Supplemental material to this article can be found at:
http://molpharm.aspetjournals.org/content/suppl/2014/11/19/mol.114.095471.DC1.html

1521-0111/87/2/197–206$25.00
MOLECULAR PHARMACOLOGY

http://dx.doi.org/10.1124/mol.114.095471
Mol Pharmacol 87:197–206, February 2015

(4-(Bis(4-Fluorophenyl)Methyl)Piperazin-1-yl)(Cyclohexyl) Methanone Hydrochloride (LDK1229): A New Cannabinoid CB_1 Receptor Inverse Agonist from the Class of Benzhydryl Piperazine Analogs[S]

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Received August 25, 2014; accepted November 19, 2014

ABSTRACT

Some inverse agonists of cannabinoid receptor type 1 (CB_1) have been demonstrated to be anorectic antiobesity drug candidates. However, the first generation of CB_1 inverse agonists, represented by rimonabant (SR141716A), otenabant, and taranabant, are centrally active, with a high level of psychiatric side effects. Hence, the discovery of CB_1 inverse agonists with a chemical scaffold distinct from these holds promise for developing peripherally active CB_1 inverse agonists with fewer side effects. We generated a new CB_1 inverse agonist, (4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)(cyclohexyl)methanone hydrochloride (LDK1229), from the class of benzhydryl piperazine analogs. This compound binds to CB_1 more selectively than cannabinoid receptor type 2, with a K_i value of 220 nM. Comparable CB_1 binding was also observed by analogs 1-[bis(4-fluorophenyl)methyl]-4-cinnamylpiperazine dihydrochloride (LDK1203) and 1-[bis(4-fluorophenyl)methyl]-4-tosylpiperazine hydrochloride (LDK1222), which differed by the substitution on the piperazine ring where the piperazine of LDK1203 and LDK1222 are substituted by an alkyl group and a tosyl group, respectively. LDK1229 exhibits efficacy comparable with SR141716A in antagonizing the basal G protein coupling activity of CB_1, as indicated by a reduction in guanosine 5′-*O*-(3-thio)triphosphate binding. Consistent with inverse agonist behavior, increased cell surface localization of CB_1 upon treatment with LDK1229 was also observed. Although docking and mutational analysis showed that LDK1229 forms similar interactions with the receptor as SR141716A does, the benzhydryl piperazine scaffold is structurally distinct from the first-generation CB_1 inverse agonists. It offers new opportunities for developing novel CB_1 inverse agonists through the optimization of molecular properties, such as the polar surface area and hydrophilicity, to reduce the central activity observed with SR141716A.

Introduction

The cannabinoid receptors are members of the class A superfamily of G protein–coupled receptors (GPCRs). The cannabinoid receptor 1 (CB_1) is present in high abundance throughout the central nervous system (Howlett, 1995) but is also expressed in a number of peripheral tissues, such as the cardiovascular and reproductive systems as well as the gastrointestinal tract (Croci et al., 1998; Batkai et al., 2001; Engeli et al., 2005), and is involved in substance addiction, chronic pain, memory, and metabolic and inflammatory disorders (Howlett et al., 2004; Mackie, 2006; Pertwee, 2006). A second subtype of the cannabinoid receptors, the cannabinoid receptor 2 (CB_2), is predominantly found in immune cells and non-neuronal tissues (Galiegue et al., 1995) and is implicated in a variety of modulatory functions, including immune suppression, induction of apoptosis, and induction of cell migration (Basu and Dittel, 2011).

The CB_1 receptor preferentially couples to the $G_{i/o}$ type of G proteins (Howlett and Fleming, 1984) and has been functionally linked to the inhibition of adenylate cyclase (Slipetz et al., 1995) and the activation of mitogen-activated protein kinases, including extracellular signal-regulated kinase-1 and -2, p38 mitogen-activated protein kinase, and c-Jun *N*-terminal kinase

This work was supported in part by the National Institutes of Health [Grants R01-DA020763, R01-DA003934, and K05-DA021358] and Faculty Development Fund of Texas A&M Health Sciences Center. The authors declare no conflict of interest.
dx.doi.org/10.1124/mol.114.095471.
[S] This article has supplemental material available at molpharm.aspetjournals.org.

ABBREVIATIONS: [^{35}S]GTPγS, guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; CP55,940, 5-(1,1-dimethylheptyl)-2-(5-hydroxy-2-(3-hydroxypropyl)cyclohexyl)phenol; EI, electron impact; GFP, green fluorescent protein; GPCR, G-protein–coupled receptor; GTPγS, guanosine 5′-*O*-(3-thio)triphosphate; HEK293, human embryonic kidney 293 cells; MS, mass; SR141716A, rimonabant; TMH, transmembrane helix; WIN55,212-2, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-*d*,*e*]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone.