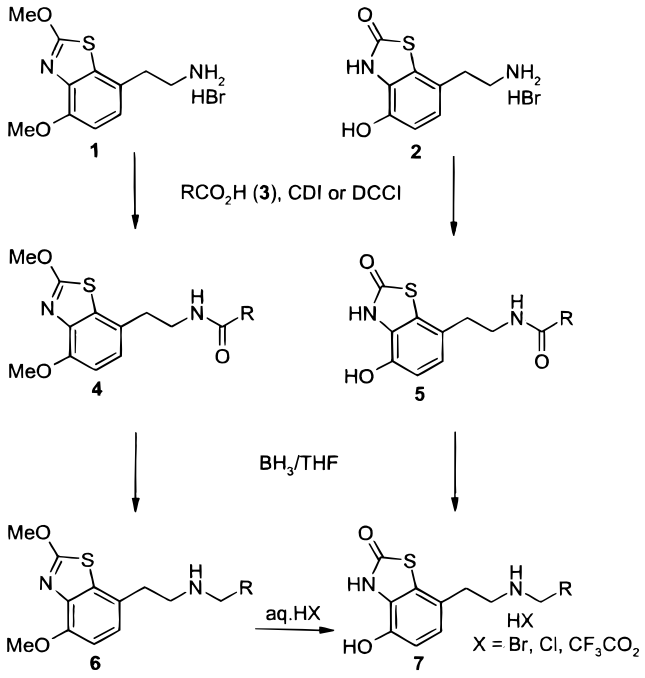
10.1021/jm980421f CCC: $15.00
Published on Web 11/13/1998

Table 1. Biological Activity of D_2-Receptor and β_2-Adrenoceptor Agonists

	D_2(LB)[a]		D_2(REA)[b]	β_2(GPT)[c]	α_1(REA)[d]
entry	pK_H	pK_L	$p[A]_{50}$ (IA)[e]	$p[A]_{50}$ (IA)[e]	$p[A]_{50}$ (IA)[e]
7a	8.99 ± 0.10	6.44 ± 0.07	*f*	7.19 ± 0.05 (0.18 ± 0.05)	6.81 ± 0.11 (0.82 ± 0.07)
7b	9.01 ± 0.02	6.93 ± 0.02	*f*	7.89 ± 0.05 (0.40 ± 0.05)	7.56 ± 0.07 (0.96 ± 0.07)
7c	9.33 ± 0.24	7.11 ± 0.01	*f*	7.33 ± 0.14 (0.11 ± 0.02)	6.85 ± 0.15 (0.82 ± 0.06)
7d	8.61 ± 0.13	6.48 ± 0.05	8.94 ± 0.07 (0.90 ± 0.04)	7.95 ± 0.05 (0.69 ± 0.03)	6.08 ± 0.15 (0.08 ± 0.03)
8	9.34 ± 0.08	6.85 ± 0.04	9.60 ± 0.04 (0.81 ± 0.05)	<5.00	6.03 ± 0.15 (0.63 ± 0.06)
10	nt[g]	nt[g]	nt[g]	7.52 ± 0.04 (0.61 ± 0.04)	5.49 ± 0.04 (0.44 ± 0.03)

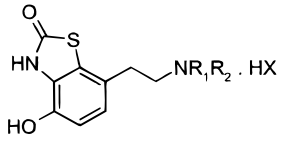
[a] See ref 12; pK_H, pK_L ± SEM. [b] See ref 13; $p[A]_{50}$ ± SEM. [c] See ref 14; $p[A]_{50}$ ± SEM. [d] See ref 15; $p[A]_{50}$ ± SEM. [e] IA, intrinsic activity versus standard agonist = 1.0. [f] Data compromised by high α_1-adenoceptor agonist activity. [g] nt, not tested.

Scheme 1. Synthetic Routes to Dual D_2-Receptor and β_2-Adrenoceptor Agonists

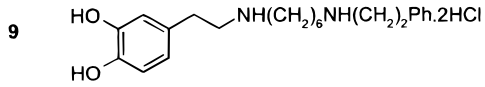


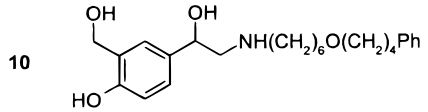
agonist activity at D_1-receptors and β_2-adrenoceptors and is a relatively weak D_2-receptor agonist.[17] During our search for compounds that were more potent as D_2-receptor agonists, we prepared hybrids of **9** and the potent D_2-receptor agonist **8**.[12] A representative example of these compounds (**7a**) is indeed a potent D_2-receptor agonist (D_2(LB): pK_H 8.99, pK_L 6.44; see Table 1; where $pK_H - pK_L \geq 1.5$, compounds have been shown to be receptor agonists when evaluated in the field stimulated rabbit ear artery preparation). For compounds **7a**−**c** α_1-adrenoceptor agonist activity (vasoconstriction) does not allow a reliable estimate of D_2-receptor agonist activity to be made using the field stimulated REA. **7a** is also a β_2-adrenoceptor partial agonist with low intrinsic activity (β_2(GPT): $p[A]_{50}$ 7.19, IA 0.18). Although salmeterol (**10**) is a β_2-adrenoceptor partial agonist (β_2(GPT): $p[A]_{50}$ 7.52, IA 0.61), it is also an efficacious antibronchoconstrictor agent;[18] consequently we required compounds with an intrinsic activity of ≥0.5. **7a** is also an α_1-adrenoceptor agonist (α_1(REA): $p[A]_{50}$ 6.81, IA 0.82), and in the context of the treatment of respiratory diseases α-adrenoceptor

Chart 1. Structures of D_2-Receptor and β_2-Adrenoceptor Agonists



Entry	R_1	R_2	HX
7a	H	$(CH_2)_6NH(CH_2)_2Ph$	2HBr
7b	H	$(CH_2)_6O(CH_2)_2Ph$	HCl
7c	H	$(CH_2)_6O(CH_2)_4Ph$	CF_3CO_2H
7d	H	$(CH_2)_2SO_2(CH_2)_3O(CH_2)_2Ph$	HCl
8	^{n}Pr	^{n}Pr	HBr





agonist activity is undesirable, given that it could produce bronchoconstriction[19] and/or pulmonary vasoconstriction.[20] Among the analogues of **7a** that were prepared, the ether (**7b**) possesses a more encouraging profile of activity. **7b** is a potent D_2-receptor agonist (D_2(LB): pK_H 9.01, pK_L 6.93) and β_2-adrenoceptor partial agonist with an increased intrinsic activity (β_2-(GPT): $p[A]_{50}$ 7.89, IA 0.40); however, it is still a potent α_1-adrenoceptor agonist (α_1(REA): $p[A]_{50}$ 7.56, IA 0.96). Salmeterol (**10**) had been described as a long-acting β_2-adrenoceptor partial agonist;[18] consequently the analogue of **10** in the benzothiazolone series was prepared, i.e., **7c**. While **7c** is a potent D_2-receptor agonist (D_2-(LB): pK_H 9.33, pK_L 7.11), it, like **7b**, is a β_2-adrenoceptor partial agonist with low intrinsic activity (β_2-(GPT): $p[A]_{50}$ 7.33, IA 0.11) and is also an α_1-adrenoceptor agonist (α_1(REA): $p[A]_{50}$ 6.85, IA 0.82).

Intensive studies in the area around **7b**,**c** were carried out with the aim of removing the unwanted α_1-adrenoceptor agonist activity. The strategy was to produce

