Dual Kinase-Bromodomain Inhibition of Brd4 and P38α using Trisubstituted-Imidazoles

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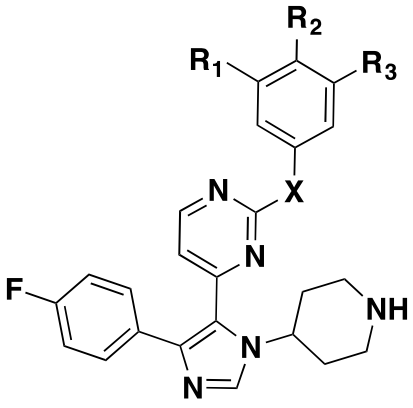
As regulators of transcription, proteins that interpret post-translational modifications to N-terminal histone tails through molecular recognition are known to be essential for maintaining cellular homeostasis. When dysregulated, these 'reader' proteins become drivers of disease. In the case of bromodomains, which recognize N-ε-acetylated-lysine, the development of isoform selective inhibitors has been a significant challenge to the field. Here we present the development of a tri-substituted imidazole scaffold with selectivity for the first bromodomain of Brd4 (Brd4(D1), Kd = 1.2 μM) and potent MAP kinase inhibition (P38α, Kd = 390 pM). Screening of the Published Kinase Inhibitor Set using a protein labelled ^{19}F NMR assay initially identified selectivity of SB-284851-BT for Brd4 over other bromodomains. Further analogs were synthesized and affinity for the Bromodomain and Extra Terminal (BET) family of bromodomains was characterized using a fluorescence anisotropy method to displace fluorescently labeled pan-BET inhibitor, BI-6727. Co-crystal structures of Brd4(D1) with our most potent molecules, 3,4- and 3,5-dimethylphenyl substituted imidazole, confirmed engagement with a conserved asparagine (N140) in the N-ε-acetylated-lysine binding pocket. Further, these molecules inhibited NF-κB signaling (50% @ 10 μM, compared to 20μM for (+)-JQ1), as measured by a luciferase reporter assay, and suppressed production of inflammatory cytokine IL-8 as measured by an immunosorbent assay. The cellular interaction of these molecules with protein targets Brd4 and P38α was verified using a CEllular Thermal Shift Assay (CETSA). In further support of bromodomain inhibition, this series of molecules suppressed production of the c-Myc oncoprotein in multiple myeloma cells. From these studies, we conclude a tri-substituted imidazole scaffold represents a valuable starting point for synergistic therapeutic inhibition of both Brd4 and P38α, the aberrant functions of which play a key role in cancer and inflammatory signaling.



X = O,N
R_1, R_2, R_3 = H, CH_3