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Optimization of Chemical Functionalities of Indole-2-carboxamides To Improve Allosteric Parameters for the Cannabinoid Receptor 1 (CB1)

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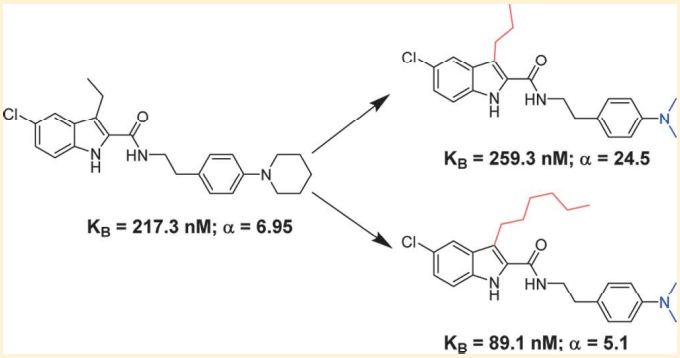
ABSTRACT: 5-Chloro-3-ethyl-*N*-(4-(piperidin-1-yl)-phenethyl)-1*H*-indole-2-carboxamide (**1**; ORG27569) is a prototypical allosteric modulator for the cannabinoid type 1 receptor (CB1). Here, we reveal key structural requirements of indole-2-carboxamides for allosteric modulation of CB1: a critical chain length at the C3-position, an electron withdrawing group at the C5-position, the length of the linker between the amide bond and the phenyl ring B, and the amino substituent on the phenyl ring B. These significantly impact the binding affinity (K_B) and the binding cooperativity (α). A potent CB1 allosteric modulator 5-chloro-*N*-(4-(dimethylamino)phenethyl)-3-propyl-1*H*-indole-2-carboxamide (**12d**) was identified. It exhibited a K_B of 259.3 nM with a strikingly high binding α of 24.5. We also identified 5-chloro-*N*-(4-(dimethylamino)phenethyl)-3-hexyl-1*H*-indole-2-carboxamide (**12f**) with a K_B of 89.1 nM, which is among the lowest K_B values obtained for any allosteric modulator of CB1. These positive allosteric modulators of orthosteric agonist binding nonetheless antagonized the agonist-induced G-protein coupling to the CB1 receptor, yet induced β-arrestin mediated ERK1/2 phosphorylation.



INTRODUCTION

The cannabinoid type 1 (CB1) receptor is the most abundant G-protein coupled receptor (GPCR) expressed in the central nervous system (CNS), where it attenuates the release of excitatory and inhibitory neurotransmitters.[1−3] The CB1 receptor is also present in lower concentrations in a variety of peripheral tissues, including, spleen, tonsil, gastrointestinal tract, liver, kidney, and heart.[4−6] It regulates a variety of physiological functions including neuronal development, neuromodulatory processes, metabolism, nociception, and cardiovascular as well as reproductive functions.[1,7,8] While CB1 preferentially couples to $G_{i/o}$ type G proteins, it can interact with G_s[9] or G_q[10] under some conditions. The CB1 receptor also modulates the activation of mitogen-activated protein kinases (MAPKs),[11] inhibits N- and P/Q-type voltage-gated Ca^{2+} channels, and activates A-type and inwardly rectifying K^+ channels.[12] Moreover, the CB1 receptor can interact with non-G protein partners such as β-arrestins, adaptor protein AP-3, GPCR-associated sorting protein 1 (GASP1), and the adaptor protein FAN to control receptor signaling or trafficking.[13,14] The complex signaling network of the CB1 receptor suggests the existence of finely controlled modulatory mechanisms of receptor functions.

Traditionally, the functions of the CB1 receptor is regulated through various agonists, partial agonists, antagonists, and inverse agonists,[15] which bind to the orthosteric site where the endogenous cannabinoids bind. Recently, several allosteric modulators of the CB1 receptor have been identified, which bind to sites that are topologically distinct from the orthosteric binding site. These include 5-chloro-3-ethyl-*N*-(4-(piperidin-1-yl)phenethyl)-1*H*-indole-2-carboxamide (**1**, ORG27569),[16] 1-(4-chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)-phenyl)urea (PSNCBAM-1),[17] 3-(4-chlorophenyl)-5-(8-methyl-3-*p*-tolyl-8-azabicyclo[3.2.1]octan-2-yl)isoxazole (RTI-371),[18] and the endogenous ligand (5*S*,6*R*,7*E*,9*E*,11*Z*,13*E*,15*S*)-5,6,15-trihydroxyicosa-7,9,11,13-tetraenoic acid (lipoxin A4).[19] Allosteric modulators typically work cooperatively with orthosteric ligands and stabilize the receptor in various biological conformations that may be difficult to achieve by the orthosteric ligands.[20] This increases the possibility of regulating receptor activities in more sophisticated ways than with orthosteric ligands. Thus, allosteric modulation can significantly expand the pharmacological repertoire for a given receptor.[21,22] Additionally, allosteric sites are less structurally conserved than the corresponding orthosteric site and thus provide new opportunities for the development of more selective therapeutics.[8,23]

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