3-Arylimino-2-indolones Are Potent and Selective Galanin GAL_3 Receptor Antagonists

Michael J. Konkel,*[,‡] Bharat Lagu,[‡,§] Lakmal W. Boteju,[‡,#] Hermogenes Jimenez,[‡] Stewart Noble,[‡,||] Mary W. Walker,[‡] Gamini Chandrasena,[‡] Thomas P. Blackburn,[‡,†] Sham S. Nikam,[⊥] Jon L. Wright,[⊥,∞] Brian E. Kornberg,[⊥] Tracy Gregory,[⊥] Thomas A. Pugsley,[⊥] Hyacinth Akunne,[⊥] Kim Zoski,[⊥] and Lawrence D. Wise[⊥]

Lundbeck Research USA, Inc., 215 College Road, Paramus, New Jersey 07652, and Pfizer Global Research and Development, 2800 Plymouth Road, Ann Arbor, Michigan 48105

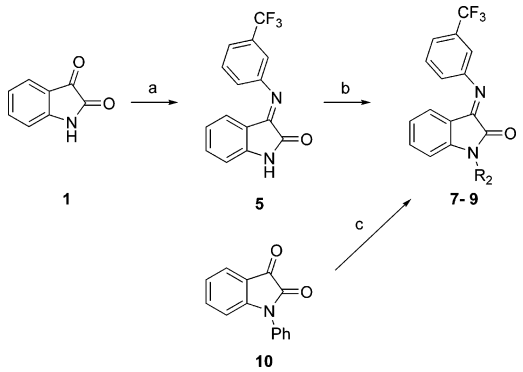
Received January 1, 2006

Abstract: A series of 3-imino-2-indolones are the first published, high-affinity antagonists of the galanin GAL_3 receptor. One example, 1,3-dihydro-1-phenyl-3-[[3-(trifluoromethyl)phenyl]imino]-2*H*-indol-2-one (**9**), was shown to have high affinity for the human GAL_3 receptor (K_i = 17 nM) and to be highly selective for GAL_3 over a broad panel of targets, including GAL_1 and GAL_2. Compound **9** was also shown to be an antagonist in a human GAL_3 receptor functional assay (K_b = 29 nM).

Galanin is a 30 amino acid residue neuropeptide in human, but it is a 29 amino acid residue neuropeptide in at least 13 other species.[1] In the rat, galanin is present in the spinal cord, in specific neuronal systems in the brain, and in neuroendocrine cells of a variety of peripheral tissues.[2] Galanin activates G-protein-coupled receptors (GPCRs) to regulate a variety of functions. In the rat, galanin has been shown to have physiological effects in feeding, insulin release, lactation, gut contractility, and growth and has effects on central functions such as spinal reflex, learning, and memory and in rodent models of depression.[3] Three cloned galanin GPCRs have been identified and are designated as GAL_1, GAL_2, and GAL_3.[4] In rat, mRNA encoding GAL_3 has been localized in the hypothalamus, pituitary, spinal cord, pancreas, liver, kidney, stomach, and adrenal gland, suggesting possible roles in feeding, digestion, pituitary hormone release, nociception, insulin homeostasis, and glucose homeostasis.[5]

To further the understanding of the pharmacological roles that galanin receptors play in disease states, we set out to find ligands that are specific for the GAL_3 receptor. Weak to moderate affinity nonpeptidic antagonists have been reported for the GAL_1 receptor.[6] However, to date there are no published papers describing nonpeptidic antagonists for GAL_2 or GAL_3 receptors.[7] Indeed, as has been recently pointed out at the third international symposium on galanin,[8] there are no reported effective small-molecule galanin receptor antagonists that can cross the blood−brain barrier. Herein, we give a preliminary report describing 3-arylimino-2-indolones as antagonists for the GAL_3 receptor.

Scheme 1. Synthesis of 3-Arylimino-2-indolones[a]



[a] Reagents and conditions: (a) 3-(trifluoromethyl)aniline (5 equiv), 140 °C, 6 h; (b) K_2CO_3 (1.5 equiv), R_2Cl or R_2Br (1.5 equiv), DMF, 16 h; (c) 3-(trifluoromethyl)aniline (1.0 equiv), 90 °C, 3 h.