compounds where the steric and/or electronic properties of the nitrogen substituent were modified such that activity at the α_1-receptor was reduced while maintaining, or possibly enhancing, activity at the D_2-receptor and β_2-adrenoceptor. This was achieved by the incorporation of an O, S, or SO_2 moiety into the $(CH_2)_6$ portion of the ethylamine nitrogen substituent. A representative compound (**7d**) is, as required, a weak α_1-adrenoceptor agonist (α_1(REA): p[*A*]$_{50}$ 6.08, IA 0.08). **7d** is a dual D_2-receptor agonist (D_2(REA): p[*A*]$_{50}$ 8.94, IA 0.90; essentially a full agonist at the D_2-receptor) and β_2-adrenoceptor partial agonist (β_2(GPT): p[*A*]$_{50}$ 7.95, IA 0.69) that is 3-fold more potent than **10**. An important structural feature of **7d** is that it is achiral, as it does not contain the benzylic hydroxy group present in the phenylethanolamine component of established β_2-adrenoceptor agonists (e.g., **10**), despite this **7d** is still a potent β_2-adrenoceptor agonist.

While there are no convincing in vivo animal models of COPD, compounds such as **7d** do show good activity in a number of in vivo D_2-receptor and β_2-adrenoceptor models that are relevant to COPD and asthma. When dosed to the lung (nebulized aqueous aerosol) **7d** reduces histamine-induced tachypnea in the β-blocked anesthetized dog (D_2), ammonia-induced mucus production in the β-blocked anesthetized dog (D_2), and histamine-induced bronchoconstriction in the anesthetized dog (β_2) with an ED_{50} dose of $\sim$1 $\mu g \cdot kg^{-1}$. These studies will be described in more detail in subsequent communications.

Conclusions. Our search for compounds that are able to modulate the hyperreactivity of pulmonary sensory nerves and provide an effective therapy for COPD and asthma has resulted in the discovery of some dual D_2-receptor and β_2-adrenoceptor agonists. Initially these 7-(2-aminoethyl)-4-hydroxybenzothiazol-2(3*H*)-one derivatives also possessed an inappropriate level of α_1-adrenoceptor agonist activity; however, this was reduced by modification of the substituent present on the ethylamine nitrogen atom. The most promising compound (**7d**, AR-C68397AA) has been selected for further development as a potential treatment for COPD and asthma. These, and other, studies will be described in subsequent communications.

Acknowledgment. We would like to thank Larry Coe, Bob Jewell, Carol Manners, Hemant Mistry, Malbinder Fagura, Diane Harper, Simon Lydford, Carolyn Taylor, Shahad Mohammed, Joe Sempik, Carol Weyman-Jones, and Keith Vendy for their expert technical assistance. We would also like to thank Nigel Gensmantel, Darren R. Flower, Allan Wallace, Alan Blackham, and Roy Eady for valuable discussions.

Supporting Information Available: Reaction schemes, synthetic procedures, and characterization data for the compounds described (15 pages). Ordering information is given on any current masthead page.

References

(1) Sibbald, B. Genetics. In *Asthma Basic Mechanisms and Clinical Management*; Barnes, P. J., Rodger, I. W., Thomson, N. C., Eds.; Academic Press: London, 1992; pp 21−31. Du Toit, J.; Woolcock, A. J.; Salome, C. M. Characteristics of bronchial hyperreactivity in smokers with chronic airflow limitation. *Am. Rev. Respir. Dis.* **1986**, *134*, 498−501.

(2) Widdicombe, J. G.; Fillenz, M. Receptors of the lungs and airways. In *Enteroceptors*; Neil, E., Ed.; Springer Verlag: Berlin, 1972; pp 81−113.

(3) Andersen, G. P. Long acting inhaled beta adrenoceptor agonists. The comparative pharmacology of formoterol and salmeterol. In *New Drugs for Asthma Therapy. Agents and Actions Supplement* 34; Anderson, G. P., Chapman, I. D., Morley, J., Eds.; Birkhauser Verlag: Basel, 1991; pp 97−115.

(4) Barnes, P. J. Molecular mechanisms of glucocorticoid action in asthma. *Pulmonary Pharmacol.* **1997**, *10*, 3−19.

(5) Barnes, P. J. Airway NANC Nerves and Neuropeptides. In *Asthma Basic Mechanisms and Clinical Management*; Barnes, P. J., Rodger, I. W., Thomson, N. C., Eds.; Academic Press: London, 1992; pp 359−391. Widdicombe, J. G. The neuronal component of asthma. *Agents Actions - Supplements* **1989**, *28*, 213−224.

