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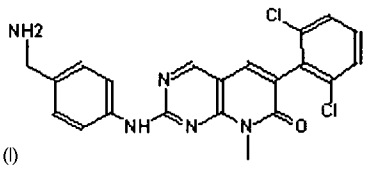
(54) Title: METHODS FOR THE IDENTIFICATION OF ZAP-70 INTERACTING MOLECULES AND FOR THE PURIFICATION OF ZAP-70



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(57) Abstract: The invention provides in a first aspect a method for the identification of an ZAP-70 interacting compound, comprising the steps of a) providing a protein preparation containing phosphorylated ZAP-70, b) contacting the protein preparation with aminopyrido-pyrimidine ligand 24 immobilized on a solid support under conditions allowing the formation of an aminopyrido-pyrimidine ligand 24 - phosphorylated ZAP-70 complex, c) incubating the aminopyrido-pyrimidine ligand 24 — phosphorylated ZAP-70 complex with a given compound, and d) determining whether the compound is able to separate phosphorylated ZAP-70 from the immobilized aminopyrido-pyrimidine ligand 24. In a second aspect, the present invention relates to A method for the identification of an ZAP-70 interacting compound, comprising the steps of a) providing a protein preparation containing phosphorylated ZAP-70, b) contacting the protein preparation with ligand aminopyrido-pyrimidine ligand 24 immobilized on a solid support and with a given compound under conditions allowing the formation of an aminopyrido-pyrimidine ligand 24 - phosphorylated ZAP-70 complex, and c) detecting the aminopyrido-pyrimidine ligand 24 - phosphorylated ZAP-70 complex formed in step b). hi a third aspect, the invention provides a method for the identification of an ZAP-70 interacting compound, comprising the steps of: a) providing two aliquots of a protein preparation containing phosphorylated ZAP-70, b) contacting one aliquot with aminopyrido-pyrimidine ligand 24 immobilized on a solid support under conditions allowing the formation of an aminopyrido-pyrimidine ligand 24 - phosphorylated ZAP-70 complex, c) contacting the other aliquot with aminopyrido-pyrimidine ligand 24 immobilized on a solid support and with a given compound under conditions allowing the formation of an aminopyrido-pyrimidine ligand 24 - phosphorylated ZAP-70 complex, and d) determining the amount of aminopyrido- pyrimidine ligand 24 - phosphorylated ZAP-70 complex formed in steps b) and c). In a forth aspect, the invention relates to a method for the identification of an ZAP-70 interacting compound, comprising the steps of: a) providing two aliquots comprising each at least one cell containing phosphorylated ZAP-70, b) incubating one aliquot with a given compound, c) harvesting the cells of each aliquot, d) lysing the cells in order to obtain protein preparations, e) contacting the protein preparations with aminopyrido-pyrimidine ligand 24 immobilized on a solid support under conditions allowing the formation of an aminopyrido-pyrimidine ligand 24 - phosphorylated ZAP-70 complex, and f) determining the amount of aminopyrido-pyrimidine ligand 24 - phosphorylated ZAP-70 complex formed in each aliquot in step e).

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