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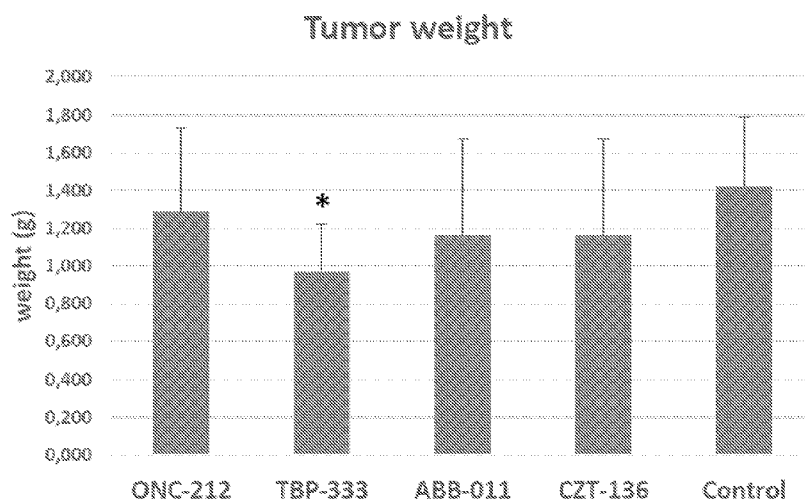
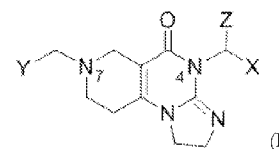


Figure 64



(57) Abstract: The present invention relates to compounds of formula (I) (I) or pharmaceutically acceptable salts, and stereoisomers thereof, including enantiomers, racemic mixtures, mixtures of enantiomers, or combinations thereof, which are applicable for use in treating cancer diseases. The present invention further relates to a pharmaceutical composition comprising the above compounds.

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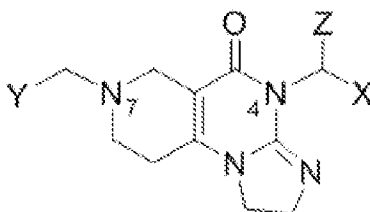
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**SYNTHESIS OF NOVEL IMIPRIDONE DERIVATIVES AND THEIR EVALUATION FOR THEIR
ANTICANCER ACTIVITY**

FIELD OF THE INVENTION

The present invention relates to compounds of formula **(I)**

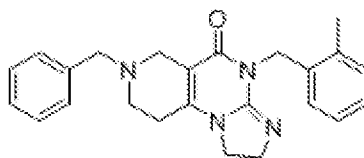


(I)

or pharmaceutically acceptable salts and stereoisomers thereof, including single enantiomers, racemic mixtures, mixtures of enantiomers, or combinations thereof, which are applicable for use in treating cancer diseases. The present invention further relates to a pharmaceutical composition comprising the above compounds.

BACKGROUND OF THE INVENTION

Imipridones, a first-in-class small molecule anti-cancer compounds, comprising an angular tricyclic heterocyclic framework of enhanced basicity with two skeletal nitrogen atoms carrying substituents [cf. general structure represented by formula **(I)**]. This well-defined angularly condensed skeletal structure, comprising suitably positioned ideal number of basic centers and lactam moiety with pending tunable aromatic rings as evenly distributed potential binding sites, render multitargeting character and ideal drug properties to imipridones being essential to their distinct mechanisms of action. Specific G protein-coupled receptors (GPCRs) controlling critical signaling pathways in cancer cells are important targets of ONC201 (Reference Compound 1), the first imipridone implicated in clinical development directly antagonizing a GPCR called DRD2 dopamine receptor [1].



ONC 201

Reference Compound 1

This molecule has also emerged as an efficient activator of the proapoptotic protein tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its receptors with a wide

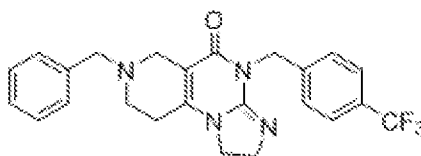
therapeutic index [2-4]. *Kline et al.* disclosed that ONC201 triggered dual inhibition of AKT and extracellular signal-regulated kinase (ERK) pathways in a number of malignant cell lines (e.g. HCT-116, HEPG-2, MCF-7, and MDA-MB-468) and demonstrated that, besides apoptosis measured by sub-G1 fraction and caspase activation, ONC201 also induced cell cycle arrest in the cell lines which were tested as early as 24 h after treatment [2].

The forgoing cited references [1-4] regard to only ONC201, and do not disclose other imipridone derivatives.

By means of bromodeoxyuridine (BrdU) labelling experiments the authors also confirmed that the proliferation of the cells was inhibited by ONC201 and, as a response to the treatment with this imipridone, the early cell cycle arrest caused a significant decrease in a number of viable cells within 48 h, even including those (e.g. A-549 and SNV-449) that did not undergo apoptosis [2]. Preclinical studies have demonstrated its potency as an exceptionally promising apoptotic anticancer agent having pronounced activity against a large variety of cancer cell lines (including e.g. PANC-1, HCT116, MDA-MB-23, U87, HFF, MRC5 and WI-38) [5-8]. Moreover, in phase II clinical trials this compound has proved to be beneficial in the treatment of patients with a wide range of advanced malignances [9].

With respect to the above mentioned references [5-9] it can also be stated that they relate to only ONC201 and do not disclose other imipridone derivatives. Besides the ONC201, *Wagner et al.* discloses an isomer of ONC201, which isomer has a linear [4,3-d] structure, therefore said isomer is not covered by the present invention [5]. *Zhe-Zhu Jin et al.* disclose the uses of ONC201 in combination with AZD-8055, which is a pyrido[2,3-d]pyrimidine derivative, therefore it does not covered by the present invention [7].

An intense search for analogues [10] identified a trifluoromethylated derivative named as ONC212 (Reference Compound 2) as a more potent imipridone with intensified and selective involvement in GPCR targeting and in tumor cell death.



ONC 212

Reference Compound 2

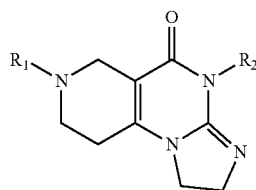
This compound produced significantly enhanced activity at nanomolar concentrations against a number of different malignant cell lines, solid tumours, and hematological malignancies [10]. The cited reference [10] does not disclose such ONC201 derivatives, which has substituted benzyl group on the N-7 position.

It is also of importance that ONC212 showed improved preclinical efficacy on pancreatic cancer, melanoma, and hepatocellular carcinoma in a few *in vivo* models including ONC201-resistant tumours, e.g. PANC-1 and Capan-2 human pancreatic cancer xenograft models [11]. Said reference [11] regards to only ONC201 and its derivatives ONC212 and does not disclose other imipridones. *Graves et al.* demonstrated that ONC201 and some related analogues are highly potent activators of ClpP [12]. The cited reference [12] does not disclose ONC201 derivatives having di- or trisubstituted benzyl group on the N-7 position.

Since convincing preclinical evidences have been disclosed about the interplay between TRAIL and redox signalling pathways implicated in cancer [17], the inventors of the present invention in an earlier work [21] attempted to identify antiproliferative imipridones with ferrocene-containing substituents capable of generating reactive oxygen species (ROS) such as nitric oxide, superoxide anion and other forms of free radicals [18, 19] that have been shown to be involved in biological regulatory processes leading to programmed cell death (apoptosis) [20]. Based on the reference [21], starting from ferrocene-containing primary amines and a selection of benzylamines a small library of ferrocene-containing derivatives and purely organic analogues were synthesized and tested, including ONC201 and ONC212 to serve as reference models without the potential of ROS-generation [21]. The results of the *in vitro* antiproliferative tests indicate that – although the organometallic imipridones, particularly the compounds with two ferrocene units (e.g. **7de**), display marked cytotoxicity on human malignant cell lines HT-29, HEPG2, PANC1, COLO205, A2058 and EBC1, comparable to that of ONC201 – their effects are substantially smaller than those exerted by ONC212. On the other hand, the effects of organic imipridones **7ah** and **7ai** proved to be comparable to those of ONC212 [21].

U.S. patent No. *US 10,239,877* discloses a new class of 4,7-benzyl-substituted imipridone derivatives, including the lead compound known as ONC201 and some another substituted analogues thereof. The disclosed compounds are potent TRAIL inducers which can be used in cancer therapy.

U.S. patent No. *US 9,376,437* discloses new substituted imipridone derivatives of the following formula (Reference Formula 1)



(Reference Formula 1),

wherein R_1 and R_2 independently represent hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxyacetyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals, and wherein when R_1 represents $\text{CH}_2\text{-Ph}$, R_2 does not represent $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$. In the preferred compounds

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{-(2-Cl-Ph)}$,

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{-(2-thienyl)}$,

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{CH}_2\text{-Ph}$,

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{CH}_2\text{-(4-N-benzyl-piperazine)}$,

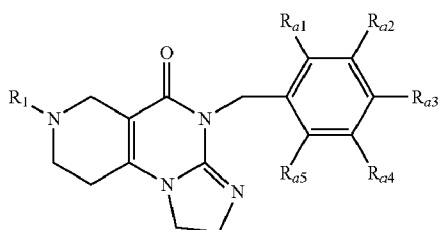
R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{-(2,4-di-F-Ph)}$,

R_1 is H and R_2 is $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$,

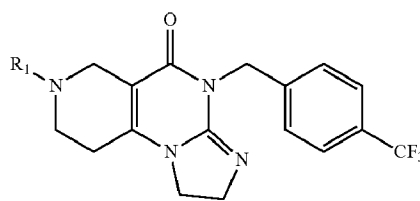
R_1 is CH_3 and R_2 is $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$,

R_1 is $\text{CH}_2\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$.

U.S. patent No. *US 9,845,324* discloses other new substituted imipridone derivatives of above Reference Formula 1, wherein R_1 is selected from the group consisting of H, alkyl, alkylphenyl, alkylphenylketone, benzyl piperazine, alkylthienyl, alkylpyridinyl, alkylisoxazolidinyl, alkylmorpholinyl, alkylthiazolyl and alkylpyrazinyl, wherein alkyl, alkylphenyl, alkylphenylketone, benzyl piperazine, alkylthienyl, alkylpyridinyl, alkylisoxazolidinyl, alkylmorpholinyl, alkylthiazolyl and alkylpyrazinyl are optionally substituted with alkyl, alkoxy, hydroxyl, perhalogenated alkyl or halogen, and wherein R_2 is a substituted or an unsubstituted heterocycloalkylalkyl, preferably a morpholinoalkyl or a piperazinylalkyl group, or wherein R_2 is a substituted heteroarylalkyl, preferably a pyridylalkyl or isoxazolidinylalkyl group. Furthermore, the cited document discloses the compounds of Reference Formula 2 and Reference Formula 3, as defined in the cited document:



(Reference Formula 2)



(Reference Formula 3)

U.S. patent application No. *US 2016/0264574* discloses new substituted imipridone derivatives of above Reference Formula 1, wherein R_1 and R_2 independently represent hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxyacetyl, aralkoxy, aralkylthio, alkanoyl,

mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals, and wherein when R_1 represents $\text{CH}_2\text{-Ph}$, R_2 does not represent $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$. In the preferred compounds

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{-(2-Cl-Ph)}$,

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{-(2-thienyl)}$,

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{CH}_2\text{-Ph}$,

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{CH}_2\text{-(4-N-benzyl-piperazine)}$,

R_1 is $\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{-(2,4-di-F-Ph)}$,

R_1 is H and R_2 is $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$,

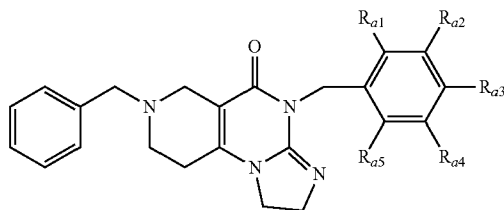
R_1 is CH_3 and R_2 is $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$,

R_1 is $\text{CH}_2\text{CH}_2\text{-Ph}$ and R_2 is $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$,

R_1 is $\text{CH}_2\text{CH}_2\text{NHCOOC(CH}_3\text{)}_3$ and R_2 is $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$,

R_1 is $\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ and R_2 is $\text{CH}_2\text{-(2-CH}_3\text{-Ph)}$.

U.S patent No. *US 10,266,533* discloses substituted imipridone derivatives of above formulae (Reference Formula 1, Reference Formula 2 and Reference Formula 3), where in the Reference Formula 1 R_1 and R_2 are independently selected from the group consisting of H, alkyl, alkylphenyl, alkylphenylketone, benzyl piperazine, alkylthienyl, alkylpyridinyl, alkylisoxazolidinyl, alkylmorpholinyl, alkylthiazolyl and alkylpyrazinyl, wherein alkyl, alkylphenyl, alkylphenylketone, benzyl piperazine, alkylthienyl, alkylpyridinyl, alkylisoxazolidinyl, alkylmorpholinyl, alkylthiazolyl and alkylpyrazinyl are optionally substituted with alkyl, alkoxy, hydroxyl, perhalogenated alkyl or halogen, and wherein R_2 is a substituted or an unsubstituted heteroarylalkyl; or where in the Reference Formula 2 R_1 is a hydrogen; and R_{a1} , R_{a2} , R_{a3} , R_{a4} and R_{a5} are each independently selected from the group consisting of hydrogen, X, $-\text{CH}_3$, $-\text{NO}_2$, $-\text{OCH}_3$, $-\text{CN}$, $-\text{CXH}_2$, $-\text{CX}_2\text{H}$, $\text{C}_2\text{-C}_4$ alkyl, $-\text{CX}_3$, $-\text{CH}_2(\text{CX}_3)$, $-\text{CH}(\text{CX}_3)_2$, $-\text{C}(\text{CX}_3)_3$, $-\text{C}_p\text{X}_{2p+1}$, $-\text{OCX}_3$, $-\text{OC}_p\text{H}_{2p+1}$, $-\text{OC}_p\text{X}_{2p+1}$, OR_m , SR_m , NR_mR_n , $\text{NR}_m\text{C(O)R}_n$, SOR_m , SO_2R_m , C(O)R_m and C(O)OR_m ; where R_m and R_n are independently selected from hydrogen or a $\text{C}_1\text{-C}_4$ alkyl; and X represents a halogen; or derivatives of Reference Formula 4



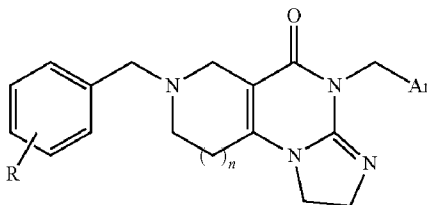
(Reference Formula 4)

wherein R_{a1} , R_{a4} and R_{a5} are each hydrogen; and R_{a2} and R_{a1} are each chlorine, or

R_{a1} , R_{a3} and R_{a5} are each hydrogen; and wherein R_{a2} and R_{a4} are fluorine, or
 R_{a2} , R_{a4} and R_{a5} are each hydrogen; and wherein R_{a1} and R_{a3} are chlorine, or
 R_{a2} , R_{a4} and R_{a5} are each hydrogen; and wherein R_{a1} is a methyl and R_{a3} is a fluorine, or
 R_{a2} , R_{a4} and R_{a5} are each hydrogen; and wherein R_{a1} is a fluorine and R_{a3} is a CF_3 .

U.S. patent No. *US 2019/0194201* is a divisional application of U.S. patent No. *US 10,266,533* and discloses essentially the same substituted imipridone derivatives as its parent application.

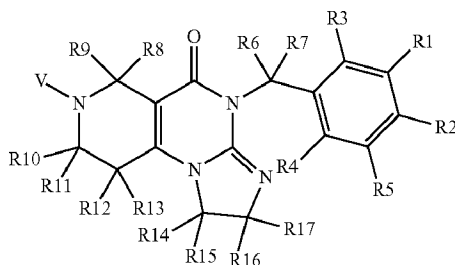
U.S. patent No. *US 2018/0141946* discloses imidazole pyrimidine ketones of Reference Formula 5



(Reference Formula 5)

wherein $n=0$ or 1 ; R is selected from the group consisting of H , mono- or multi halos, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, halo-substituted C_1 - C_6 alkyl, hetero substituent such as nitrogen or oxygen and six-membered heterocyclic ring with zero, one or two hetero atom substitutions; Ar is selected from the group consisting of mono- or di-substituted aryl groups, with at least one substituent selected from the group consisting of halogen, C_1 - C_6 alkyl and halo-substituted C_1 - C_4 alkyl group; and wherein when $n=1$ and R is H , Ar is not phenyl, 2-chlorophenyl, 2,4-difluorophenyl, or o-methyl-phenyl. The preferred meaning of R is F , Cl , Br , methyl, isobutyl, methoxy, trifluoromethyl, morpholinyl or piperazinyl group. The cited document discloses the preparation and efficacy data of 58 specific compounds.

International patent application No. *WO 2018/031987* discloses further substituted 4,7-di-benzyl- and 4-benzyl-7-(thiophenyl-methyl)-imipridone of formula (Reference Formula 6)



(Reference Formula 6)

wherein V means substituted benzyl, (thiophen-2-yl)-methyl or (thiophen-3-yl)-methyl group, and the other substituents are as defined in the cited document. The application includes the preparation and efficacy data of 27 specific compounds.

U.S. patent application No. *US 2018/016277* relates to novel deuterated imidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one compounds, such as ONC201 and analogues. This document also discloses compositions comprising a compound of the cited document and the use of such compositions alone or in combination with other therapeutics in the treatment of diseases and conditions that are beneficially treated by administering an inducer of the gene encoding tumor necrosis factor (TNF) related apoptosis-inducing ligand (TRAIL) superfamily member 10. Further, the cited patent documents disclose the use of imipridone derivatives in cancer therapies. So, the use of compound ONC201 in the treatment of genitourinary cancer is disclosed in U.S. patent No. *US10,456,402*.

U.S. patent No. *US 9,688,679* discloses the use of ONC 201 in the treatment of leukemia.

International patent application No. *WO2017/132661* discloses the use of a compound of above mentioned Reference Formula 1 for treating or preventing a disease, disorder, or condition in a subject in need of selective modulation of the activity of a Class A G protein-coupled receptor (GPCR) or a Class A GPCR signaling pathway, in particular, for treating the cancer is selected from the group consisting of a central nervous system tumor, a brain tumor, a peripheral nervous system tumor, a pheochromocytoma, a paraganglioma, a neuroendocrine tumor, a pancreatic cancer, a prostate cancer, an endometrial cancer, a hematological malignancy, and a lymphatic system tumor. The preferred compounds are ONC 201, 206, 212, 2013 and 236.

U.S. patent No. *US 10,172,862* and No. *US 10,369,154* disclose the use of compound ONC201 treatment of midline gliomas.

THE PROBLEM TO BE SOLVED BY THE INVENTION

There is a need for novel compounds, which has enhanced anticancer activity as compared to the state of the art, which property makes these compounds suitable for use in medicine.

THE DISCOVERY ACCORDING TO THE PRESENT INVENTION

By means of a close inspection of the structural features of the residual members of imipridones, we have discovered that novel imipridone derivatives, which contain 3,5-disubstituted benzyl group attached to position 7, related to the present invention, have enhanced anticancer activity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: IC₅₀ curves created based on the results of *in vitro* test performed on PC3 human prostate cancer cell line.

Figure 2: IC₅₀ curves created based on the results of *in vitro* test performed on LNCap human prostate cancer cell line.

Figure 3: IC₅₀ curves created based on the results of *in vitro* test performed on BxPC3 pancreatic cancer cell line.

Figure 4: IC₅₀ curves created based on the results of *in vitro* test performed on MiaPaCa2 pancreatic cancer cell line.

Figure 5: IC₅₀ curves created based on the results of *in vitro* test performed on Panc1 pancreatic cancer cell line.

Figure 6: IC₅₀ curves created based on the results of *in vitro* test performed on A549 lung carcinoma cell line.

Figure 7: IC₅₀ curves created based on the results of *in vitro* test performed on HCC827 lung carcinoma cell line.

Figure 8: IC₅₀ curves created based on the results of *in vitro* test performed on H1993 lung carcinoma cell line.

Figure 9: IC₅₀ curves created based on the results of *in vitro* test performed on H520 lung carcinoma cell line.

Figure 10: IC₅₀ curves created based on the results of *in vitro* test performed on MDA-MB-453 breast cancer cell line.

Figure 11: IC₅₀ curves created based on the results of *in vitro* test performed on MDA-MB-231 breast cancer cell line.

Figure 12: Dose-response curve obtained by compound I/1 (ONC 212) (reference) on DU 145, LNCaP and PC-3 cell lines.

Figure 13: Dose-response curve obtained by compound I/7 (ABB-011) on DU 145, LNCaP and PC-3 cell lines.

Figure 14: Dose-response curve obtained by compound I/3 (CZT-021) (reference) on DU 145, LNCaP and PC-3 cell lines.

Figure 15: Dose-response curve obtained by compound I/124 (TBP-333) on DU 145, LNCaP and PC-3 cell lines.

Figure 16: Dose-response curve obtained by compound I/6 (TBP-218) (reference) on DU 145, LNCaP and PC-3 cell lines.

Figure 17: Dose-response curve obtained by compound I/111 (TBP-272) on DU 145, LNCaP and PC-3 cell lines.

Figure 18: Dose-response curve obtained by compound I/149 (TBP-353) (reference) on DU 145, LNCaP and PC-3 cell lines.

Figure 19: Dose-response curve obtained by compound I/133 (TBP-400) on DU 145, LNCaP and PC-3 cell lines.

Figure 20: Dose-response curve obtained by compound I/30 (TBP-301) on DU 145, LNCaP and PC-3 cell lines.

Figure 21: Dose-response curve obtained by compound I/107 (CZT-136) on DU 145, LNCaP and PC-3 cell lines.

Figure 22: Dose-response curve obtained by compound I/1 (ONC 212) (reference) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 23: Dose-response curve obtained by compound I/7 (ABB-011) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 24: Dose-response curve obtained by compound I/3 (CZT-021) (reference) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 25: Dose-response curve obtained by compound I/124 (TBP-333) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 26: Dose-response curve obtained by compound I/6 (TBP-218) (reference) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 27: Dose-response curve obtained by compound I/111 (TBP-272) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 28: Dose-response curve obtained by compound I/149 (TBP-353) (reference) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 29: Dose-response curve obtained by compound I/133 (TBP-400) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 30: Dose-response curve obtained by compound I/30 (TBP-301) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 31: Dose-response curve obtained by compound I/107 (CZT-136) on Panc-1, Capan-1 and MIA PaCa-2 cell lines.

Figure 32: Dose-response curve obtained by compound I/1 (ONC 212) (reference) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 33: Dose-response curve obtained by compound I/7 (ABB-011) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 34: Dose-response curve obtained by compound I/3 (CZT-021) (reference) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 35: Dose-response curve obtained by compound I/124 (TBP-333) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 36: Dose-response curve obtained by compound I/6 (TBP-218) (reference) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 37: Dose-response curve obtained by compound I/111 (TBP-272) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 38: Dose-response curve obtained by compound I/149 (TBP-353) (reference) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 39: Dose-response curve obtained by compound I/133 (TBP-400) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 40: Dose-response curve obtained by compound I/30 (TBP-301) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 41: Dose-response curve obtained by compound I/107 (CZT-136) on Detroit 562, SCC-25 and Fadu cell lines.

Figure 42: Dose-response curve obtained by compound I/1 (ONC 212) (reference) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 43: Dose-response curve obtained by compound I/7 (ABB-011) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 44: Dose-response curve obtained by compound I/3 (CZT-021) (reference) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 45: Dose-response curve obtained by compound I/124 (TBP-333) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 46: Dose-response curve obtained by compound I/6 (TBP-218) (reference) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 47: Dose-response curve obtained by compound I/111 (TBP-272) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 48: Dose-response curve obtained by compound I/149 (TBP-353) (reference) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 49: Dose-response curve obtained by compound I/133 (TBP-400) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 50: Dose-response curve obtained by compound I/30 (TBP-301) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 51: Dose-response curve obtained by compound I/107 (CZT-136) on EBC-1, MDA-MB-231 and MDA-MB-453 cell lines.

Figure 52: Cytotoxic effect of compound I/1 (ONC 212) (reference) on Panc-1 cell line.

Figure 53: Cytotoxic effect of compound I/3 (CZT-021) (reference) on Panc-1 cell line.

Figure 54: Cytotoxic effect of compound I/6 (TBP-218) (reference) on Panc-1 cell line.

Figure 55: Cytotoxic effect of compound I/149 (TBP-353) (reference) on Panc-1 cell line.

Figure 56: Cytotoxic effect of compound I/30 (TBP-301) on Panc-1 cell line.

Figure 57: Cytotoxic effect of compound I/7 (ABB-011) on Panc-1 cell line.

Figure 58: Cytotoxic effect of compound I/124 (TBP-333) on Panc-1 cell line.

Figure 59: Cytotoxic effect of compound I/111 (TBP-272) on Panc-1 cell line.

Figure 60: Cytotoxic effect of compound I/133 (TBP-400) on Panc-1 cell line.

Figure 61: Cytotoxic effect of compound I/107 (CZT-136) on Panc-1 cell line.

Figure 62: Change of the animal weight in the experiment disclosed in Example 23.

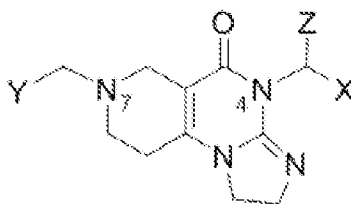
Figure 63: Change of tumor volumes [*: $p < 0.05$ (Student's t-test)] in the experiment disclosed in Example 23.

Figure 64: Tumor size after termination (weight) [*: $p < 0.05$ (Student's t-test)] in the experiment disclosed in Example 23.

Figure 65: Tumors after termination in the experiment disclosed in Example 23.

BRIEF DESCRIPTION OF THE INVENTION

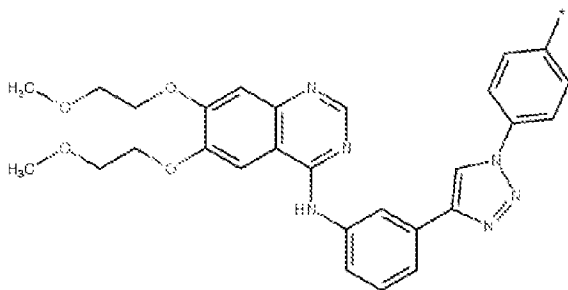
1. Compounds of formula (I)



(I)

wherein

if **Y is phenyl and Z is H**, then X is 3-fluorophenyl-methyl (Compound I/44), 3-(aminomethyl)phenyl (Compound I/58), 4-(aminomethyl)phenyl (Compound I/62), 3-azetidiny (Compound I/90), 4-piperidiny (Compound I/96), 3-azidophenyl (Compound I/102), 4-azidophenyl (Compound I/104), (4-ferrocenyl-1H-1,2,3-triazol-1-yl)phenyl (Compound I/121) or:



(Compound I/123); or

if Y is 3-fluorophenyl and Z is H, then X is 2-iodoferrocenyl (Compound I/52), 3-(aminomethyl)phenyl (Compound I/60), 4-(aminomethyl)phenyl (Compound I/63), 3-azidophenyl (Compound I/105) or 4-azidophenyl (Compound I/106); or

if Y is 3,5-difluorophenyl and Z is H, then X is 3-(aminomethyl)phenyl (Compound I/61), 4-(aminomethyl)phenyl (Compound I/64), 4-azidophenyl (Compound I/107), 3-azidophenyl (Compound I/127), (4-ferrocenyl-1H-1,2,3-triazol-1-yl)phenyl (Compound I/132); or

if Y is 4-(trifluoromethyl)phenyl and Z is H, then X is or 3-(aminomethyl)phenyl (Compound I/68); or

if Y is 3-fluoro-4-(trifluoromethyl)phenyl and Z is H, then X is 3-(aminomethyl)phenyl (Compound I/73); or

if Y is 3-fluorophenylmethyl and Z is H, then X is 2-methylphenyl (Compound I/45); or

if Y is 3-(aminomethyl)phenyl and Z is H, then X is 4-iodophenyl (Compound I/59), ferrocenyl (Compound I/67), 4-(trifluoromethyl)phenyl (Compound I/69) or ferrocenylmethyl (Compound I/70); or

if Y is ferrocenyl and Z is H, then X is 3-(aminomethyl)phenyl (Compound I/65); or

if Y is ferrocenylmethyl and Z is H, then X is 3-(aminomethyl)phenyl (Compound I/66); or

if Y is 3-(methoxycarbonylaminomethyl)phenyl and Z is H, then X is ferrocenylmethyl (Compound I/71) or ferrocenyl (Compound I/72); or

if Y is 4-aminophenyl and Z is H, then X is 3-azetidiny (Compound I/91) or 4-piperidiny (Compound I/93); or

if Y is 3-azetidiny and Z is H, then X is 2-methylphenyl (Compound I/92); or

if Y is 4-piperidiny and Z is H, then X is 2-methylphenyl (Compound I/94) or 4-fluorophenyl (Compound I/95); or

if Y is 3-pyrrolidiny and Z is H, then X is 2-methylphenyl (Compound I/97) or 4-fluorophenyl (Compound I/98); or

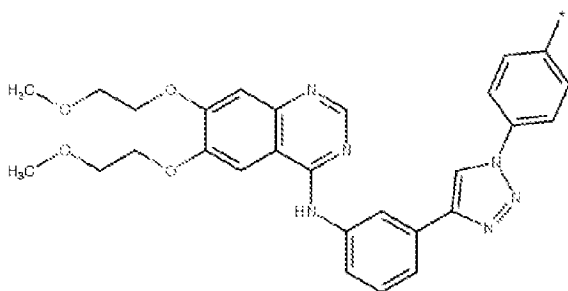
if Y is 2-pyrrolidiny and Z is H, then X is 2-methylphenyl (Compound I/99) or 4-fluorophenyl (Compound I/100); or

if **Y is 4-azidophenyl and Z is H**, then X is 2-methylphenyl (Compound I/101); 3-fluorophenyl (Compound I/108), 4-fluorophenyl (Compound I/109), 4-(trifluoromethyl)phenyl (Compound I/110), 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/114), 4-iodophenyl (Compound I/115), 3,4,5-trimethoxy-phenyl (Compound I/116), 4-azidophenyl (Compound I/117), 3-azetidiny (Compound I/118) or 4-piperidiny (Compound I/119); or

if **Y is 3-azidophenyl and Z is H**, then X is 2-methylphenyl (Compound I/103), 4-(trifluoromethyl)phenyl (Compound I/111), 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/112), 4-iodophenyl (Compound I/113) or 4-chlorophenyl (Compound I/133); or

if **Y is (4-ferrocenyl-1H-1,2,3-triazol-1-yl)phenyl and Z is H**, then X is 2-methylphenyl (Compound I/120); or

if **Y is**



then X is 2-methylphenyl (Compound I/122); or

if **Y is 3,5-diazidophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/138) or 4-(trifluoromethyl)phenyl (Compound I/139); or

if **Y is 3-thiocyanatophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/142) or 4-(trifluoromethyl)phenyl (Compound I/143); or

if **Y is 3-selenocyanatophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/145) or 4-(trifluoromethyl)phenyl (Compound I/146);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

2. The compounds according to Point 1, wherein

if **Y is 3-fluorophenyl and Z is H**, then X is 4-azidophenyl (Compound I/106); or

if **Y is 3,5-difluorophenyl and Z is H**, then X is 4-azidophenyl (Compound I/107); or

if **Y is 3-azidophenyl and Z is H**, then X is 4-(trifluoromethyl)phenyl (Compound I/111) or 4-chlorophenyl (Compound I/133); or

if **Y is 3,5-diazidophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/138) or 4-(trifluoromethyl)phenyl (Compound I/139);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

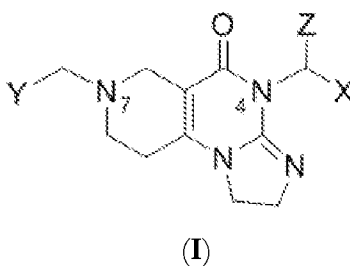
3. The compounds according to Point 1, wherein

if Y is 3,5-difluorophenyl and Z is H, then X is 4-azidophenyl (Compound I/107); or

if Y is 3-azidophenyl and Z is H, then X is 4-(trifluoromethyl)phenyl (Compound I/111) or 4-chlorophenyl (Compound I/133);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

4. The compounds of formula (I)



wherein

if Y is phenyl and Z is H, then X is 4-fluorophenyl (Compound I/5), 3-fluorophenyl (Compound I/8), 3,4,5-trifluorophenyl (Compound I/9), 2,3,4-trifluorophenyl (Compound I/29), 2-fluoro-4-nitrophenyl (Compound I/48) or 3-aminophenyl (Compound I/75); or

if Y is 3-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/31), 4-iodophenyl (Compound I/46), 2-fluoro-4-nitrophenyl (Compound I/49), 2-methylphenyl (Compound I/53) or 4-aminophenyl (Compound I/84); or

if Y is 3,5-difluorophenyl and Z is H, then X is 4-fluorophenyl (Compound I/7), 4-(trifluoromethyl)phenyl (Compound I/30), 4-iodophenyl (Compound I/38), 4-bromophenyl (Compound I/39), 2-fluoro-4-nitrophenyl (Compound I/50), 4-chlorophenyl (Compound I/124) or 3-aminophenyl (Compound I/126) or 2-methylphenyl (Compound I/37); or

if Y is 2-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/26) or 4-iodophenyl (Compound I/54); or

if Y is 4-(trifluoromethyl)phenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/27); or

if Y is 4-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/28) or 4-iodophenyl (Compound I/55); or

if **Y is 4-aminophenyl and Z is H**, then X is 4-(trifluoromethyl)phenyl (Compound I/81), 4-fluorophenyl (Compound I/86), 4-iodophenyl (Compound I/87) or 3,4,5-trimethoxyphenyl (Compound I/89); or

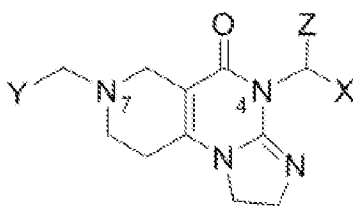
if **Y is 3,5-difluorophenyl and Z is hydroxymethyl**, then X is 4-chlorophenyl (racemic) (Compound I/128), 4-fluorophenyl (R-enantiomer) (Compound I/129 (R)) or 4-fluorophenyl (S-enantiomer) (Compound I/129 (S));

if **Y is 3,5-dicyanophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/136) or 4-(trifluoromethyl)phenyl (Compound I/137); or

if **Y is phenyl and Z is H**, then X is 2-iodophenyl (Compound I/11);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof for use as a medicament.

5. The compounds of formula (I)



(I)

wherein

if **Y is phenyl and Z is H**, then X is 4-fluorophenyl (Compound I/5), 3-fluorophenyl (Compound I/8), 3,4,5-trifluorophenyl (Compound I/9), 2,3,4-trifluorophenyl (Compound I/29), 2-fluoro-4-nitrophenyl (Compound I/48) or 3-aminophenyl (Compound I/75); or

if **Y is 3-fluorophenyl and Z is H**, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/31), 4-iodophenyl (Compound I/46), 2-fluoro-4-nitrophenyl (Compound I/49), 2-methylphenyl (Compound I/53) or 4-aminophenyl (Compound I/84); or

if **Y is 3,5-difluorophenyl and Z is H**, then X is 4-fluorophenyl (Compound I/7), 4-(trifluoromethyl)phenyl (Compound I/30), 4-iodophenyl (Compound I/38), 4-bromophenyl (Compound I/39), 2-fluoro-4-nitrophenyl (Compound I/50), 4-chlorophenyl (Compound I/124) or 3-aminophenyl (Compound I/126) or 2-methylphenyl (Compound I/37); or

if **Y is 2-fluorophenyl and Z is H**, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/26) or 4-iodophenyl (Compound I/54); or

if **Y is 4-(trifluoromethyl)phenyl and Z is H**, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/27); or

if Y is 4-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/28) or 4-iodophenyl (Compound I/55); or

if Y is 4-aminophenyl and Z is H, then X is 4-(trifluoromethyl)phenyl (Compound I/81), 4-fluorophenyl (Compound I/86), 4-iodophenyl (Compound I/87) or 3,4,5-trimethoxyphenyl (Compound I/89); or

if Y is 3,5-difluorophenyl and Z is hydroxymethyl, then X is 4-chlorophenyl (racemic) (Compound I/128), 4-fluorophenyl (R-enantiomer) (Compound I/129 (R)) or 4-fluorophenyl (S-enantiomer) (Compound I/129 (S));

if Y is 3,5-dicyanophenyl and Z is H, then X is 4-chlorophenyl (Compound I/136) or 4-(trifluoromethyl)phenyl (Compound I/137); or

if Y is phenyl and Z is H, then X is 2-iodophenyl (Compound I/11);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof for use in the treatment of cancer.

6. The compounds for use according to the Point 5, wherein the cancer is selected from the group consisting of prostate carcinoma, pancreatic cancer, lung carcinoma, breast cancer, glioma, cancers of head and neck, colon cancer, skin cancer.

7. The compounds for use according to Points 4 to 6, wherein

if Y is 3-fluorophenyl and Z is H, then X is 2-methylphenyl (Compound I/53); or

if Y is 3,5-difluorophenyl and Z is H, then X is 4-fluorophenyl (Compound I/7), 4-(trifluoromethyl)phenyl (Compound I/30), 4-bromophenyl (Compound I/39), 4-chlorophenyl (Compound I/124) or 2-methylphenyl (Compound I/37); or

if Y is 3,5-dicyanophenyl and Z is H, then X is 4-chlorophenyl (Compound I/136) or 4-(trifluoromethyl)phenyl (Compound I/137); or

if Y is phenyl and Z is H, then X is 2-iodophenyl (Compound I/11);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

8. The compounds for use according to Points 4 to 6, wherein

if Y is 3,5-difluorophenyl and Z is H, then X is 4-fluorophenyl (Compound I/7), 4-(trifluoromethyl)phenyl (Compound I/30) or 4-chlorophenyl (Compound I/124);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

9. The compounds according to the Points 1-3 for use as a medicament.
10. The compounds according to the Points 1-3 for use in the treatment of cancer.
11. The compounds for use according to the Point 10, wherein the cancer is selected from the group consisting of prostate carcinoma, pancreatic cancer, lung carcinoma, breast cancer, glioma, cancers of head and neck, colon cancer, skin cancer.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compounds of formula (I) as described above in Item 1, wherein the identifiers in the formula are as defined in Item 1, and pharmaceutically acceptable salts, stereoisomers thereof, including single enantiomers, , racemic mixtures, mixtures of enantiomers, or combinations thereof.

A group of compounds of the present invention is a compound of formula (I) as described above, wherein the identifiers in the formula are as defined in Item 2, and pharmaceutically acceptable salts, stereoisomers thereof, including single enantiomers, racemic mixtures, mixtures of enantiomers, or combinations thereof.

Another group of compounds of the present invention are compounds of formula (I) as described above in Item 3, wherein the identifiers in the formulae are as defined in Item 3, and the pharmaceutically acceptable salts, stereoisomers thereof, including single enantiomers, racemic mixtures, mixtures of enantiomers, or combinations thereof.

A further group of compounds of the present invention is a compound of formula (I) as described above in Item 4, wherein the identifiers in the formula are as defined in Item 4, and the pharmaceutically acceptable salts, stereoisomers thereof, including single enantiomers, racemic mixtures, mixtures of enantiomers, or combinations thereof.

A further group of compounds of the present invention is a compound of formula (I) as described above in Item 5, and the pharmaceutically acceptable salts, stereoisomers thereof, including enantiomers, racemic mixtures, mixtures of enantiomers, or combinations thereof. As noted above, the compounds of the present invention may exist as racemic mixtures and as optical isomers, in the form of one enantiomer, which is pure or predominantly present. It will be appreciated that both racemic mixtures and enantiomers in pure form or in the mixture predominantly as compared to the other enantiomer belong to the subject matter of the invention.

The invention also relates to the compounds of the invention for use in the treatment of cancer diseases selected from the group consisting of prostate carcinoma, pancreatic cancer, lung carcinoma, breast cancer, glioma, cancers of head and neck, colon cancer, skin cancer.

By means of a close inspection of the structural features of the residual members of imipridones described in the prior art, it can be established that besides the limited versatility of the substituents bonded to N-4 and N-7, except for ONC234 (Reference Compound 1), the known imipridones do not contain di- or trisubstituted benzyl group attached to position 7 indicating that the accessible chemical space is underexplored. This realization has led to identify novel lead compounds of more pronounced potential in chemotherapeutic applications which, in addition to enhanced anticancer activity, have additional beneficial properties, e.g. in terms of therapeutic window and bioavailability superior to those of the state of the art collection.

Accordingly, following our convergent synthetic route presented on Reaction Scheme 1 we performed a diversity oriented synthesis of novel imipridones with a variety of mono-, di- and trisubstituted benzyl groups and amine-based molecular fragment on both terminals of the heterocyclic skeleton (see Table 1).

To expand the scope of the functional groups on the pending skeletal substituents, the compounds prepared from commercially available building blocks containing Boc-protected aromatic amine residue(s), were converted into azido derivatives (see Table 1) by a one-pot procedure comprising simultaneous acid-catalyzed *N*-deprotection and diazotation followed by diazonium→azide exchange (see Reaction Scheme 2). By means of copper(I)-catalysis two azides were coupled with ethynylferrocene and erlotinib to get triazole-tethered hybrids (see Table 1).

In the course of inventive work the results of biological tests, disclosing characteristic structure activity relationships (SAR), were continuously taken into account for the structural refinement in the design and synthesis of the further members of novel imipridones that displayed enhanced cytotoxic effects. Since only a limited set of data resulted from the bioassays of imipridones known from the prior art are accessible (WO 2018031987 A1), besides ONC212 three additional known halogenated analogues [**I/2** (ONC217), **I/3** (2185824-99-9P) and **I/4** (2185824-98-8P) in Table 1] were also prepared to serve as references in our biological tests.

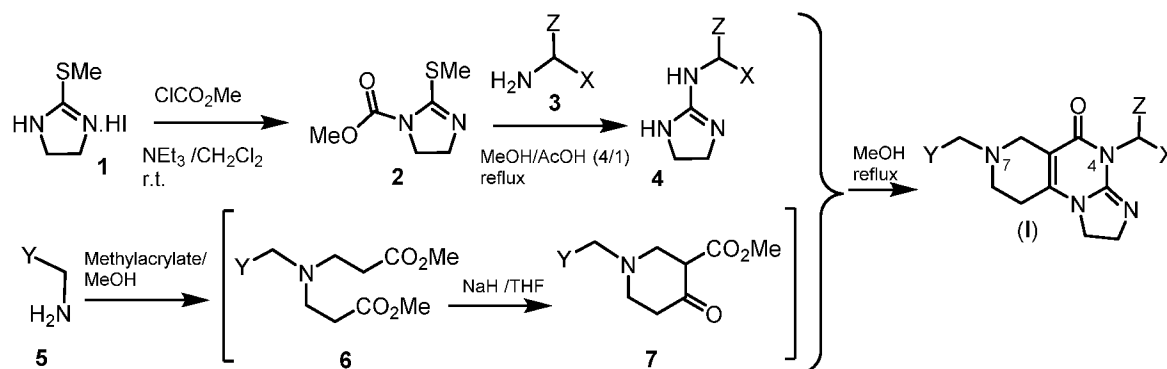
As a result of stepwise refinement we identified three novel halogenated imipridones [**I/7** (ABB-011), **I/30** (TBP-301) and **I/39** (TBP-302)] as the most potent drug candidates displaying extremely strong cytotoxicity on representative cell lines characterized by IC₅₀ values in a range of ca. 3-8 nM unambiguously indicating their superiority over each on reference compounds known from the prior art (Tables 2 and 3) We have also disclosed that the R-enantiomer of a

hydroxymethyl substituted imipridone **I/129(R)** [TBP-339(R)] exhibits much stronger antiproliferative effect on PANC1 cell line that does the S-enantiomer **I(129(S))** [(TBP-339(S))] ($IC_{50} = 15$ nM vs 265 nM: Table 2).

A clear structure-activity relationship, associated with the stepwise introduction of fluorine substituents in the “*meta*” position(s) of the N-7-benzyl group, can be established from the tendency observed in the gradual increase in the cytotoxicity produced by ONC212, 2185824-99-9P and **I/30** (TBP-301). The very same tendency is discernible in the antiproliferative effects of the series **I/5** (TBP-134), **I/6** (TBP-218), **I/7** (ABB-011) and **I/41** (TBP-285), 2185824-98-8P **I/39**(TBP-302) the 3,5-difluorobenzyl-substituted derivatives being far the most active ones.

Preparation of compounds of the invention

The convergent synthesis of the novel imipridones of general formula (**I**) (except for the compounds containing N_3 , CN, SCN and SeCN substituents in the side chains pending on positions 4 and 7) (Reaction scheme 1) are based on straightforward coupling reactions and annulations utilizing readily available precursors, such as 2-(methylthio)-4,5-dihydro-1*H*-imidazole (**1**), methylacrylate and primary amines types **3** and **5**.



Reaction Scheme 1: The convergent synthetic route to compounds of formula (**I**)

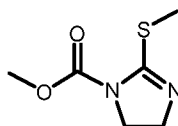
Materials and Methods

All fine chemicals were obtained from commercially available sources (Merck, Fluorochem, Molar Chemicals, VWR) and used without further purification. Dioxane was distilled from sodium benzophenone. Merck Kieselgel (230-400 mesh, 60 Å) was used for flash column chromatography. Melting points (uncorrected) were determined with a Büchi M-560. The 1H - and ^{13}C NMR spectra of all compounds were recorded in $CDCl_3$ solution in 5 mm tubes at RT, on a Bruker DRX-500 spectrometer at 500 (1H) and 125 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as the internal standard. The HSQC, HMBC, COSY and

NOESY spectra, which support the exact assignments the of ^1H - and ^{13}C NMR signals were obtained by using the standard Bruker pulse programs.

General procedures of the synthetic steps finally leading to compounds of formula (I) as presented on Reaction Scheme 1

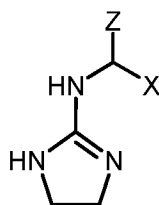
1. Methyl 2-(methylthio)-4,5-dihydro-1H-imidazole-1-carboxylate (**2**)



(2)

Commercially available 2-methylthio-4,5-dihydroimidazolium iodide (12.21 g, 50 mmol) and triethylamine (TEA, 16 mL, 11.62 g, 115 mmol) were dissolved in DCM (50 mL). Methylchloroformate (5 mL, 6.12 g, 65 mmol) was added dropwise to the solution previously cooled down to 0 °C. The reaction mixture was allowed to warm up to 25 °C and was stirred overnight. After addition of EtOAc (200 mL) and stirring for 15 min, the precipitated ammonium salts were filtered off and washed through with EtOAc (50 mL). The combined solution was evaporated to dryness. The solid residue was triturated with water filtered off and dried under vacuo to obtain **5** as white solid. Yield: 5.55 g (64%).

2. N-substituted-4,5-dihydro-1H-imidazol-2-amine (**4**)



(4)

To the solution of primary amine type **3** (2 mmol) dissolved in a mixture of MeOH:AcOH (4 mL:1 mL) methyl 2-(methylthio)-4,5-dihydro-1H-imidazole-1-carboxylate **2** (0.47g, 2.4 mmol) was added and the resulting solution was stirred at reflux for 20 h. After cooling down the reaction was concentrated in *vacuo* and the oily residue was dissolved in DCM (30 mL). The solution was washed with 3 M NaOH (10 mL), brine (10 mL), dried over Na_2SO_4 and evaporated to dryness. The colorless oil was crystallised from ether and used without further purification for the cyclisation to imipridone framework.

3. *Imipridone forming cyclisation*

Primary amine **5** (1 mmol), dissolved in MeOH (4 mL), methylacrylate (0.23 mL, 2.5 mmol) was added and the mixture was stirred for 24 h at room temperature, concentrated *in vacuo* and the obtained crude dipropionate type **6** was dissolved in anhydrous THF (4 mL). Under argon atmosphere NaH (0.12 g, 5 mmol) was added in small portions to the intensively stirred solution that previously was cooled down to 0 °C. The resulting suspension was stirred for additional 2 h at reflux temperature and concentrated to dryness under *vacuo*. The resulting solid residue containing the crude sodium salt of methyl *N*-substituted oxopiperidine-3-carboxylate (**7**) was dissolved in anhydrous MeOH (5 mL). To this solution was added *N*-substituted-4,5-dihydro-1*H*-imidazol-2-amine (**4**) (1 mmol) prepared in separate steps as described above. The basic solution was stirred at a reflux for 12 h, under argon atmosphere then cooled down by ice-water. The cooled reaction mixture was stirred for 1 h and the precipitated solid was collected by filtration, washed with cold methanol and dried to produce the pure imipridone product of formula (**I**).

4. *Synthesis of amine-containing imipridones.*

For the synthesis of the target compounds with pending primary amine or cyclic secondary amine moiety, the corresponding diamine (**3** or **5**) *mono*-Boc-protected either in X or in Y group was used as coupling component in the general procedures described above. The isolated Boc-protected imipridone (2 mmol) was dissolved in cc HCl (5 mL) and the resulting solution was heated at reflux for 5 minutes and cooled down to room temperature. The pH of this solution was then set to ca. 13-14 by concentrated aqueous potassium hydroxide. The precipitated amine product was collected by filtration, thoroughly washed with cold water and dried in desiccator over potassium hydride pellets.

5. *Synthesis of azidobenzyl imipridones*

The corresponding aminobenzyl imipridone (1 mmol) was dissolved in cc HCl (5 mL). To this solution cooled down to 0 °C was dropwise added an aqueous solution of NaNO₂ (137.8 mg, 2 mmol dissolved in 2.5 mL of water). The diazotation reaction was monitored by TLC. After completion, NaN₃ (324 mg, 5 mmol) was added at 0 °C to the reaction mixture which was then stirred at room temperature for 1 h. The pH of the solution was adjusted to 10-11 by a careful addition of solid Na₂CO₃. The resulting mixture was extracted by CH₂Cl₂ (2 × 40 mL) and the organic phase was dried over Na₂SO₄ then evaporated to dryness. The oily residue was crystallized by *n*-hexane to obtain the product as colourless solid.

6. *Synthesis of aryltriazolylbenzyl imipridones from azidobenzyl imipridones*

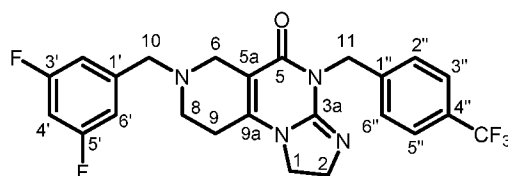
The corresponding azidobenzyl imipridone (1 mmol), the terminal alkyne component (1 mmol) and CuI (29.3 mg, 0.15 mmol) were dissolved in DMSO (5 mL). In a closed vessel the reaction mixture was stirred for 24 h at room temperature and poured on water (50 mL). The precipitated solid was collected by filtration, washed with water (100 mL) and suspended in aqueous ammonia solution (20 mL). The suspension was stirred for 20 min and filtered. The residue was washed with water (50 mL), dried and dissolved in a 9:1 mixture of CH₂Cl₂ and MeOH (10 mL). The solution was passed through silica and evaporated. The solid residue was crystallized with ether.

In the following, the invention will be illustrated by means of exemplary embodiments which, however, are not to be construed as limiting the invention.

EXAMPLES

Example 1:

7-(3,5-Difluorobenzyl)-4-(4-(trifluoromethyl)benzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-301) (Compound I/30)

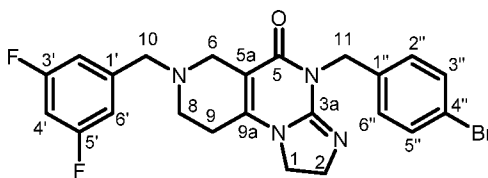


(Compound I/30)

Yield: 387 mg (81%). Mp.: 168.0 °C. ¹H-NMR (CDCl₃): 7.53 and 7.50 (A and B part of an AA'BB' spin system, J_{AB} =8.9 Hz, 2x2H, H-3'',5'' and H-2'',6'', resp.); 6.84 (br dt, J ~7 Hz and ~2 Hz, 2H, H-2',6'); 6.67 (tt, J =9.0 Hz and 2.3 Hz, 1H, H-4'); 5.06 (s, 2H, H-11); 3.88 (s, 4H, H-1 and H-2); 3.60 (s, 2H, H-10); 3.24 (br s, 2H, H-6); 2.64 (t, 2H, J =5.7 Hz, H-8); 2.46 (t, 2H, J =5.7 Hz, H-9). ¹³C-NMR (CDCl₃): 163.1 (dd, J =250.2 Hz and 15.6 Hz, C-3',5'); 161.3 (C-5); 152.9 (C-3a); 145.8 (C-9a); 142.2 (t, J =8.4 Hz, C-1'); 140.8 (C-1''); 129.6 (qa, J =32.5 Hz, C-4''); 128.8 (C-2'',6''); 125.3 (qa, J =3.8 Hz, C-3'',5''); 124.4 (qa, J =272.5 Hz, CF₃); 111.3 (dd, J =19.3 Hz and 4.9 Hz, C-2',6'); 102.8 (t, J =25.8 Hz, C-4'); 101.6 (C-5a); 61.4 (C-10); 50.6 (C-2); 49.3 (C-6); 48.4 (C-8); 46.9 (C-1); 45.0 (C-11); 26.8 (C-9).

Example 2:

4-(4-Bromobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-302) (Compound I/39)

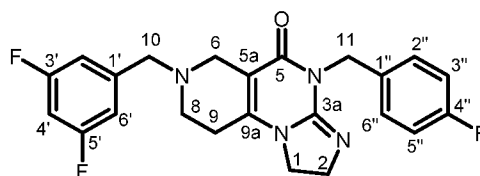


(Compound I/39)

Yield: 271 mg (56%). Mp.: 173.5 °C. ¹H-NMR (CDCl₃): 7.36 (d, *J*=8.3 Hz, 2H, H-3'',5''); 7.32 (d, *J*=8.3 Hz, 2H, H-2'',6''); 6.84 (br dt, *J*~7 Hz and ~2 Hz, 2H, H-2',6'); 6.66 (tt, *J*=9.0 Hz and 2.3 Hz, 1H, H-4'); 4.95 (s, 2H, H-11); 3.87 (s, 4H, H-1 and H-2); 3.60 (s, 2H, H-10); 3.23 (br s, 2H, H-6); 2.62 (t, 2H, *J*=5.7 Hz, H-8); 2.44 (t, 2H, *J*=5.7 Hz, H-9). ¹³C-NMR (CDCl₃): 163.1 (dd, *J*=250.2 Hz and 15.6 Hz, C-3',5'); 161.3 (C-5); 152.9 (C-3a); 145.8 (C-9a); 142.2 (t, *J*=8.4 Hz, C-1'); 135.9 (C-1''); 131.4 (C-3'',5''); 130.6 (C-2'',6''); 121.4 (C-4''); 111.3 (dd, *J*=19.3 Hz and 4.9 Hz, C-2',6'); 102.8 (t, *J*=25.7 Hz, C-4'); 101.6 (C-5a); 61.4 (C-10); 50.6 (C-2); 49.4 (C-6); 48.4 (C-8); 46.9 (C-1); 44.8 (C-11); 26.8 (C-9).

Example 3:

4-(4-Fluorobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (ABB-011) (Compound I/7)

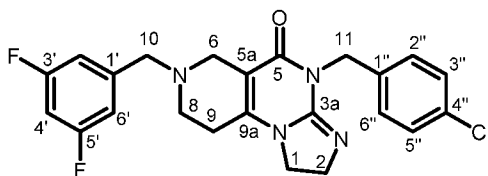


(Compound I/7)

Yield: 290 mg (68%). Mp.: 190.5 °C. ¹H-NMR (CDCl₃): 7.44 (dd, *J*=8.7 Hz and 5.7 Hz, 2H, H-2'',6''); 6.92 (t, *J*=8.7 Hz, 2H, H-3'',5''); 6.84 (br dt, *J*~7 Hz and ~2 Hz, 2H, H-2',6'); 6.66 (tt, *J*=8.9 Hz and 2.3 Hz, 1H, H-4'); 4.97 (s, 2H, H-11); 3.92-3.83 (m, 4H, H-1 and H-2); 3.59 (s, 2H, H-10); 3.23 (br s, 2H, H-6); 2.62 (t, 2H, *J*=5.7 Hz, H-8); 2.44 (t, 2H, *J*=5.7 Hz, H-9). ¹³C-NMR (CDCl₃): 162.2 (d, *J*=162.2 Hz, C-4''); 163.1 (dd, *J*=250.0 Hz and 15.2 Hz, C-3',5'); 161.4 (C-5); 152.9 (C-3a); 145.5 (C-9a); 142.2 (t, *J*=8.4 Hz, C-1'); 132.7 (C-1''); 130.7 (d, *J*=7.9 Hz, C-2'',6''); 115.0 (d, *J*=21.3 Hz, C-3'',5''); 111.4 (dd, *J*=19.3 Hz and 4.9 Hz, C-2',6'); 102.7 (t, *J*=25.7 Hz, C-4'); 101.6 (C-5a); 61.4 (C-10); 50.6 (C-2); 49.4 (C-6); 48.4 (C-8); 46.9 (C-1); 44.7 (C-11); 26.7 (C-9).

Example 4:

4-(4-Chlorobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-333) (Compound I/124)

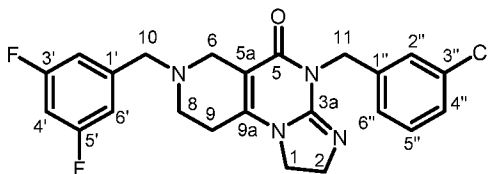


(Compound I/124)

Yield: 332 mg (75%). Mp.: 162.4 °C. ¹H-NMR (CDCl₃): 7.38 (d, *J*=8.3 Hz, 2H, H-2'',6''); 7.20 (d, *J*=8.3 Hz, 2H, H-3'',5''); 6.84 (br dt, *J*~7 Hz and ~2 Hz, 2H, H-2',6'); 6.66 (tt, *J*=9.0 Hz and 2.3 Hz, 1H, H-4'); 4.97 (s, 2H, H-11); 3.90-3.83 (m, 4H, H-1 and H-2); 3.59 (s, 2H, H-10); 3.22 (br s, 2H, H-6); 2.62 (t, 2H, *J*=5.7 Hz, H-8); 2.44 (t, 2H, *J*=5.7 Hz, H-9). ¹³C-NMR (CDCl₃): 163.1 (dd, *J*=248.2 Hz and 14.6 Hz, C-3',5'); 161.3 (C-5); 152.9 (C-3a); 145.6 (C-9a); 142.2 (t, *J*=8.4 Hz, C-1'); 135.4 (C-1''); 133.2 (C-4''); 130.6 (C-2'',6''); 128.4 (C-3'',5''); 111.7 (dd, *J*=19.3 Hz and 4.9 Hz, C-2',6'); 102.7 (t, *J*=25.7 Hz, C-4'); 101.6 (C-5a); 61.4 (C-10); 50.6 (C-2); 49.4 (C-6); 48.4 (C-8); 46.9 (C-1); 44.7 (C-11); 26.7 (C-9).

Example 5:

4-(3-Chlorobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-344) (Compound I/125)

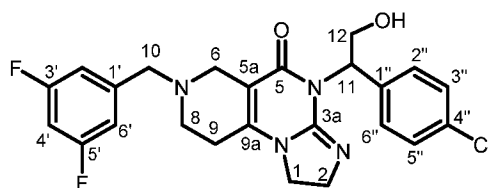


(Compound I/125)

Yield: 302 mg (68%). Mp.: 204.8 °C. ¹H-NMR (CDCl₃): 7.40 (br s, 1H, H-2''); 7.30 (m, 1H, H-5''); 7.18-7.15 (m, 2H, H-4'',6''); 6.84 (br dt, *J*~7 Hz and ~2 Hz, 2H, H-2',6'); 6.66 (tt, *J*=8.9 Hz and 2.2 Hz, 1H, H-4'); 4.98 (s, 2H, H-11); 3.87 (br ~s, 4H, H-1 and H-2); 3.59 (s, 2H, H-10); 3.24 (br s, 2H, H-6); 2.63 (t, 2H, *J*=5.6 Hz, H-8); 2.45 (t, 2H, *J*=5.6 Hz, H-9). ¹³C-NMR (CDCl₃): 163.1 (dd, *J*=248.6 Hz and 14.0 Hz, C-3',5'); 161.3 (C-5); 152.9 (C-3a); 145.7 (C-9a); 142.3 (t, *J*=8.4 Hz, C-1'); 138.8 (C-1''); 134.1 (C-3''); 128.4 (two coalesced lines, C-2'' and C-4''); 127.6 (C-6''); 126.8 (C-5''); 111.3 (dd, *J*=19.6 Hz and 5.1 Hz, C-2',6'); 102.7 (t, *J*=25.3 Hz, C-4'); 101.6 (C-5a); 61.3 (C-10); 50.6 (C-2); 49.4 (C-6); 48.4 (C-8); 46.9 (C-1); 44.9 (C-11); 26.8 (C-9).

Example 6:

rac-4-(4-Chlorobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-342) (Compound I/128 (racemate))

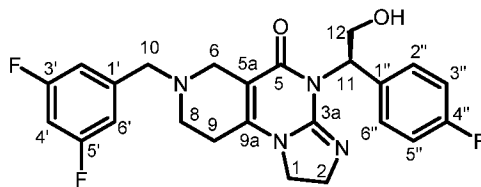


(Compound I/128 (racemate))

Yield: 38 mg (8%). Mp.: 86 °C. ¹H-NMR (CDCl₃): 7.28 (d, *J*=8.3 Hz, 2H, H-2'',6''); 7.23 (d, *J*=8.3 Hz, 2H, H-3'',5''); 6.84 (br dt, *J*~7 Hz and ~2 Hz, 2H, H-2',6'); 6.67 (tt, *J*=9.0 Hz and 2.3 Hz, 1H, H-4'); 6.08 (dd, *J*=5.1 Hz and 1.6 Hz, 1H, H-11); 4.31 (dd, *J*=12.8 Hz and 5.1 Hz, 1H, H-12_A); 4.06 (dd, *J*=12.8 Hz and 1.6 Hz, 1H, H-12_B); 3.93-3.85 (m, 3H, H-1 and H-2_A); 3.78 (m, 1H, H-2_B); 3.60 (s, 2H, H-10); 3.24 and 3.21 (A and B parts of an AB spin system, *J*_{AB}=14.9 Hz, 2x1H, H-6_A and H-6_B); 2.66 (m, 2H, H-8); 2.50 (m, 2H, H-9). ¹³C-NMR (CDCl₃): 163.1 (dd, *J*=248.2 Hz and 13.9 Hz, C-3',5'); 161.6 (C-5); 153.5 (C-3a); 146.0 (C-9a); 142.2 (t, *J*=8.4 Hz, C-1'); 135.2 (C-1''); 133.1 (C-4''); 139.0 (C-2'',6''); 128.4 (C-3'',5''); 111.4 (dd, *J*=19.3 Hz and 4.9 Hz, C-2',6'); 102.8 (t, *J*=25.7 Hz, C-4'); 102.4 (C-5a); 62.9 (C-12); 61.3 (C-10); 57.6 (C-11); 49.5 (C-2); 49.4 (C-6); 48.4 (C-8); 46.5 (C-1); 26.8 (C-9).

Example 7:

R-4-(4-Fluorobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-339) (Compound I/129 (R-enantiomer))



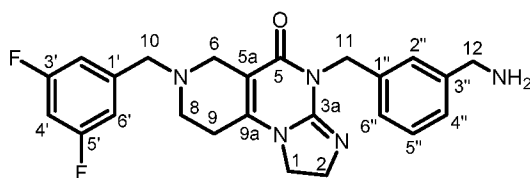
(Compound I/129 (R-enantiomer))

Yield: 51 mg (11%). Mp.: 76 °C. ¹H-NMR (CDCl₃): 7.36 (dd, *J*=8.8 Hz and 5.8 Hz, 2H, H-2'',6''); 6.96 (t, *J*=8.3 Hz, 2H, H-3'',5''); 6.85 (br dt, *J*~7 Hz and ~2 Hz, 2H, H-2',6'); 6.67 (tt, *J*=9.0 Hz and 2.3 Hz, 1H, H-4'); 6.09 (dd, *J*=5.3 Hz and 1.6 Hz, 1H, H-11); 4.32 (dd, *J*=12.8 Hz and 5.3 Hz, 1H, H-12_A); 4.07 (dd, *J*=12.8 Hz and 1.6 Hz, 1H, H-12_B); 3.94-3.85 (m, 3H, H-1 and H-2_A); 3.81 (m, 1H, H-2_B); 3.61 (s, 2H, H-10); 3.25 and 3.21 (A and B parts of an AB spin system,

$J_{AB}=14.9$ Hz, 2x1H, H-6_A and H-6_B); 2.66 (m, 2H, H-8); 2.50 (m, 2H, H-9). ¹³C-NMR (CDCl₃): 163.1 (dd, $J=248.2$ Hz and 13.9 Hz, C-3',5'); 162.0 (d, $J=245.8$ Hz, C-4''); 161.6 (C-5); 153.5 (C-3a); 145.8 (C-9a); 142.1 (t, $J=8.4$ Hz, C-1'); 132.5 (br s, C-1''); 129.4 (d, $J=7.8$ Hz, C-2'',6''); 115.1 (d, $J=21.4$ Hz, C-3'',5''); 111.3 (dd, $J=19.6$ Hz and 5.2 Hz, C-2',6'); 102.8 (t, $J=25.6$ Hz, C-4); 102.5 (C-5a); 63.1 (C-12); 61.3 (C-10); 57.6 (C-11); 49.5 (two coalesced lines, C-2 and C-6); 48.3 (C-8); 46.5 (C-1); 26.8 (C-9).

Example 8:

4-(3-(Aminomethyl)benzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-324) (Compound I/61)

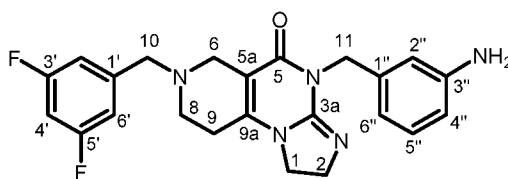


(Compound I/61)

Yield: 376 mg (86%). Mp.: 129.5 °C. ¹H-NMR (CDCl₃): 7.33 (br s, 1H, H-2''); 7.30 (br d, $J=7.6$ Hz, 1H, H-6''); 7.22 (t, $J=7.6$ Hz, 1H, H-5''); 7.15 (br d, $J=7.6$ Hz, 1H, H-4''); 6.84 (br dt, $J\sim 7$ Hz and ~ 2 Hz, 2H, H-2',6'); 6.66 (tt, $J=8.9$ Hz and 2.3 Hz, 1H, H-4'); 5.00 (s, 2H, H-11); 3.92-3.83 (m, 4H, H-1 and H-2); 3.79 (s, 2H, H-12); 3.59 (s, 2H, H-10); 3.24 (br s, 2H, H-6); 2.63 (t, 2H, $J=5.7$ Hz, H-8); 2.45 (t, 2H, $J=5.7$ Hz, H-9); 1.77 (br s, 2H, NH₂). ¹³C-NMR (CDCl₃): 163.1 (dd, $J=248.4$ Hz and 14.1 Hz, C-3',5'); 161.5 (C-5); 153.2 (C-3a); 145.6 (C-9a); 143.2 (C-3''); 142.3 (t, $J=8.4$ Hz, C-1'); 137.2 (C-1''); 128.6 (C-5''); 127.3 (C-4''); 127.1 (C-2''); 126.2 (C-4''); 111.4 (dd, $J=20.0$ Hz and 5.0 Hz, C-2',6'); 102.7 (t, $J=25.7$ Hz, C-4'); 101.7 (C-5a); 61.4 (C-10); 50.7 (C-2); 49.4 (C-6); 48.4 (C-8); 46.9 (C-1); 46.4 (C-12); 45.4 (C-11); 26.7 (C-9).

Example 9:

4-(3-Aminobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-346) (Compound I/126)

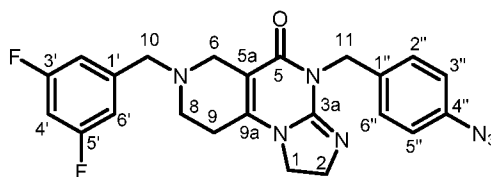


(Compound I/126)

Yield: 347 mg (82%). Mp.: 114.5 °C. ¹H-NMR (CDCl₃): 7.03 (t, *J*=7.9 Hz, 1H, H-5^{''}); 6.85 (br dt, *J*~7 Hz and ~2 Hz, 2H, H-2^{''},6^{''}); 6.82 (br d, *J*=7.9 Hz, 1H, H-6^{''}); 6.75 (br s, 1H, H-2^{''}); 6.66 (tt, *J*=8.9 Hz and 2.3 Hz, 1H, H-4^{''}); 6.52 (dd, *J*=7.6 Hz and 2.2 Hz, 1H, H-4^{''}); 4.93 (s, 2H, H-11); 3.92-3.79 (m, 4H, H-1 and H-2); 3.59 (s, 2H, H-10); 3.24 (br s, 2H, H-6); 2.62 (t, 2H, *J*=5.7 Hz, H-8); 2.44 (t, 2H, *J*=5.7 Hz, H-9); ¹³C-NMR (CDCl₃): 163.1 (dd, *J*=248.4 Hz and 14.1 Hz, C-3^{''},5^{''}); 161.5 (C-5); 153.0 (C-3a); 146.4 (C-3^{''}); 145.3 (C-9a); 142.3 (t, *J*=8.4 Hz, C-1^{''}); 138.0 (C-1^{''}); 129.2 (C-5^{''}); 119.0 (C-6^{''}); 115.2 (C-2^{''}); 114.3 (C-4^{''}); 111.4 (dd, *J*=20.1 Hz and 5.4 Hz, C-2^{''},6^{''}); 102.7 (t, *J*=25.7 Hz, C-4^{''}); 101.7 (C-5a); 61.4 (C-10); 50.6 (C-2); 49.4 (C-6); 48.4 (C-8); 46.8 (C-1); 45.3 (C-11); 26.7 (C-9).

Example 10:

4-(4-Azidobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (CZT-136)

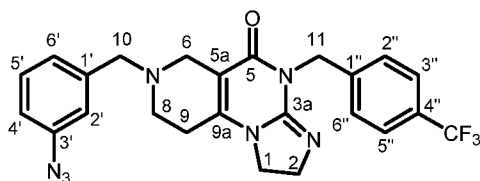


(Compound I/107)

Yield: 270 mg (60%). mp.: 155.5 °C. ¹H-NMR (CDCl₃): 7.44 (d, *J*=8.3 Hz, 2H, H-2^{''},6^{''}); 6.90 (d, *J*=8.3 Hz, 2H, H-3^{''},5^{''}); 6.84 (br ~dt, *J*~7 Hz and ~2 Hz, 2H, H-2^{''},6^{''}); 6.66 (tt, *J*=9.0 Hz and 2.3 Hz, 1H, H-4^{''}); 4.97 (s, 2H, H-11); 3.91-3.84 (m, 4H, H-1 and H-2); 3.59 (s, 2H, H-10); 3.23 (br s, 2H, H-6); 2.63 (t, 2H, *J*=5.7 Hz, H-8); 2.44 (t, 2H, *J*=5.7 Hz, H-9). ¹³C-NMR (CDCl₃): 163.1 (dd, *J*=250.2 Hz and 15.6 Hz, C-3^{''},5^{''}); 161.4 (C-5); 152.9 (C-3a); 145.5 (C-9a); 142.2 (t, *J*=8.4 Hz, C-1^{''}); 139.1 (C-4^{''}); 133.8 (C-1^{''}); 130.4 (C-2^{''},6^{''}); 118.9 (C-3^{''},5^{''}); 111.3 (dd, *J*=19.3 Hz and 4.9 Hz, C-2^{''},6^{''}); 102.7 (t, *J*=25.7 Hz, C-4^{''}); 101.7 (C-5a); 61.4 (C-10); 50.6 (C-2); 49.4 (C-6); 48.4 (C-8); 46.9 (C-1); 44.8 (C-11); 26.8 (C-9).

Example 11:

7-(3-Azidobenzyl)-4-(4-(trifluoromethyl)benzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (IBP-272)

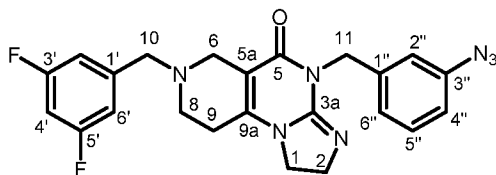


(Compound I/111)

Yield: 313 mg (65%). mp.: 128.8 °C. ¹H-NMR (CDCl₃): 7.52 and 7.50 (A and B part of an AA'BB' spin system, J_{AB} =8.9 Hz, 2x2H, H-3'',5'' and H-2'',6'', resp.); 7.26 (t, J =7.7 Hz, 1H, H-5'); 7.06 (d, J =7.7 Hz, 1H, H-6'); 6.99 (t, J =1.5 Hz, 1H, H-2'); 6.90 (dd, J =7.7 Hz and 1.5 Hz, 1H, H-4'); 5.06 (s, 2H, H-11); 3.87 (s, 4H, H-1 and H-2); 3.61 (s, 2H, H-10); 3.24 (br s, 2H, H-6); 2.64 (t, 2H, J =5.7 Hz, H-8); 2.45 (t, 2H, J =5.7 Hz, H-9). ¹³C-NMR (CDCl₃): 161.7 (C-5); 153.0 (C-3a); 145.9 (C-9a); 140.8 (C-1''); 140.2 (C-3'); 140.0 (C-1'); 129.8 (C-5'); 129.6 (qa, J =32.5 Hz, C-4''); 128.9 (C-2'',6''); 125.5 (C-6'); 125.3 (qa, J =3.5 Hz, C-3'',5''); 124.4 (qa, J =272.5 Hz, C-F₃); 119.4 (C-2'); 118.1 (C-4'); 101.7 (C-5a); 61.9 (C-10); 50.6 (C-2); 49.4 (C-6); 48.4 (C-8); 46.9 (C-1); 45.0 (C-11); 26.8 (C-9).

Example 12:

4-(3-Azidobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (TBP-347) (Compound I/127)

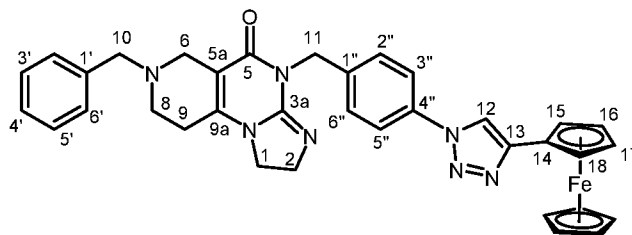


(Compound I/127)

Yield: 355 mg (79%). Mp.: 158 °C. ¹H-NMR (CDCl₃): 7.23 (t, J =7.9 Hz, 1H, H-5''); 7.18 (br ~d, J ~8 Hz 1H, H-6''); 7.10 (br s, 1H, H-2''); 6.87 (br ~d, J ~8 Hz 1H, H-4''); 6.84 (br dt, J ~7 Hz and ~2 Hz, 2H, H-2',6'); 6.66 (tt, J =8.9 Hz and 2.3 Hz, 1H, H-4'); 5.00 (s, 2H, H-11); 3.90-3.81 (m, 4H, H-1 and H-2); 3.60 (s, 2H, H-10); 3.24 (br s, 2H, H-6); 2.63 (t, 2H, J =5.7 Hz, H-8); 2.45 (t, 2H, J =5.7 Hz, H-9); ¹³C-NMR (CDCl₃): 163.1 (dd, J =248.4 Hz and 13.7 Hz, C-3',5'); 161.3 (C-5); 153.0 (C-3a); 145.6 (C-9a); 142.3 (t, J =9.0 Hz, C-1'); 140.0 (C-3''); 138.9 (C-1''); 129.7 (C-5''); 125.1 (C-6''); 121.2 (C-2''); 118.0 (C-4''); 111.4 (dd, J =20.1 Hz and 5.4 Hz, C-2',6'); 102.7 (t, J =25.7 Hz, C-4'); 101.7 (C-5a); 61.3 (C-10); 50.6 (C-2); 49.4 (C-6); 48.4 (C-8); 46.9 (C-1); 45.0 (C-11); 26.8 (C-9).

Example 13:

7-Benzyl-4-(4-(4-ferrocenyl-1H-1,2,3-triazol-1-yl)benzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one (ABB-030)



(Compound I/121)

Yield: 443 mg (71%). mp.: 165.4 °C. ¹H-NMR (CDCl₃): 7.81 (s, 1H, H-12); 7.65 (d, *J*=7.9 Hz, 2H, H-3'',5''); 7.59 (d, *J*=7.9 Hz, 2H, H-2'',6''); 7.31-7.26 (m, 4H, H-2',3',5',6'); 7.22 (m, 1H, H-4'); 5.11 (s, 2H, H-11); 4.74 (t, *J*=1.8 Hz, 2H, H-15,18); 4.29 (t, *J*=1.8 Hz, 2H, H-16,17); 4.07 (s, η⁵-C₅H₅); 3.87 (br s, 4H, H-1 and H-2); 3.63 (s, 2H, H-10); 3.27 (br s, 2H, H-6); 2.63 (t, 2H, *J*=5.7 Hz, H-8); 2.44 (t, 2H, *J*=5.7 Hz, H-9). ¹³C-NMR (CDCl₃): 161.3 (C-5); 153.0 (C-3a); 147.5 (C-4); 145.9 (C-9a); 130.1 (C-2'',6''); 128.4 (C-2',6'); 127.4 (C-4'); 126.1 (C-3',5'); 120.1 (C-3'',5''); 116.6 (C-12); 102.2 (C-5a); 75.0 (C-14); 69.6 (η⁵-C₅H₅); 68.8 (C-16,17); 66.8 (C-15,18); 62.3 (C-10); 50.6 (C-2); 49.5 (C-6); 48.3 (C-8); 46.8 (C-1); 44.9 (C-11); 26.8 (C-9).

Example 14:

Synthesis of 4-arylmethyl-substituted 7-(3,5-diazidobenzyl)-2,4,6,7,8,9-hexahydro-imidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-ones (Compounds I/138 and I/139)

Sodium azide (0.260 g, 4 mmol), sodium ascorbate (0.119 g, 0.60 mmol), N,N-dimethylethylenediamine (0.080 g, 0.9 mmol), NaOH (0.012 g, 0.30 mmol), the corresponding 7-(3,5-dibromobenzyl) imipridone **I/134** or **I/135** (1 mmol) and CuI (0.057 g, 0.30 mmol) are dissolved in 20 mL of a degassed (argon for 30 min) EtOH/H₂O solvent mixture (7:3) and heated at reflux temperature for 3 h. The reaction mixture is concentrated under reduced pressure to ca. third of its original volume and extracted with CH₂Cl₂ (3 × 4 mL). The combined organic phases are washed with water (3 × 50 mL), dried over Na₂SO₄, filtered through paper and concentrated. Final purification is achieved by column chromatography using CH₂Cl₂/MeOH (99:1) as the eluent.

Example 15:

Synthesis of 4-arylmethyl-substituted 7-(3,5-dicyanobenzyl)-2,4,6,7,8,9-hexahydro-imidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-ones (Compounds I/136 and I/137)

To a stirred solution of the corresponding 7-(3,5-dibromobenzyl) imipridone **I/134** or **I/135** (1 mmol) in DMF (2 mL), are sequentially added zinc cyanide (0.181 g, 1.2 mmol), 1,1'-bis(diphenylphosphino)ferrocene ligand (DPPF, 0.065 g, 0.117 mmol) and finally Pd₂dba₃ (45.8 mg, 0.05 mmol). The flask is flushed through with nitrogen and stirred in an oil bath at 110-120°C for 20 h. After cooling down to room temperature, the reaction mixture is evaporated under vacuo. The resulting crude product is subjected to flash silica gel column chromatography (eluent, ethyl acetate:hexane (1:4)) to obtain the dicyanobenzyl-substituted imipridone as a pure product.

Example 16:

Synthesis of 4-arylmethyl-substituted 7-(3-thiocyanatobenzyl)-2,4,6,7,8,9-hexahydro-imidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-ones (Compounds I/142 and I/143)

Under an argon atmosphere, a mixture of the corresponding 7-(3-iodobenzyl) imipridone (**I/140** or **I/141**) (1.0 mmol), CuSCN (0.12 g, 1.0 mmol), KSCN (0.095 g, 1.0 mmol) and DMF (3 mL) is heated with stirring in an oil bath and maintained at 140 °C for 12 h. After cooling, the mixture is diluted with toluene (5 mL) and water (5 mL) then filtered through a Celite bed. The aqueous phase is extracted with toluene (2×5 mL) and the combined organic phase is washed with water, dried over Na₂S₂O₄, and concentrated. The residue is chromatographed over silica gel (hexane as eluent) affording the thiocyanate product that is further purified by recrystallization from hexane.

Example 17:

Synthesis of 4-arylmethyl-substituted 7-(3-selenocyanatobenzyl)-2,4,6,7,8,9-hexahydro-imidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-ones (Compounds I/145 and I/146)

To a solution of the corresponding 7-(3-iodobenzyl) imipridone (**I/79** and **I/144**) (5 mmol) in dioxane (4 mL) is added 6 N HCl (10 mL). The resulting suspension is cooled to 0 °C, then NaNO₂ (0.415 g, 6 mmol) in water (2 mL) is slowly added. After stirring for 30 min, saturated NaOAc solution (ca. 30 mL) is added in portions to adjust the pH of the reaction mixture to 5-6. The resulting suspension is poured into a solution of KSeCN (0.793 g, 5.5 mmol) in water (25 mL) at 0 °C. After stirring for 30 min, the reaction mixture was warmed to room temperature and extracted with ether. The organic layer was washed with water and brine, dried over Na₂SO₄, and concentrated. The residue is purified by column chromatography over silica using ethyl acetate – hexane (1:5) as eluent.

Example 18:

In this example we list the compounds of formula (I) according to the present invention and demonstrate the results of their *in vitro* antiproliferative assays for representative compounds investigated during our systematic experimental work along with relevant references known from prior art.

Cell Cultures for long treatment experiments

The PANC-1 (human pancreatic carcinoma of ductal origin), COLO 205 (human colorectal adenocarcinoma), A2058 (human metastatic melanoma) obtained from European Collection of Authenticated Cell Cultures (ECACC, Salisbury, UK) and EBC-1 (human lung squamous cell carcinoma) purchased from Japanese Research Resources Bank (Tokyo, Japan) were used to determine the tumor growth inhibitory effects of the imipridone derivatives. PANC-1 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Lonza, Basel, Switzerland); for the culturing of COLO-205 cell line DMEM medium formulated with 4500 mg/L d-glucose was used; EBC-1 cells were cultured in DMEM medium containing 1% non-essential amino acids (NEAA, Gibco®/Invitrogen Corporation, New York, NY, USA), 1 mM sodium pyruvate (Sigma-Aldrich, St. Louis, MO, USA), while A2058 cell line was grown in RPMI 1640 (Lonza, Basel, Switzerland). In case of all cell lines, the aforementioned basal media were supplemented with 10% fetal bovine serum (FBS, Gibco®/Invitrogen Corporation, New York, NY, USA), L-glutamine (2 mmol/L) (Lonza, Basel, Switzerland) and 100 µg/mL penicillin/streptomycin (Gibco®/Invitrogen Corporation, New York, NY, USA). (Gibco®/Invitrogen Corporation, New York, NY, USA). All cell lines were cultivated under standard conditions (37 °C, humidified 5% CO₂ atmosphere) in plastic culture dishes (Sigma-Aldrich, St. Louis, MO, USA or Eppendorf AG, Hamburg, Germany).

*Viability Assays (long treatment experiments)**Impedance-Based Assay*

The cytotoxicity experiments on PANC-1 cells was conducted using the impedance-based xCELLigence SP System (ACEA Biosciences, San Diego, CA, USA). A more detailed description of the basis of impedimetric measurement is given in our previous paper [22]. Monitoring the impedance change, which is proportional to the number of adhered cells on an electrode surface, provides a sensitive way for cytotoxicity studies [23]. The change in the impedance is expressed as in the form of Cell Index (CI) calculated by the software (RTCA 2.0, ACEA Biosciences, San

Diego, CA, USA) integrated to xCELLigence System. For determination of IC_{50} (a concentration that decreases the cell viability by 50%) values the tested imipridones were solved in DMSO and further diluted in supplemented DMEM medium to prepare a concentration range from 2.5×10^{-4} to 5×10^{-7} M. The steps of our impedimetric experiment proceeded in the same way as what was indicated in [24]. In brief, after gaining a constant CI value during the background measurement, the PANC-1 cells (1.5×10^4 cells/well) were added to the so-called E-plate, and their adhesion/spreading was monitored for 24 h in order to settle the plateau phase of cell culture. In the last step, the cells in this balanced state were treated with the test compounds (final concentrations: 2.5×10^{-5} to 5×10^{-8} M) and the changes in CI were monitored for at least 72 hours at 10 kHz. In case of the control wells, the adequate volume ratio of DMSO was added. Three parallels were measured for each measurement. The CI values of each concentration obtained at 72 h after the treatment were normalized to that of the DMSO control. The IC_{50} value was calculated for these normalized CI values by fitting a sigmoidal dose-response curve with the nonlinear regression function of OriginPro 8 (OriginLab Corporation, Northampton, MA, USA).

Colorimetric Assay

The antiproliferative/cytotoxic effects of the imipridones on COLO-205 and A2058 cell lines were measured by the alamarBlue-assay. This colometric assay proved to be the more suitable method for the analysis of these cell lines than the xCELLigence System, because COLO-205 cells show weak/negligible adhesion, while A2058 cell line fails to establish a stable plateau phase during the impedimetric analysis. The inoculation of the cells and the procedure of alamarBlue-assay were analogous to the description reported in our previous paper [24]. The main steps are the following: (i) cell seeding on 96-well plates (Sarstedt AG, Nümbrecht, Germany) at 10^4 cells/well concentration, (ii) treatment with the test substances at 2.5×10^{-5} to 5×10^{-8} M final concentrations for 72 h, (iii) addition of alamarBlue reagent (0.15 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) solved in PBS (phosphate-buffered saline, pH = 7.2) and (iv) reading the fluorescence intensity of the samples after 6-8 h incubation with alamarBlue reagent. LS-50B Luminescence Spectrometer (Perkin Elmer Ltd., Buckinghamshire United Kingdom) was applied for the fluorescence measurements with the following settings: excitation wavelength = 560 nm and emission wavelength = 590 nm. Each measurement was done in triplicates. Wells containing adequate volume ratio of DMSO served as control. The fluorescence intensity of each sample was expressed as a ratio of the fluorescence of DMSO control. The nonlinear regression function of OriginPro 8 (OriginLab Corporation, Northampton, MA, USA) was used for fitting sigmoidal

dose-response curves to the normalized fluorescence intensities in order to calculate the IC_{50} values.

Statistical evaluation of data Evaluation of the results was performed using RTCA 2.0 (ACEA Biosciences, San Diego, CA, USA), MS Excel, OriginPro 8 (OriginLab Corporation, Northampton, MA, USA) software. Data obtained from each experiment represent mathematical averages. The standard deviations of IC_{50} parameters were also obtained with the sigmoidal curve fitting.

Short term cytotoxicity studies using MTT assay

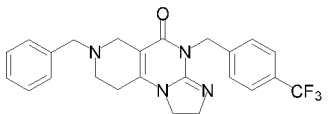
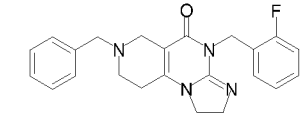
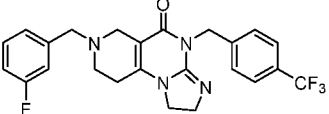
For the short term cytotoxicity studies the A-431 (human squamous carcinoma) and U-87 (human primary glioblastoma) cells were cultured in RPMI-1640 medium supplemented with 10% FCS (fetal calf serum, Sigma Ltd.), 2 mM L-glutamine, and 160 mg/mL gentamicin. Cell cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2 . The cells were grown to confluency and were distributed into 96-well plate with initial cell number of 5.0×10^3 per well. After 24 h incubation at 37 °C, the cells were treated with the compounds in 200 μ L final volume containing 1.0 v/v% DMSO. The cells were incubated with the compounds at 10^{-4} - 10^2 μ M concentration range for 1 h. Control cells were treated with serum free medium (RPMI-1640) only or with DMSO (c = 1.0 v/v %) at 37 °C for 1 h. After incubation the cells were washed twice with serum free (RPMI-1640) medium. To determine the in vitro cytostatic effect, the cells were cultured for a further 72 h in 10% serum containing medium. MTT-solution (45 mL, 2 mg/mL, final concentration: 0.37 mg/mL) was added to each well. The respiratory chain [26, 27] and other electron transport systems [28] reduce MTT and thereby form non-water-soluble violet formazane crystals within the cell [29]. The amount of these crystals can be determined spectrophotometrically and serves as an estimate for the number of mitochondria and hence the number of living cells in the well [30]. After 4 h of incubation the cells were centrifuged for 5 min (900 g) and the supernatant was removed. The obtained formazane crystals were dissolved in DMSO (100 mL) and optical density (OD) of the samples was measured at $\lambda = 540$ and 620 nm, respectively, using ELISA Reader (iEMS Reader, Labsystems, Finland). OD620 values were subtracted from OD540 values. The percent of cytostasis was calculated by using the following equation: Cytostatic effect (%) = $[1 - (OD_{treated}/OD_{control})] \times 100$ Values $OD_{treated}$ and $OD_{control}$ correspond to the optical densities of the treated and the control cells, respectively. In each case two independent experiments were carried out with 4 parallel measurements. The 50% inhibitory concentration (IC_{50}) values were determined from the dose-response curves. The curves were defined using Microcal TM Origin1 (version 7.5) software:

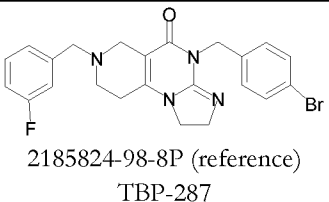
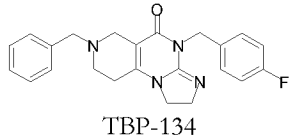
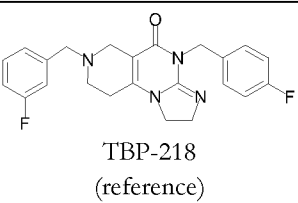
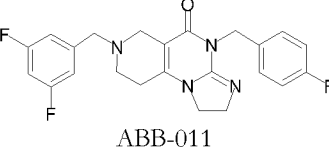
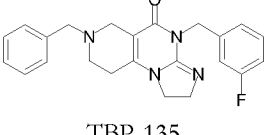
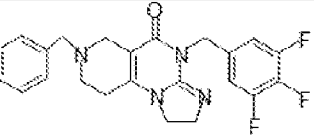
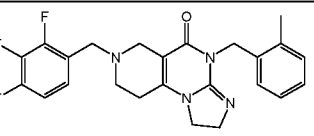
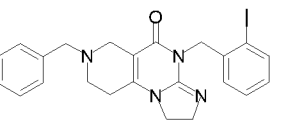
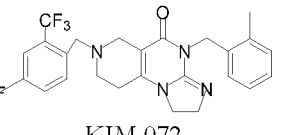
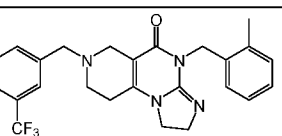
cytostasis was plotted as a function of concentration, fitted to a sigmoidal curve, and based on this curve, the half maximal inhibitory concentration (IC_{50}) value was determined. IC_{50} represents the concentration of a compound that is required for 50% inhibition *in vitro*.

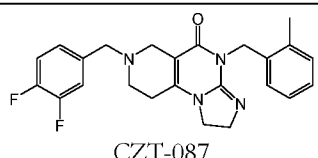
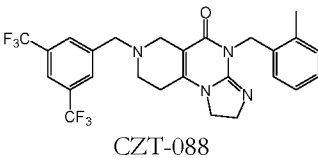
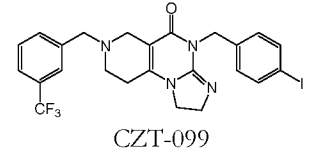
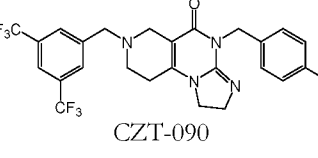
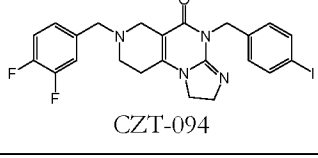
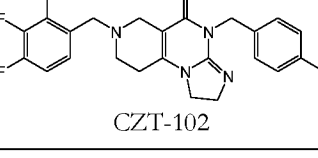
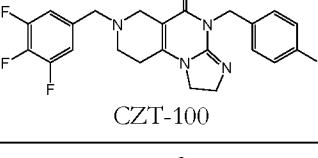
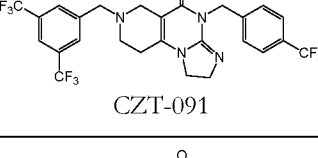
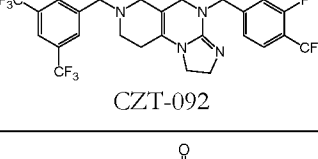
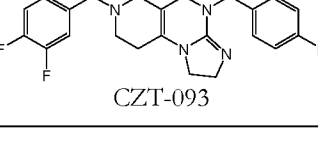
High-throughput screening on EBC-1 and H2228 lung cancer cell lines using CellTiter-Glo luminescent cell viability assay.

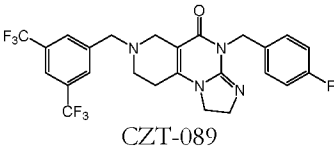
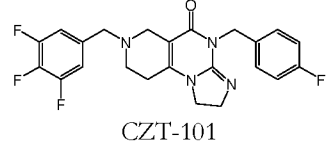
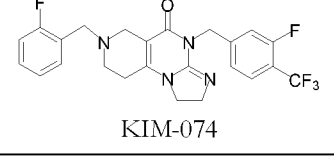
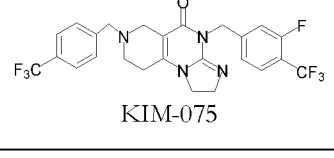
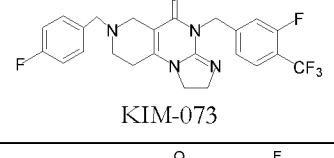
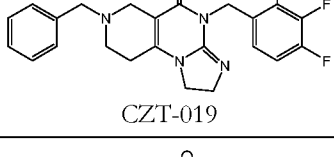
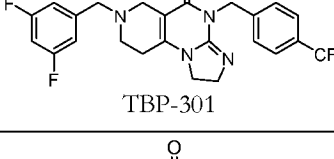
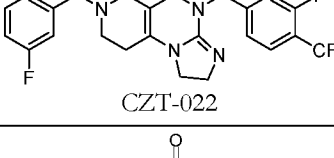
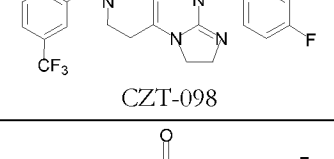
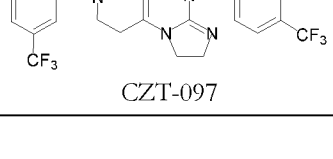
EBC-1 (obtained from JRCB, <https://cellbank.nibiohn.go.jp/english/>) and H2228 (obtained from ATCC <https://www.lgcstandards-atcc.org/>) cell lines were maintained according to the instructions provided by JRCB and ATCC in a 5% CO_2 humidified incubator maintained at 37 °C temperature.. Effect of compounds on cell viability was measured via CellTiter-Glo® luminescent cell viability assay (Promega, Madison, WI, USA). Cells were plated at 1000 cells/well onto a flat-bottomed, white 96 well plate (BRANDplates, cat. no.: 781965). After 24 h, cells were treated for 72 h with the compounds at 100 nM concentration. After the treatment, medium was removed and CellTiter-Glo® reagent was added. Untreated cells were used as control. The luminescence signal was recorded using a microplate reader (BioTek Synergy 2 Multi-Mode Reader, BioTek, Winooski, VT, USA). Cell viability data (% of untreated control cells) was evaluated with Microsoft Excel.

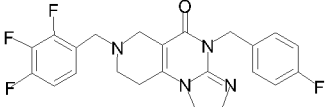
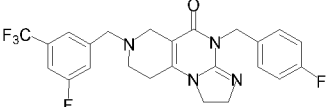
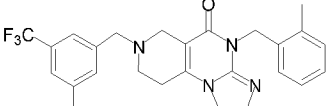
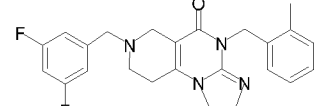
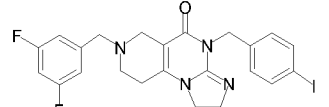
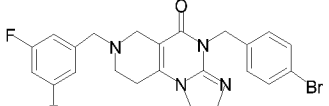
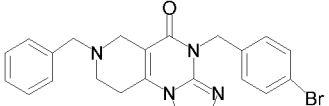
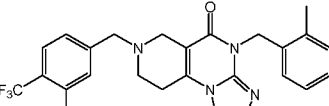
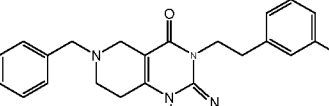
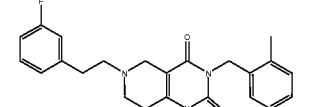
Table 1: The compounds of formula (I) according to the present invention and the results of *in vitro* antiproliferative assays for some representative compounds

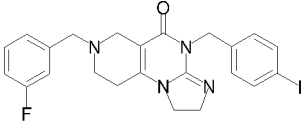
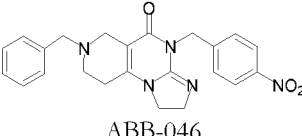
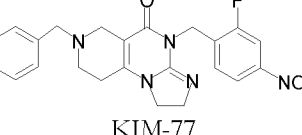
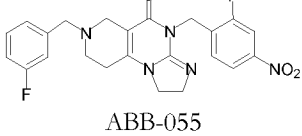
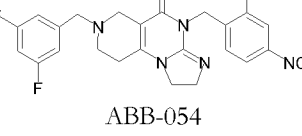
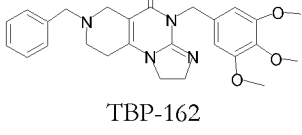
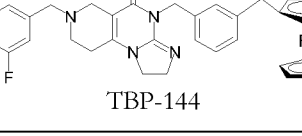
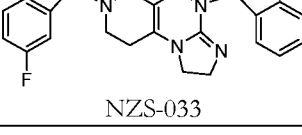
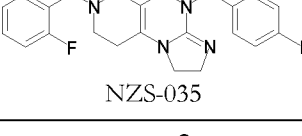
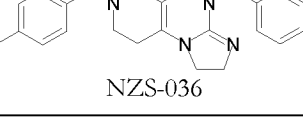
No. of compound	structure and code	Studied cell lines and measured cytotoxicity expressed in IC_{50}						
		A-431	U-87	PANC-1	COLO-205	A-2058	EBC-1 ^a	H2228 ^a
I/1	 ONC-212 (reference)	n/a	n/a	48 nM	250 nM	>10 μ M	<100 nM	>100 nM
I/2	 ONC217 (reference)	n/a	n/a	4.18 μ M	9.97 μ M	2.95 μ M	>100 nM	>100 nM
I/3	 2185824-99-9P (reference) CZT-021	<200 nM	4.16 μ M	44 nM	>10 μ M	>10 μ M	<100 nM	<100 nM

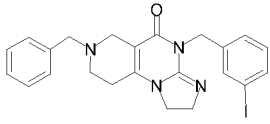
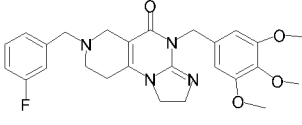
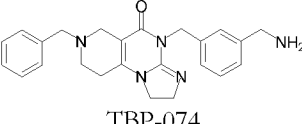
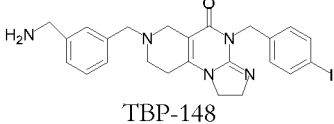
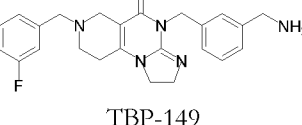
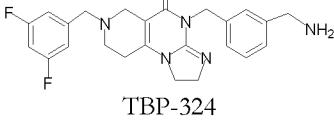
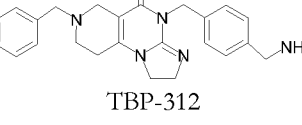
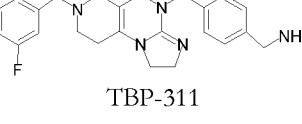
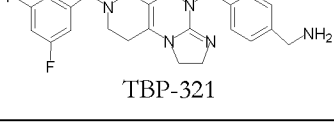
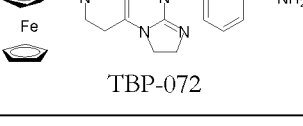
I/4	 2185824-98-8P (reference) TBP-287	n/a	n/a	n/a	n/a	n/a	<100 nM	<100 nM
I/5	 TBP-134	3.55 μ M	>25 μ M	250 nM	247 nM	250 nM	>100 nM	>100 nM
I/6	 TBP-218 (reference)	n/a	n/a	<100 nM	n/a	n/a	<100 nM	<100 nM
I/7	 ABB-011	n/a	n/a	8 nM	n/a	n/a	<100 nM	<100 nM
I/8	 TBP-135	7.29 μ M	>25 μ M	1.42 μ M	4.96 μ M	1.73 μ M	>100 nM	>100 nM
I/9	 KIM-056	1.39 μ M	6.02 μ M	2.22 μ M	>10 μ M	>10 μ M	n/a	n/a
I/10	 KIM-061	n/a	n/a	>10 μ M	>10 μ M	>10 μ M	n/a	n/a
I/11	 TBP-098	n/a	n/a	>10 μ M	>10 μ M	>10 μ M	>100 nM	>100 nM
I/12	 KIM-072	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/13	 CZT-095	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM

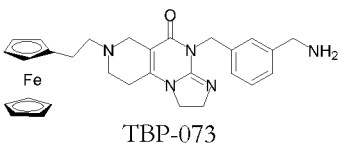
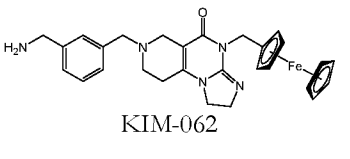
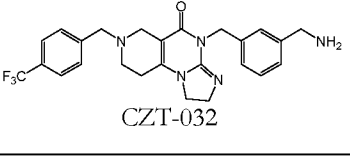
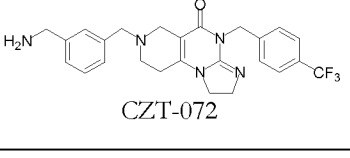
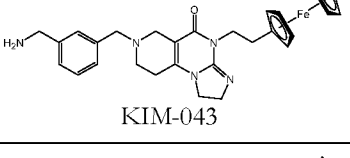
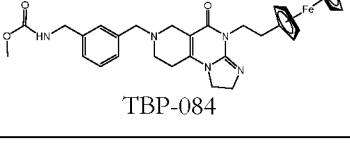
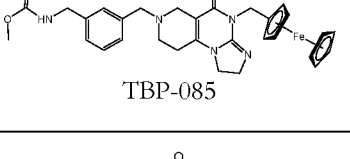
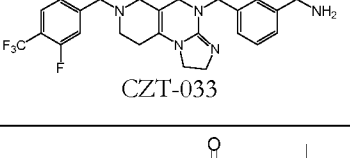
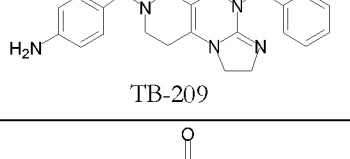
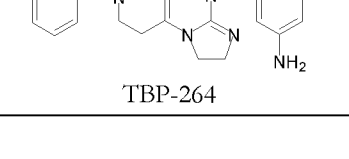
I/14	 CZT-087	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/15	 CZT-088	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/15A	 CZT-099	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/16	 CZT-090	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/17	 CZT-094	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/18	 CZT-102	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/19	 CZT-100	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/21	 CZT-091	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/22	 CZT-092	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/23	 CZT-093	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM

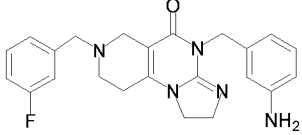
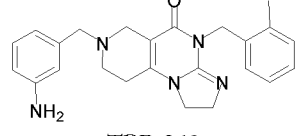
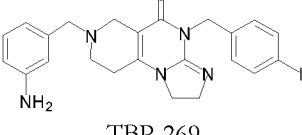
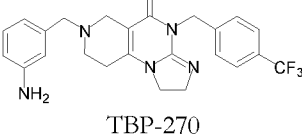
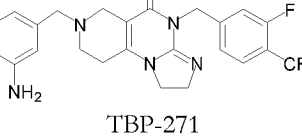
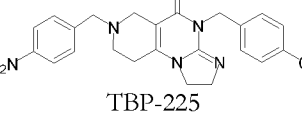
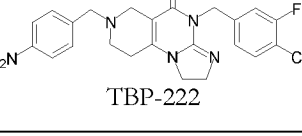
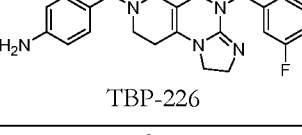
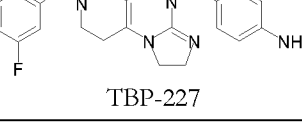
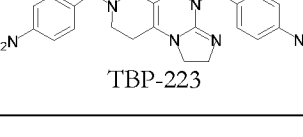
I/24	 CZT-089	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/25	 CZT-101	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/26	 KIM-074	3.63 μM	3.51 μM	920 nM	>10 μM	>10 μM	>100 nM	>100 nM
I/27	 KIM-075	8.95 μM	5.99 μM	25.0 μM	25.0 μM	12.8 μM	>100 nM	>100 nM
I/28	 KIM-073	2.66 μM	17.6 μM	960 nM	3.27 μM	>10 μM	>100 nM	>100 nM
I/29	 CZT-019	n/a	n/a	660 nM	>10 μM	>10 μM	n/a	n/a
I/30	 TBP-301	n/a	n/a	4 nM	n/a	n/a	<100 nM	<100 nM
I/31	 CZT-022	<200 nM	>25 μM	76.7 nM	n/a	n/a	<100 nM	<100 nM
I/32	 CZT-098	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/33	 CZT-097	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM

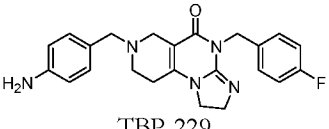
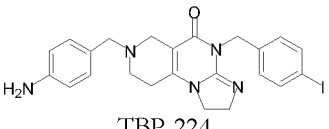
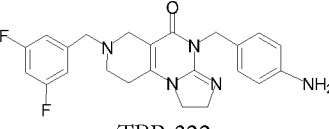
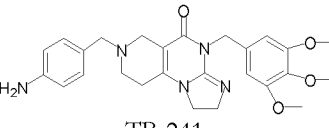
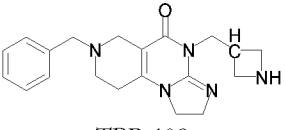
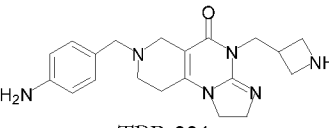
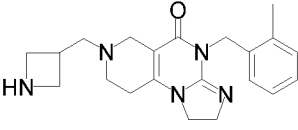
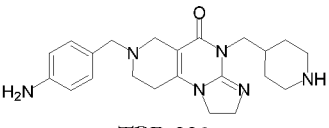
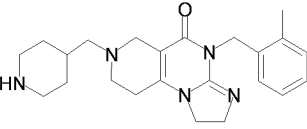
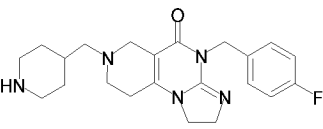
I/34	 CZT-103	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/35	 ABB-010	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/36	 ABB-008	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/37	 ABB-007	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/38	 TBP-317	n/a	n/a	n/a	n/a	n/a	<100 nM	<100 nM
I/39	 TBP-302	n/a	n/a	3 nM	n/a	n/a	<100 nM	<100 nM
I/41	 TBP-285 (reference)	n/a	n/a	n/a	n/a	n/a	>100 nM	<100 nM
I/43	 CZT-028	n/a	n/a	>10 μM	>10 μM	>10 μM	>100 nM	>100 nM
I/44	 KIM-039	n/a	n/a	>10 μM	>10 μM	>10 μM	n/a	n/a
I/45	 KIM-041	n/a	n/a	>10 μM	>10 μM	>10 μM	n/a	n/a

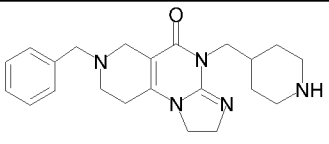
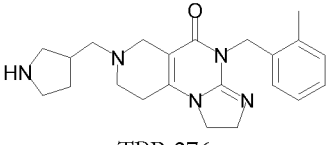
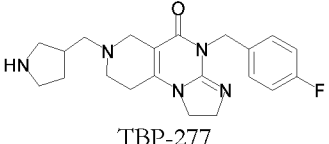
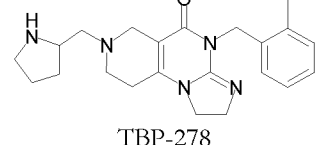
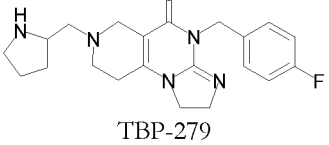
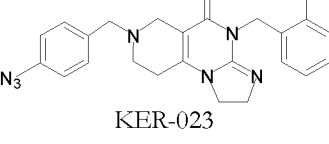
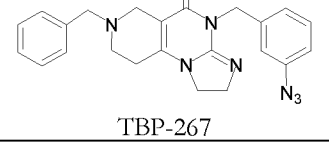
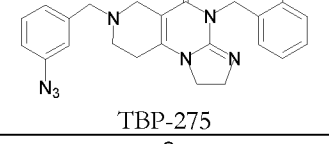
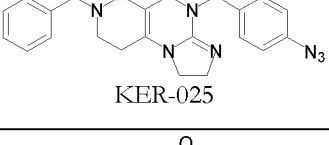
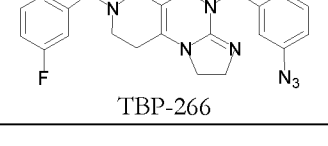
I/46	 TBP-150	6.16 μM	25 μM	25 nM	n/a	>10 μM	<100 nM	~100 nM
I/47	 ABB-046	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/48	 KIM-77	n/a	n/a	6.16 μM	25 μM	500 nM	>100 nM	>100 nM
I/49	 ABB-055	n/a	n/a	n/a	n/a	n/a	<100 nM	<100 nM
I/50	 ABB-054	n/a	n/a	n/a	n/a	n/a	<100 nM	<100 nM
I/51	 TBP-162	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/52	 TBP-144	n/a	n/a	>10 μM	>10 μM	>10 μM	>100 nM	>100 nM
I/53	 NZS-033	n/a	n/a	2.0 μM	2.0 μM	n/a	>100 nM	>100 nM
I/54	 NZS-035	n/a	n/a	1.8 μM	2.0 μM	n/a	n/a	n/a
I/55	 NZS-036	n/a	n/a	760 nM	1.0 μM	n/a	>100 nM	>100 nM

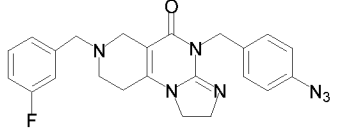
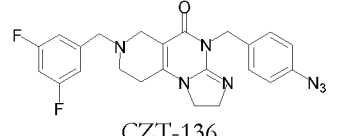
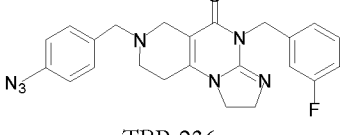
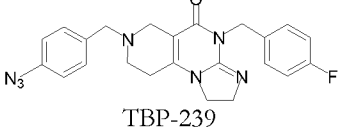
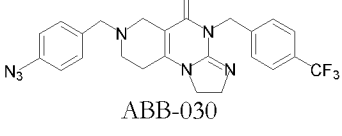
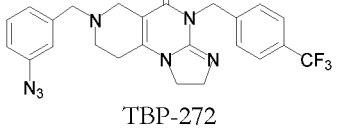
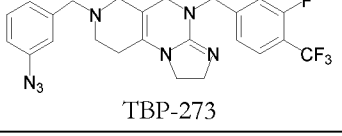
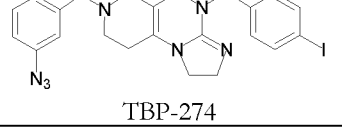
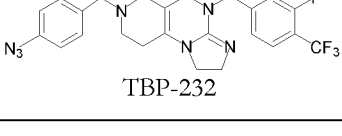
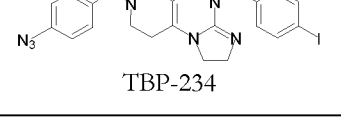
I/56	 TBP-303	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/57	 TBP-161	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/58	 TBP-074	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/59	 TBP-148	n/a	n/a	660 nM	n/a	n/a	>100 nM	>100 nM
I/60	 TBP-149	<200 nM	416 nM	430 nM	>10 μM	>10 μM	>100 nM	>100 nM
I/61	 TBP-324	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/62	 TBP-312	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/63	 TBP-311	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/64	 TBP-321	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/65	 TBP-072	n/a	n/a	>10 μM	>10 μM	21.4 μM	>100 nM	>100 nM

