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Abstract

We screened a chemolibrary of drug-like molecules for their ability to activate reactive oxygen species (ROS) production in murine phagocytes, and we identified 26 novel compounds with potent neutrophil activating properties. We used substructure screening, fragment-focusing, and structure-activity relationship analyses to further probe the parent library and defined at least two groups of activators of ROS production in murine neutrophils: t-butyl benzene and thiophene-2-amide-3-carboxylic ester derivatives. Further studies of the active compounds revealed 11 compounds that activated ROS production in human neutrophils, and six of these compounds also activated intercellular Ca2+ mobilization and chemotaxis in human neutrophils. Of the latter compounds, compound 14 (1,3-benzodioxolane-5-carboxylic acid 4′-benzyloxy-3′-methoxybenzylidene-hydrazide) activated neutrophils at nanomolar concentrations, and Ca2+ mobilization was inhibited by pertussis toxin and N-t-butoxycarbonyl-Phe-Leu-Phe-Leu-Phe (Boc-2), an antagonist of formyl peptide receptors (FPR/FPRL1). Likewise, activation by compound 14 was desensitized after N-formyl-Met-Leu-Phe pretreatment. Similar biological activities were found for compound 104 (1,3-benzodioxolane-5-carboxylic acid 3′-bromo-5′-ethoxy-4′-hydroxybenzylidene-hydrazide), an analog of compound 14. Furthermore, conformational analysis of the activators of chemotaxis and Ca2+ mobilization showed a high degree of similarity in distances between pharmacophore points of compounds 14 and 104 with a model of FPR published by Edwards et al. (Mol Pharmacol68:1301–1310, 2005), indicating that conformational features of the agonists identified here are structurally compatible with steric constraints of the ligand-binding pocket of the receptor. Based on these results, we conclude that compounds 14 and 104 represent novel small-molecule agonists of FPR. These studies enhance our understanding of FPR ligand/receptor interactions and structure/activity relationships of phagocyte agonists.