(6) Barnes, P. J. New therapies for chronic obstructive pulmonary disease. *Thorax* **1998**, *53*, 137−147.

(7) Ilhan, M.; Long, J. P. Inhibition of the sympathetic nervous system by dopamine. *Arch. Int. Pharmacodyn.* **1975**, *216*, 4−10.

(8) Sampson, S. R.; Aminoff, M. J.; Jaffe, R. A.; Vidruk, E. H. Analysis of the inhibitory effects of dopamine on carotid body chemoreceptors in cats. *Am. J. Physiol.* **1976**, *230*, 1494−1498.

(9) Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. Dopamine Receptors: From Structure to Function. *Physiol. Rev.* **1998**, *78*, 189−225. Strange, P. G. Dopamine Receptors: Studies on Structure and Function. In *Advances in Drug Research*; Testa, B., Meyer, U. A., Eds.; Academic Press: London, 1996; Vol. 28, pp 313−352.

(10) Lawrence, A. J.; Krstew, E.; Jarrott, B. Functional dopamine D_2 receptors on rat vagal afferent neurones. *Br. J. Pharmacol.* **1995**, *114*, 1329−1334.

(11) Xie, G. X.; Jones, K.; Peroutka, S. J.; Palmer, P. P. Detection of mRNA's and alternatively spliced transcripts of dopamine receptors in rat peripheral sensory and sympathetic ganglia. *Brain Res.* **1998**, *785*, 129−135.

(12) Weinstock, J.; Gaitanopoulus, D. E.; Stringer, O. D.; Franz, R. G.; Heible, J. P.; Kinter, L. B.; Mann, W. A.; Flaim, K. E.; Gessner, G. Synthesis and evaluation of non-catechol D-1 and D-2 dopamine receptor agonists: benzimidazol-2-one, benzoxazol-2-one, and the highly potent benzothiazol-2-one-7-ethylamines. *J. Med. Chem.* **1987**, *30*, 1166−1176.

(13) Sibley, D. R.; De Lean, A.; Creese, I. Anterior pituitary dopamine receptors. Demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. *J. Biol. Chem.* **1982**, *257*, 6351−6361.

(14) Heible, J. P.; Nelson, S. H.; Steinsland, O. S. Neuronal dopamine receptors of the rabbit ear artery: pharmacological characterisation of the receptor. *J. Autonom. Pharmacol.* **1985**, *5*, 115−124.

(15) Dougall, I. G.; Harper, D.; Jackson, D. M.; Leff, P. Estimation of the efficacy and affinity of the β_2-adrenoceptor agonist salmeterol in guinea-pig trachea. *Br. J. Pharmacol.* **1991**, *104*, 1057−1061.

(16) Fagura, M. S.; Lydford, S. J.; Dougall, I. G. Pharmacological classification of α_1-adrenoceptors mediating contractions of rabbit isolated ear artery: comparison with rat isolated thoracic aorta. *Br. J. Pharmacol.* **1997**, *120*, 247−258.

(17) Brown, R. A.; Dixon, J.; Farmer, J. B.; Hall, C.; Humphries, R. G.; Ince, F.; O'Connor, S. E.; Simpson, W. T.; Smith, G. W. Dopexamine: a novel agonist at peripheral dopamine receptors and β_2-adrenoceptors. *Br. J. Pharmacol.* **1985**, *85*, 599−608.

(18) Johnson, M. Salmeterol. *Med. Res. Rev.* **1995**, *15*, 225−257.

(19) Snashall, P. D.; Boother, F. A.; Sterling, G. A. The effects of alpha-adrenoceptor stimulation on the airways of normal and asthmatic man. *Clin. Sci. Mol. Med.* **1978**, *54*, 283−289.

(20) Hyman, A. L.; Lippton, H. L.; Kadowitz, P. J. Nature of alpha 1 and postjunctional alpha 2 adrenoceptors in the pulmonary vascular bed. *Fed. Proc.* **1986**, *45*, 2336−2340.

JM980421F