

[Table 35]

Example No.	MFE296 (IC ₅₀ (nM))
Example 2	10.4
Example 21	8.8
Example 22	12.2

[0387] 12. MFE280 growth inhibition assay

In this assay, the growth inhibitory activity of a test substance in a human endometrial cancer cell line expressing S252W-variant FGFR2 is measured.

[0388] It has been reported that a human endometrial cancer cell line MFE280 (DMSZ Number ACC-410) expresses S252W-variant FGFR2 (Proc Natl Acad Sci USA, 2008, 105:8713-8717). ~~SW780~~-MFE280 cells were maintenance-cultured in a 5% CO₂ incubator (37°C) by using an RPMI-1640 (Wako Pure Chemical Industries, Ltd., 187-02021) medium containing 10% FBS, penicillin and streptomycin (Wako Pure Chemical Industries, Ltd., 168-23191). To each well of a 96 well plate (Becton, Dickinson and Company, 35-3075), 150 µL of an MFE280 cell suspension adjusted to a concentration of 3.3 x 10⁴ cells/mL by using an RPMI-1640 medium containing 10% FBS, penicillin and streptomycin was added, and the cells were cultured overnight in a 5% CO₂ incubator (37°C). On the next day, 50 µL of a test substance diluted with an RPMI-1640 medium containing 10% FBS, penicillin and streptomycin was added to each well, and the resultant was cultured for 3 days in a 5% CO₂ incubator (37°C). Ten µL of Cell Counting Kit-8 (Dojindo Laboratories, CK04) was added to each well, and the resultant was cultured for 1 to 2 hours in a 5% CO₂ incubator (37°C) to cause a color reaction. ENVISION (TM) (PerkinElmer Co., Ltd.) was used for measuring absorbance at 450 nm. Assuming that the absorbance attained in a well containing the cells but not containing the test substance was 100% and that the absorbance attained in a well containing no cells was 0%, an absorbance ratio attained in the presence of the test substance was obtained. The concentration of the test substance necessary for inhibiting the cell growth by 50% (i.e., an IC₅₀ value) was calculated, and IC₅₀ values of respective test substances thus calculated are shown in Table 36.

[0389] <Data of evaluation of MFE280 growth inhibitory action>

[Table 36]