

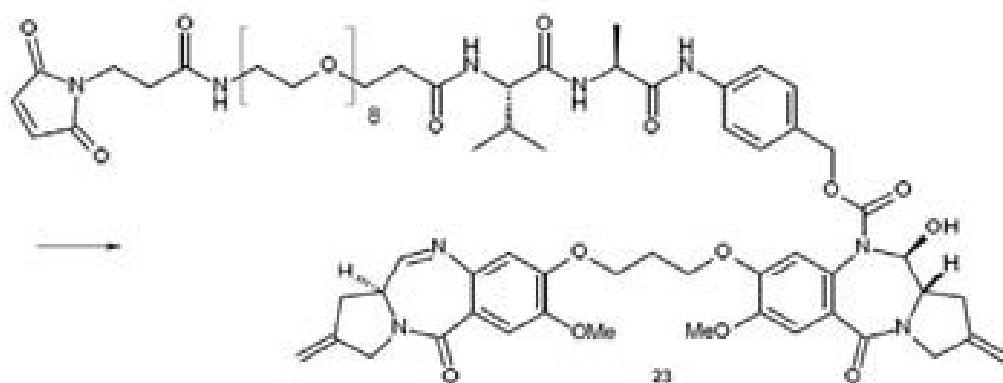
Annex 1

ADC cytotoxicity method MTS

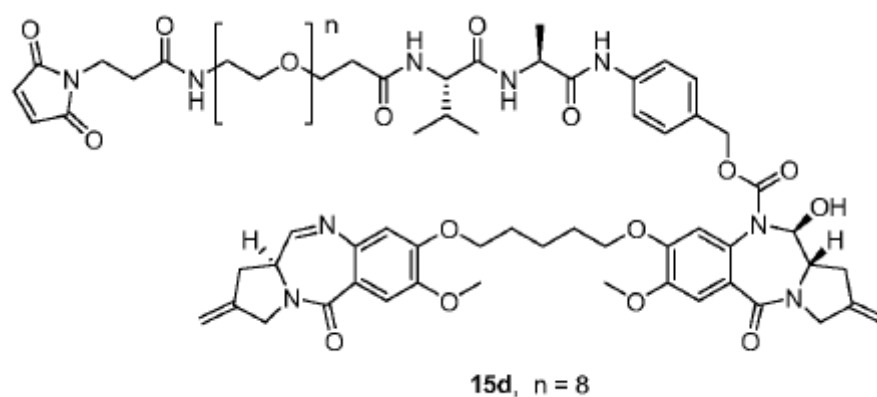
Two compounds were tested - comparative compound 1 and compound 23 of the present application. These compounds were tested in NCI-N87 cell lines (human gastric epithelial cell lines).

Compounds tested

Compound 23 of the present application is:



Comparative compound 1 is:



Comparative compound 1 is compound 15d from **D1**.

The compounds differ in that comparative compound 1 has a pentylene bridge whereas compound 23 has a propylene bridge.

Methods

The same method as Example 5 of the present application was followed.

Cells were diluted to 2×10^5 /ml, dispensed (50 μ l per well) into 96-well flat-bottom plates.

A stock solution (1 ml) of antibody drug conjugate (ADC) (20 μ g/ml) was made by dilution of filter-sterilised ADC into cell culture medium. A set of 8x 10-fold dilutions of stock ADC were made in a 24-well plate by serial transfer of 100 μ l into 900 μ l of cell culture medium. ADC dilution was dispensed (50 μ l per well) into 4 replicate wells of the 96-well plate, containing 50 μ l cell suspension seeded the previously. Control wells received 50 μ l cell culture medium. The 96-well plate containing cells and ADCs was incubated at 37°C in a CO₂-gassed incubator for the exposure time.

At the end of the incubation period, cell viability was measured by MTS assay. MTS (Promega) was dispensed (20 μ l per well) into each well and incubated for 4 hours at 37°C in the CO₂-gassed incubator. Well absorbance was measured at 490 nm. Percentage cell survival was calculated from the mean absorbance in the 4 ADC-treated wells compared to the mean absorbance in the 4 control untreated wells (100%).

Results

The EC₅₀ value of Herceptin-MAIA-comparative compound 1 of **D1** in Her2-expressing cell line NCI-N87 is 0.0006377.

The EC₅₀ value of Herceptin-MAIA-compound 23 of the **invention** in Her2-expressing cell line NCI-N87 is 0.009554.