5.5 Fluorescein-conjugated compounds are good imaging vehicles in vitro

5.5.1 Introductory remarks

Compound 13 and 15 (fluorescein-conjugated derivatives of Compound 31) were tested on a human cell panel for their ability to stain cells. In general, the chemical structure of such type of molecules should drive them toward a target-dependent internalization. The RGD scaffold of Compound 13 and 15 was then expected to be able to direct that molecules toward a RGD-binding integrin receptor. Given that both Compounds displayed a good cell-free binding affinity for integrin αvβ3 receptor, cells highly expressing integrin αvβ3 should have been able to efficiently internalize Compounds 13 and 15.

5.5.2 Compounds 13 and 15 positively stained integrin αvβ3 expressing cells

As disclosed in the **Introductory remarks**, the good cell-free binding affinity for integrin αvβ3 displayed by the fluorescein-conjugated Compounds 13 and 15 prompted us to test their possible use as tracers of cells expressing integrin αvβ3. Both molecules were synthesized starting from Compound 31, by a conjugation of fluorescein with the heteroalkyl side chain of the RGD scaffold.

In particular, fluorescein detection was measured in HUVEC, ECV-304, T98G, H460, PC-3 and Caki-1 cells. MDA-MB-231 breast adenocarcinoma cells were included as a negative control, given the results of flow cytometry mentioned in **paragraph 5.1.2**. In fact, these cells expressed almost exclusively integrin α5β1. H460 were rapidly excluded from the screening because of their *in vitro* growth characteristics. These cells tended in fact to form clumps in culture and it was really difficult to appreciate the staining given by fluorescein-conjugated compounds.

All cell lines were exposed to scalar concentrations of Compound 13 and 15 for different incubation times. A 4 hours incubation with 10 μM of each Compound was determined to be the best treatment condition, giving a good detection threshold for the fluorescein signal in vascular endothelial cells and cancer-derived cells expressing integrin αvβ3. Overall, Compound 13 gave a signal stronger than that of Compound 15. Each Compound displayed a fluorescence distributed both at the cell surface and in putative cytosolic vesicles, suggesting that both Compounds were internalized upon binding with integrin αvβ3. Moreover, the morphological analysis of cells showed no signs of toxicity or stress after 4 or 24 hours incubation with 10 μM of either Compound 13 or 15. Notably, breast adenocarcinoma cells subjected to the treatment previously described did not show any sign of fluorescence, suggesting that both compounds were highly specific for integrin αvβ3 (**Figure 24**).

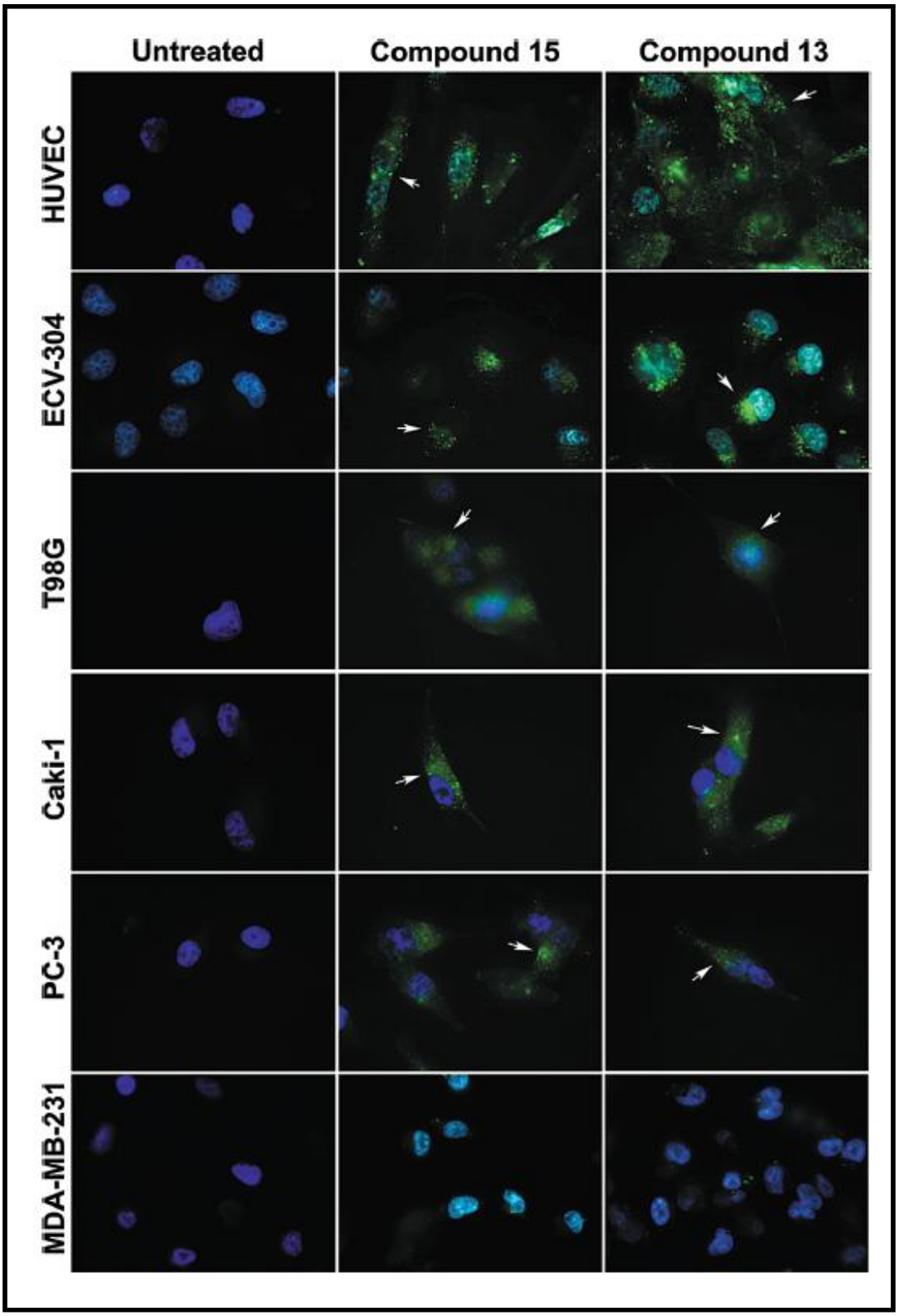

Figure 24. *Biological activity of Compounds 15 or 13 on the cell panel. HUVEC, ECV-304, T98G, Caki-1, PC3, and MDA-MB-231 cells were incubated 4 hours with treatment medium alone (left column), 10 µM Compound 15 (middle column, green), or 13 (right column, green). Nuclei were counterstained with DAPI (blue). The signal was highly detectable and located both at the cell surface and in putative cystosolic vesicles (arrows). Original magnification: 63x.*