The 3-arylimino-2-indolones were synthesized as outlined in Scheme 1. Isatin (**1**), 1-phenylisatin (**10**), **3**, **4**, and **6** were purchased from commercial sources. Isatin was allowed to react neat with 3-trifluoromethylaniline at 140 °C, giving **5** in 95% yield. Similarly, 1-phenylisatin was allowed to react neat with 3-trifluoromethylaniline at 90 °C, giving **9** in 78% yield. The 3-arylimino-2-indolones exist as *E*- and *Z*-isomers that can be seen by ^{1}H NMR but interconvert rapidly in solution at room temperature and cannot be separated by thin-layer chromatography or HPLC. The ratio of the isomers is solvent-dependent. At room temperature, **9** exists as a 4:1 ratio of isomers in $CDCl_3$ but as a 1.9:1 ratio in DMSO-d_6. Variable-temperature ^{19}F NMR of **9** in DMSO-d_6 shows that the signals for the *E*- and *Z*-isomers coalesce at 90 °C and then separate again upon cooling. It is unknown whether the *E* or the *Z* isomer is the major isomer. Although the isomers are not separable, we are not aware of any limitation of the utility of this series due to the mixtures.

Affinity of the disclosed compounds for human GAL_3 was determined using a previously described ^{125}I-galanin displacement assay.[9] The 1-unsubstituted indolone **2** (Table 1), initially identified from a high-throughput screen using a modification of this assay,[10] has modest binding affinity for the human GAL_3 receptor (K_i = 437 nM). Preliminary SAR analysis of 1-unsubstituted indolones shows that 4-chloro or 3-trifluoromethyl substitution on the phenyl ring (**3** and **5**) gives affinity (K_i = 850 and 596 nM, respectively) comparable to that of **2** while 4-methoxy substitution (**4**) gives much weaker affinity.

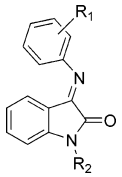
Preliminary investigations into the effects of substitution on the indole nitrogen atom indicate that substitution increases binding affinity. For instance, the allyl substituted **6** has K_i = 150 nM and 1-ethylpropyl substituted **7** and the propynyl substituted **8** have K_i < 100 nM. Substitution on the indole nitrogen atom with a phenyl group (**9**) results in an even more substantial increase in binding affinity (K_i = 17 nM).

Compound **9** was further characterized. In a functional assay it was found to be an antagonist with potency (K_b = 29 nM) that compares well with the human GAL_3 binding affinity (K_i = 17 nM).[11] Compound **9**, as well as other 3-imino-2-indolones, was tested against human GAL_1 and GAL_2 and was not found to have affinity for these receptors (K_i > 10 μM). Compound **9** did not show activity in a broad panel of enzyme, channel, and receptor assays.[12] Compound **9** was also tested for exposure

* To whom correspondence should be addressed. Phone: 201-261-1331. Fax: 201-261-0623. E-mail: pacl@Lundbeck.com.
‡ Lundbeck Research USA.
§ Current address: Johnson & Johnson PRD, 1000 Route 202, P.O. Box 300 (PC-111), Raritan, NJ 08869.
# Current address: Lexicon Pharmaceutical Corporation, 350 Carter Road, Princeton, NJ 08540.
|| Current address: Kalypsys, Inc., 11099 North Torrey Pines Road, La Jolla, CA 92037.
† Current address: Helicon Therapeutics, Inc., 1 Bioscience Park Drive, Farmingdale, NY 11735.
⊥ Pfizer Global Research and Development.
∞ Current address: Celgene-Signal Research Division, 4550 Towne Centre Court, San Diego, CA 92121.

10.1021/jm060001n CCC: $33.50
Published on Web 06/07/2006

Table 1. Binding Affinity of 3-Imino-2-indolones for Human GAL_3[a]



compd	R^1	R^2	GAL_3 K_i, nM
2	2,3-di-Cl	H	437
3	4-Cl	H	850
4	4-OMe	H	>10000
5	3-CF_3	H	596
6	3,4-di-Cl	allyl	150
7	3-CF_3	1-ethylpropyl	52
8	3-CF_3	2-propynyl	89
9	3-CF_3	Ph	17

[a] Binding affinity was determined using a previously described ^{125}I-galanin displacement assay.[8] K_i values are the averages from two or more individual determinations: for $N = 2$, the mean values are within 3-fold of their individual determinations; for $N > 2$, the values for the standard error of the mean are less than 60% of their mean values.

