Chromenopyrazole as Versatile Cannabinoid Scaffold: from CB_1 towards CB_2 Selectivity

Paula Morales[1], Maria Gómez-Cañas[2], Dow P. Hurst[3], Moisés Garcia-Arencibia[2], Javier Fernández-Ruiz[2], Patricia Reggio[3], Pilar Goya[1], Nadine Jagerovic[1]. [1]Consejo Superior de Investigaciones Científicas, Instituto de Química Médica, Madrid, Spain, [2]Facultad de Medicina, Universidad Complutense de Madrid, Departamento de Bioquímica y Biología Molecular. Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Ins, Madrid, Spain, [3]Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, USA

Background. A series of chromenopyrazoles has been described by us as non-psychoactive and selective CB_1 cannabinoid agonists with peripheral antinociceptive properties (Cumella J et al, ChemMedChem 7:452, 2012).

Purpose. In an attempt to target the CB_2 type receptor, we propose different structural modifications of the chromenopyrazole scaffold. Structural features required for CB_1/CB_2 affinity and selectivity are explored by molecular modelling. Affinity of new compounds is evaluated using competitive binding assays. Then, compounds with the highest CB_2 affinity profiles were tested in CB2-mediated functional assays using BV2 cultured cells (a mouse microglia cell line).

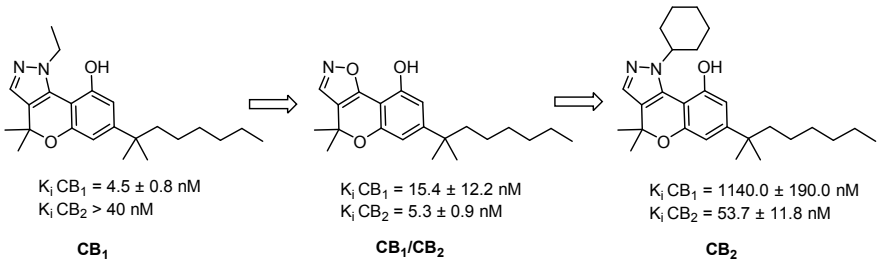
Methods.

Synthesis. The 7-alkyl-1,4-dihydro-4,4-dimethylchromenopyrazol-9-oles were synthesized from the corresponding resorcinol following the procedure described in Cumella J et al. The preparation of the 9-alkoxy-7-alkyl-1,4-dihydro-4,4-dimethylchromenopyrazoles was achieved by alkylation of the corresponding chromenopyrazol-9-oles.

Molecular modelling. Conformational analysis of selected new chromenopyrazoles was first performed to determine the global minimum-energy conformation. Then, they were docked by using models of active state CB_1 (CB_1R^*) and CB_2 (CB_2R^*) receptors (Pei Y et al, Chem Biol 15: 1207, 2008; Hurst DP et al, J Biol Chem 285:17954, 2010) that include extracellular and intracellular loops and N and C terminus.

Pharmacological evaluation. The affinity of the new compounds was evaluated measuring their ability to displace [^{3}H]CP55940 from human cannabinoid CB_1 and CB_2 receptors transfected into HEK293 EBNA cells. The effects of the selected CB_2 chromenopyrazole ligands on lipopolysaccharide (LPS)-induced production of prostaglandin E_2 (PGE_2) were examined in BV2 microglia. The PGE_2 release was measured using an ELISA kit. This CB_2-mediated functional assay was performed on new compounds alone or in combination with WIN 55,212,2 or SR144528. (Oh YT et al, Neurosci Lett 474:148, 2010).

Results. A new library of chromenopyrazoles was synthesized, characterized, and tested at cannabinoid CB_1 and CB_2 receptors by radioligand binding and functional activity assays. Fine-tuning of affinity and selectivity was achieved by structural modifications performed on the scaffold (figure 1). The docking studies using the CB_1 and CB_2 receptors models (CB_1R^* and CB_2R^*) validate the structure activity relationships. The different ligand-receptor interactions provide detailed structural information.



K_i CB_1 = 4.5 ± 0.8 nM
K_i CB_2 > 40 nM

CB_1

K_i CB_1 = 15.4 ± 12.2 nM
K_i CB_2 = 5.3 ± 0.9 nM

CB_1/CB_2

K_i CB_1 = 1140.0 ± 190.0 nM
K_i CB_2 = 53.7 ± 11.8 nM

CB_2

Figure 1. Examples of chromenopyrazoles.

Conclusion. Chromenopyrazoles were found to constitute a versatile scaffold for obtaining potent cannabinoid receptor ligands with selectivity at either CB_1 or CB_2 receptor types.

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