Characterization of compound 584, an Abl kinase inhibitor with lasting effects

Miriam Puttini,[1] Sara Redaelli,[1] Loris Moretti,[2] Stefania Brussolo,[3] Rosalind H Gunby,[1] Luca Mologni,[1] Edoardo Marchesi,[4] Loredana Cleris,[4] Arianna Donella-Deana,[5] Peter Drueckes,[6] Elisa Sala,[1] Vittorio Lucchini,[3] Michael Kubbutat,[6] Franca Formelli,[4] Alfonso Zambon,[3] Leonardo Scapozza,[2] and Carlo Gambacorti-Passerini[1,7]

[1]Department of Clinical Medicine, University of Milano-Bicocca-S. Gerardo Hospital, Monza, Italy; [2]Pharmaceutical Biochemistry Group, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland; [3]Department of Environmental Sciences, University of Ca' Foscari, Venezia, Italy; [4]Department of Experimental Oncology, National Cancer Institute, Milan, Italy; [5]Department of Biological Chemistry University of Padova, Padova, Italy; [6]ProQinase GmbH, Freiburg, Germany and [7]Department of Oncology, McGill University, Montreal, Quebec, Canada

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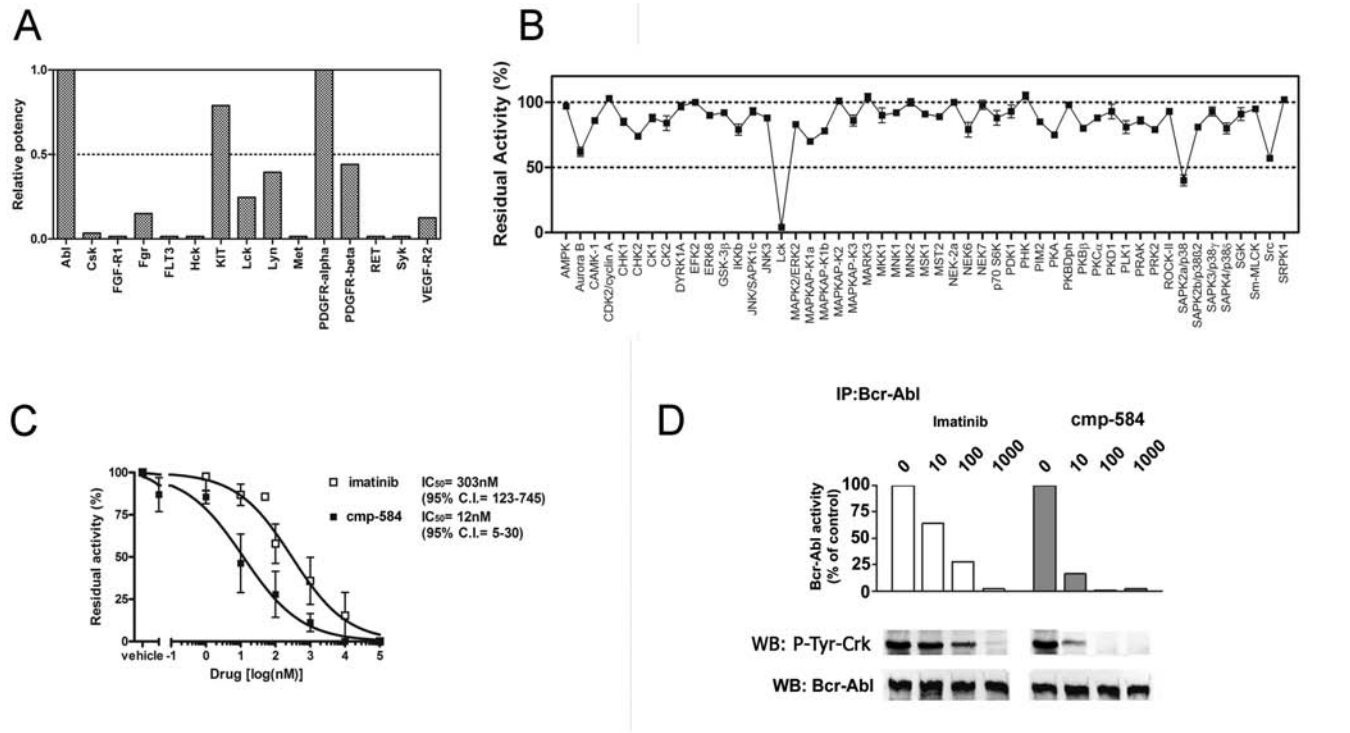

Supplementary Figure S1. In vitro effects of imatinib and cmp-584 on several protein kinases. (A) Relative potency of cmp-584 against a selected panel of tyrosine kinase. IC_{50} values were determined using the 33Pankinase® radioactive kinase assay against purified kinases and are expressed as relative potency, compared to the IC50 value calculated for ABL1 kinase (150 nM), which was arbitrarily set at 1. (B) Residual activity (mean ± SD, % of control) of the listed protein kinases in the presence of 1 μM cmp-584. Kinase activity was measured with a radioactive kinase assay using exogenous peptide as described in the experimental section. (C) 10^7 Lama 84 cells were lysed and Bcr-Abl kinase was immunoprecipitated using anti-Abl antibody. Immunocomplexes were subjected to an *in vitro* radioactive kinase assay using an exogenous Abl peptide in the presence of cmp-584 or imatinib as described in the experimental section. (D) Bcr-Abl-immunocomplexes were assayed in a cold kinase assay using 0.2 μg of purified GST-Crk as a substrate in the presence of the indicated concentrations of cmp-584 in nM. Reaction products were analyzed by western blot with anti-phosphotyrosine antibody and Tyr-phosphorylated Crk bands (P-Tyr-Crk) were quantified by densitometric analysis and expressed as a percentage relative to the untreated control (upper columns of panel D). In parallel, an aliquot of the immunocomplexes was analyzed by western blot with anti-Bcr-Abl antibody as a loading control.