Table 2. Rat Exposure Levels with Compound **9**[a]

route (dose, mg/kg)	time, h	plasma level,[a] μg/mL	brain level, μg/g
pp (10)	2	0.09	NT[b]
sc (10)	2	0.99	0.35
ip (30)	2	0.78	1.06
ip (30)	4	1.68	1.21

[a] Values determined from an average of two animals with variability of <25% around the average value. [b] Not tested.

levels in rat by ip, sc, and po administration. The results of these experiments are shown in Table 2.

In conclusion, 3-imino-2-indolones bind to the GAL_3 receptor. *N*-Aryl substitution is critical for obtaining high affinity. Compound **9** is a high-affinity ligand for the GAL_3 receptor ($K_i = 17$ nM), and it was demonstrated to be an antagonist in an in vitro functional assay. It was further characterized and shown to be highly selective for the GAL_3 receptor against a panel of 75 cross-reactivity targets. It was also shown to give exposure levels to rat in plasma and brain above its K_i. These properties make it a good tool to further characterize the pharmacology of galanin receptors. The use of this tool for elucidating some pharmacological effects of GAL_3 receptor antagonism will be reported shortly.

Acknowledgment. We thank the following people for their support of this research: Deborah Hurtado, Anastatia Kokkinakis, and Tracy Johnson Blake for cell culture and membrane preparation; Marc Dizon and Kiho Han for radioligand displacement assays and in vitro functional assay; QingPing Han and John Wetzel for ^{19}F NMR studies and discussion; Rui Li and Asanthi Pieris-Gunatilaka for the pharmacokinetic studies of **9**.

Supporting Information Available: Detailed experimental and characterization data for **5** and **7**−**9** and dosing solutions for the pharmacokinetic studies of **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Langel, Ü.; Bartfai, T. Chemistry and Molecular Biology of Galanin Receptor Ligands. *Ann. N. Y. Acad. Sci*. **1998**, *863*, 86−93.

(2) Reviews: (a) Rökaeus, Å. Galanin: A Newly Isolated Biologically Active Neuropeptide. *Trends Neurosci.* **1987**, *10*, 158−164. (b) Crawley, J. N. Biological Actions of Galanin. *Regul. Pept.* **1995**, *59*, 1−16. (c) Rökaeus, Å.; Jiang, K.; Spyrou, G.; Waschek, J. Transcriptional Control of the Galanin Gene: Tissue-Specific Expression and Induction by NGF, Protein Kinase C, and Estrogen. *Ann. N. Y. Acad. Sci.* **1998**, *863*, 1−13

(3) (a) Bartfai, T.; Hökfelt, T.; Langel, Ü. Galanin: A Neuroendocrine Peptide. *Curr. Rev. Neurobiol*. **1993**, *7*, 229−274. (b) Kask, K.; Langel, Ü.; Bartfai, T. Galanin−A Neuropeptide with Inhibitory Actions. *Cell. Mol. Neurobiol.* **1995**, *15*, 653−673. (c) Bedecs, B.; Berthold, M.; Bartfai, T. Int. Galanin−10 Years with a Neuroendocrine Peptide. *Int. J. Biochem. Cell Biol.* **1995**, *27*, 337−349. (d) Kask, K.; Berthold, M.; Bartfai, T. Galanin Receptors: Involvement in Feeding, Pain, Depression and Alzheimer's Disease. *Life Sci.* **1995**, *60*, 1523−1533.

(4) (a) The official nomenclature of the International Union of Pharmacology for the three galanin receptors are to be referred to as GAL_1, GAL_2, and GAL_3, and the respective genes from which they are derived are referred to as GALR1, GALR2, and GALR3, respectively. Foord, S. M.; Bonner, T. I.; Neubig, R. R.; Rosser, E. M.; Pin, J.-P.; Davenport, A. P.; Spedding, M.; Harmar, A. J. International Union of Pharmacology. XLVI. G Protein-Coupled Receptor List. *Pharmacol. Rev.* **2005**, *57*, 279−288. (b) Review of galanin receptor subtypes: Branchek, T. A.; Smith, K. E.; Gerald, C.; Walker, M. W. Galanin Receptor Subtype. *Trends Pharmacol. Sci.* **2000**, *21*, 109−116.

(5) Smith, K. E.; Walker, M. W.; Artymyshyn, R.; Bard, J.; Borowsky, B.; Tamm, J. A.; Yao, W.-J.; Vaysse, P. J.-J.; Branchek, T. A.; Gerald, C.; Jones, K. A. Cloned Human and Rat Galanin GALR3 Receptors. Pharmacology and Activation of G-Protein Inwardly Rectifying K^+ Channel. *J. Biol. Chem.* **1998**, *273*, 23321−23326.

(6) Scott, M. K.; Ross, T. M.; Lee, D. H. S.; Wang, H.-Y.; Shank, R. P.; Wild, K. D.; Davis, C. B.; Crooke, J. J.; Potocki, A. C.; Reitz, A. B. 2,3-Dihydrodithiin and -Dithiepine-1,1,4,4-tetroxides: Small molecule Non-peptide Antagonists of the Human Galanin hGAL-1 Receptor. *Bioorg. Med. Chem.* **2000**, *8*, 1383−1391.

(7) Our laboratory has presented two posters at a recent American Chemical Society meeting on another series of GAL_3 antagonists: (a) Topiwala, U.; Chen, H.; Jimenez, H.; Reitman, M.; Walker, M.; Han, K.; Boyle, N.; Caputo, G.; Muske, G.; Konkel, M.; Wetzel, J. 3-Phenyl-2-indolylcarbohydrazides and their Azo Analogues as Potent Antagonists of the GalR3 Receptor. Presented at the 228th National Meeting of the American Chemical Society, Philadelphia, PA, August 22−26, 2004; Paper MEDI 30. (b) Chen, H.; Topiwala, U. P.; Boteju, L. W.; Eldemenky, E.; Jimenez, H.; Reitman, M.; Walker, M.; Han, K.; Boyle, N.; Caputo, G.; Muske, G.; Yang, J.; Konkel, M. J.; Wetzel, J. M. Discovery of 3-Phenyl-2-indolylcarbohydrazides as Antagonists of the GalR3 Receptor. Presented at the 228th National Meeting of the American Chemical Society, Philadelphia, PA, August 22−26, 2004; Paper MEDI 31.

(8) Hökfelt, T. Galanin and Its Receptors: Introduction to the Third International Symposium, San Diego, California, USA, 21−22 October 2004. *Neuropeptides* **2004**, *39*, 125−142.

(9) Borowsky, B.; Walker, M. W.; Huang, L.-Y.; Jones, K. A.; Smith, K. E.; Bard, J.; Branchek, T. A.; Gerald, C. Cloning and Characterization of the Human Galanin GALR2 Receptor. *Peptides* **1998**, *19*, 1771−1781.

(10) The screening was carried out at a single concentration of 10 μM in duplicate. Hits with >50% displacement were followed up with 12 concentration points. K_i determination was done in duplicate.

(11) When the human Gal3 receptor is cotransfected with Gαz into COS-7 cells, activation by porcine galanin results in suppression of forskolin-stimulated cAMP accumulation. Pretreatment with increasing concentrations of **9** produced a rightward shift in the galanin dose response curve. Linear regression of the corresponding Schild plot yields an average slope of 0.92 ± 0.04 (mean ± SEM, $n = 4$) consistent with competitive antagonism. Constraining the slope to unity generates $pK_b = 7.54 \pm 0.10$, $K_b = 29$ nM ($n = 4$).

(12) Less than 50% inhibition in 74 out of 75 assays was observed. The single exception was the human 5-HT_4 serotonin receptor, in which case the binding affinity of **9** ($K_i = 72$ nM, $n = 2$) was within a 2-fold range of that for human Gal_3. However, when incubated with intact cells expressing the cloned human 5-HT_4 receptor, **9** displayed no 5-HT_4 agonist or antagonist activity at concentrations up to 10 μM.

JM060001N