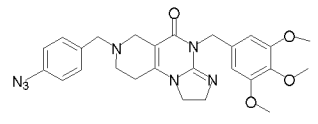
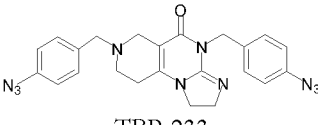
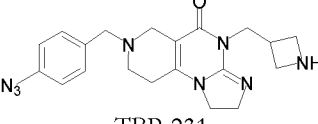
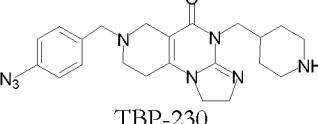
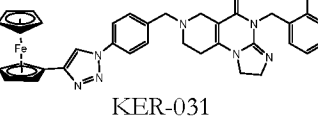
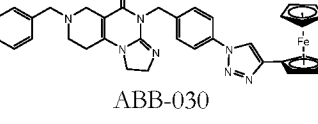
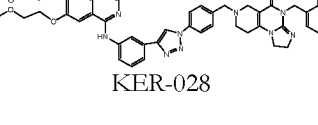
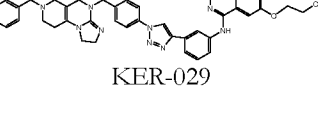
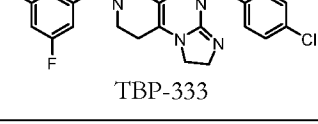
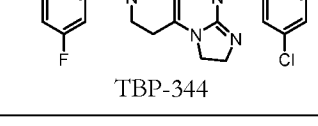
I/66	 TBP-073	n/a	n/a	>10 μM	13.1 μM	20.4 μM	n/a	n/a
I/67	 KIM-062	n/a	n/a	>10 μM	>10 μM	>10 μM	n/a	n/a
I/68	 CZT-032	n/a	n/a	>10 μM	>10 μM	>10 μM	>100 nM	>100 nM
I/69	 CZT-072	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/70	 KIM-043	n/a	n/a	>10 μM	>10 μM	>10 μM	>100 nM	>100 nM
I/71	 TBP-084	n/a	n/a	>10 μM	>10 μM	>10 μM	n/a	n/a
I/72	 TBP-085	n/a	n/a	>10 μM	>10 μM	>10 μM	>100 nM	>100 nM
I/73	 CZT-033	n/a	n/a	>10 μM	>10 μM	>10 μM	n/a	n/a
I/74	 TB-209	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/75	 TBP-264	n/a	n/a	6.1 μM	n/a	25.0 μM	>100 nM	>100 nM

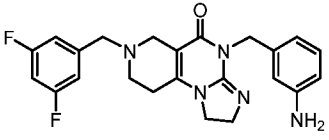
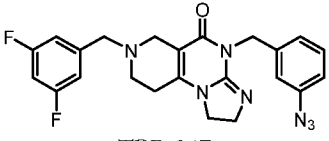
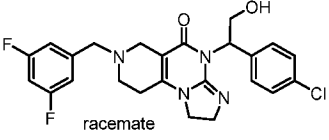
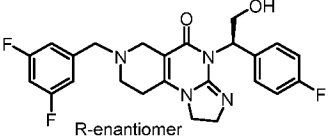
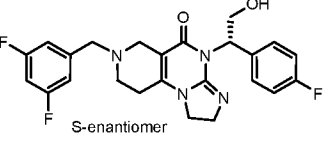
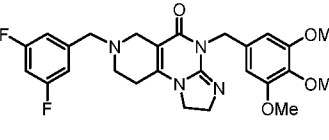
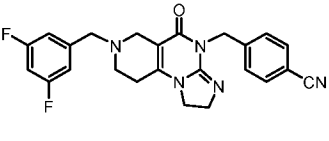
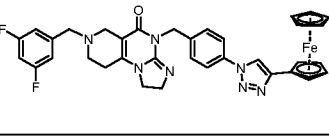
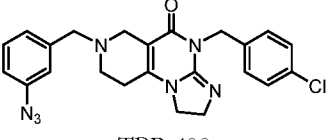
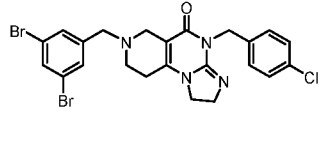
I/76	 TBP-265	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/77	 TBP-268	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/78	 TBP-269	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/79	 TBP-270	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/80	 TBP-271	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/81	 TBP-225	n/a	n/a	21.0 μ M	n/a	n/a	>100 nM	>100 nM
I/82	 TBP-222	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/83	 TBP-226	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/84	 TBP-227	n/a	n/a	23.0 μ M	n/a	n/a	>100 nM	>100 nM
I/85	 TBP-223	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM

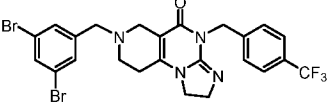
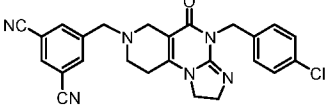
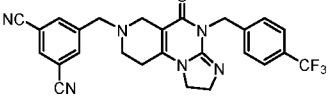
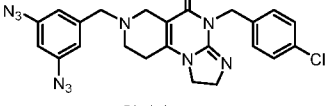
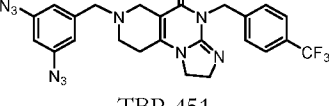
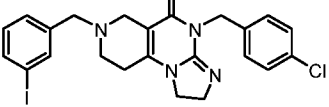
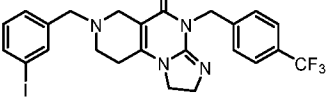
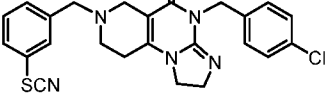
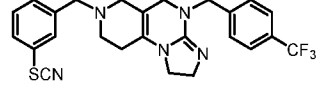
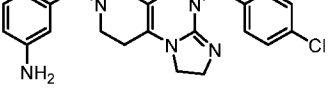
I/86	 TBP-229	n/a	n/a	25.0 μM	n/a	n/a	>100 nM	>100 nM
I/87	 TBP-224	n/a	n/a	16.0 μM	n/a	n/a	>100 nM	>100 nM
I/88	 TBP-322	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/89	 TB-241	n/a	n/a	25 μM	n/a	n/a	>100 nM	>100 nM
I/90	 TBP-109	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/91	 TBP-221	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/92	 TBP-180	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/93	 TBP-220	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/94	 TBP-247	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/95	 TBP-248	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM

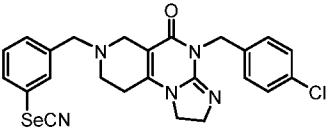
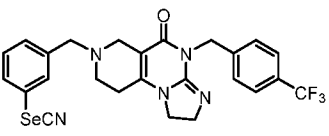
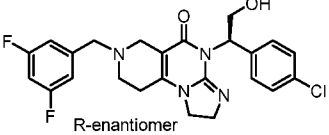
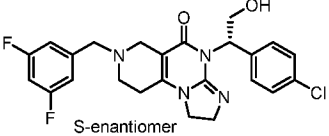
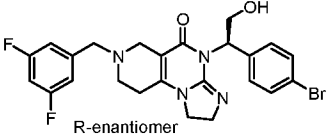
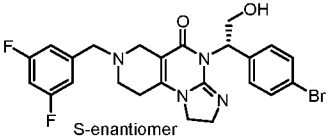
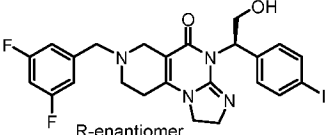
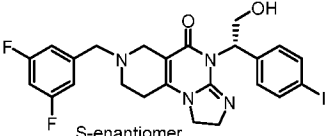
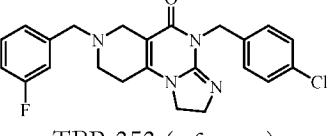
I/96	 TBP-246	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/97	 TBP-276	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/98	 TBP-277	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/99	 TBP-278	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/100	 TBP-279	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/101	 KER-023	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/102	 TBP-267	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/103	 TBP-275	n/a	n/a	0.27 μM	n/a	25.0 μM	>100 nM	>100 nM
I/104	 KER-025	n/a	n/a	500 nM	250 nM	2.5 μM	n/a	n/a
I/105	 TBP-266	n/a	n/a	n/a	n/a	n/a	<100 nM	~100 nM

I/106	 ABB-034	n/a	n/a	n/a	n/a	n/a	~100 nM	>100 nM
I/107	 CZT-136	n/a	n/a	11 nM	n/a	n/a	<100 nM	<100 nM
I/108	 TBP-236	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/109	 TBP-239	n/a	n/a	8.9 μM	n/a	n/a	n/a	n/a
I/110	 ABB-030	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/111	 TBP-272	n/a	n/a	10 nM	n/a	n/a	<100 nM	<100 nM
I/112	 TBP-273	n/a	n/a	100 nM	n/a	25.0 μM	<100 nM	<100 nM
I/113	 TBP-274	n/a	n/a	250 nM	n/a	250 nM	<100 nM	<100 nM
I/114	 TBP-232	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/115	 TBP-234	n/a	n/a	n/a	n/a	n/a	n/a	n/a

I/116	 TBP-242	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/117	 TBP-233	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/118	 TBP-231	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/119	 TBP-230	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/120	 KER-031	n/a	n/a	n/a	n/a	5.0 μM	>100 nM	>100 nM
I/121	 ABB-030	n/a	n/a	n/a	n/a	2.5 μM	>100 nM	>100 nM
I/122	 KER-028	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/123	 KER-029	n/a	n/a	n/a	n/a	n/a	>100 nM	>100 nM
I/124	 TBP-333	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/125	 TBP-344	n/a	n/a	n/a	n/a	n/a	n/a	n/a

I/126	 <p>TBP-346</p>	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/127	 <p>TBP-347</p>	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/128	 <p>racemate TBP-342</p>	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/129 (R)	 <p>R-enantiomer TBP-339</p>	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/129 (S)	 <p>S-enantiomer TBP-339</p>	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/130		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/131		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/132		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/133	 <p>TBP-400</p>	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/134		n/a	n/a	n/a	n/a	n/a	n/a	n/a

I/135		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/136	 CZT-261	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/137	 TBP-453	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/138	 CZT-224	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/139	 TBP-451	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/140		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/141		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/142		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/143		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/144		n/a	n/a	n/a	n/a	n/a	n/a	n/a

I/145		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/146		n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/128 (R)	 R-enantiomer	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/128 (S)	 S-enantiomer	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/147 (R)	 R-enantiomer	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/147 (S)	 S-enantiomer	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/148 (R)	 R-enantiomer	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/148 (S)	 S-enantiomer	n/a	n/a	n/a	n/a	n/a	n/a	n/a
I/149	 TBP-353 (reference)							

^a The results of High throughput screening (HTS) are presented on lung cancer cell lines EBC-1 and H2228. In subsequent experiments IC₅₀ values were determined for the compounds proved to be potent in the HTS (Table 3).

Example 19:

Some representative imipridones and ONC212 as reference were further tested on the following human malignant cell lines: PC3 and LNCaP (prostate carcinoma); BxPC3, MiaPaCa2 and Panc1 (pancreatic cancer); A549, HCC827, H1993 and H520 (lung carcinoma); MDA-MB-453 and MDA-MB-231 (breast cancer) (see Table 2 and Figures 1-11).

Cell lines were maintained according to the instructions provided by ATCC (<https://www.lgcstandards-atcc.org/>) in a 5% CO₂ humidified incubator maintained at 37 °C temperature. Effects of compounds on cell viability were measured via CellTiter-Glo[®] luminescent cell viability assay (Promega, Madison, WI, USA) Cells were plated at 1000 cells/well onto a flat-bottomed, white 96 well plate (BRANDplates, cat. no.: 781965). After 24 h, cells were treated for 72 h with 3-fold serial diluted compound concentrations (300 nM, 100 nM, 33.3 nM, 11.1 nM, 3.7 nM, 1.2 nM). After the treatment, medium was removed and CellTiter-Glo[®] reagent was added. The luminescence signal was recorded using a microplate reader (BioTek Synergy 2 Multi-Mode Reader, BioTek, Winooski, VT, USA).

Cell viability data (% of untreated control cells) was evaluated with Microsoft Excel. Dose-response curves (using a non-linear regression model, log (inhibitor) vs response, variable slope) were generated and IC₅₀ values were determined using Graph Pad Prism 5.02 software (GraphPad Software, San Diego, CA, USA).

In these tests our compounds proved to be extremely efficient antiproliferative agents characterized by IC₅₀ values in low nanomolar range far superior to ONC212. Particularly TBP-301 and TBP-302 can be regarded as exceptionally potent anticancer agents. On the other hand, the potent azide derivatives CZT-136 and TBP-272 provide unique possibility to identify cellular targets, thus to disclose certain mechanisms of action indispensable to drug-approval.

Table 2: The results of *in vitro* tests (IC₅₀ [nM]) of some representative compounds of the invention and ONC212 (as a reference) on a set of human malignant cell lines

No. of compound (code)	PC3	LNCaP	BxPC3	MiaPaCa2	Panc1	A549	HCC827	H1993	H520

No. of compound (code)	PC3	LNCaP	BxPC3	MiaPaCa2	Panc1	A549	HCC827	H1993	H520
I/1 (ONC212) (reference)	80	52	110	76	74	60	105	90	73
I/7 (ABB-011)	9	5	12	11	11	8	13	10	7
I/107 (CZT-136)	11	5	14	16	14	16	15	12	8
I/111 (TBP-272)	7	5	13	10	9	8	12	6	6
I/30 (TBP-301)	2	2	4	3	3	4	5	4	3
I/39 (TBP-302)	2	2	3	4	4	4	4	3	3
I/124 (TBP-333)					2				
I/61 TBP-324					300				
I/129 (R) TBP-339 (R)					15				
I/129 (S) TBP-339 (S)					265				
I/128 TBP-342					10				

Example 20:

Additional data for demonstrating the effectiveness of compounds of the invention.

Table 3: The results of additional *in vitro* tests (IC_{50} [nM]) of representative compounds of invention and ONC212 (as a reference) on a set of human malignant cell lines

No. of compound (code)	MDA-MB-453	MDA-MB-231	EBC-1	H2228	SCC-25	Detroit 562	Fadu
I/1 (ONC212) (reference)	16	24	76	105	166	32	90
I/7 (ABB-011)	1	2	9	10	13	3	10
I/107 (CZT-136)	2	3	17	24	31	7	19
I/111 (TBP-272)	<1	1	8	13	15	3	11
I/30 (TBP-301)	<1	<1	5	5	6	2	5
I/39 (TBP-302)	<1	<1	4	6	7	3	5
I/124 (TBP-333)			3	3	4	<1	2
I/61 (TBP-324)			29	104	89	84	22
I/126 (TBP-346)			288	269	>300	103	290
I/127 (TBP-347)			16	15	21	10	16
I/129 (S) (TBP-339 (S))			>300	300	>300	120	150
I/128 (TBP-342)			13	12	19	5	12

Example 21: Cell Viability Assay Protocols

Effect of the selected compounds on cancer cell viability was measured via CellTiter-Glo[®] luminescent cell viability assay (Promega, Madison, WI, USA) according to the manufacturer's instructions. Cells were plated onto a flat-bottomed, white 96 well plate (BRANDplates[®], cat. no.: 781965). Density of the seeded cells was optimized based on their size and growth rate, as the follows: Panc-1:750 cells/well; DU 145, PC-3, Capan-1, MIA PaCa-2, SCC-25, FaDu and EBC-1: 1000 cells/well; LNCaP, Detroit 562, MDA-MB-231 and MDA-MB-453 1500 cells/well. After 48 h incubation, cells were treated for 72 h with 3-fold serial diluted compound concentrations (300 nM – 1.2 nM range) Untreated cells (incubated during the 72 h treatment in the

corresponding cell culture medium) were used as control. After the treatment, the luminescence signal was recorded using a microplate reader (BioTek Synergy 2 Multi-Mode Reader, BioTek, Winooski, VT, USA). Dose-response curves were generated by GraphPad Prism 8.4.2. software using a non-linear regression model (variable slope, four parameters), (see Figures 12-51).

Table 4: Cell viability measurements on DU 145 cell line

DU 145 (prostate, carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	8.1 ± 2.2	17.5 ± 4.2	103.1 ± 2.5	101.3 ± 4.3	99.0 ± 4.6	98.2 ± 6.9
I/3 (CZT-021) (ref.)	7.2 ± 1.2	7.1 ± 1.6	15.7 ± 4.1	95.9 ± 5.1	102.0 ± 3.5	100.3 ± 7.5
I/6 (TBP-218) (ref.)	7.9 ± 1.6	8.0 ± 1.4	18.7 ± 2.8	93.4 ± 2.2	96.8 ± 3.7	99.0 ± 6.3
I/149 (TBP-353) (ref.)	8.1 ± 0.2	8.0 ± 1.6	7.8 ± 1.2	59.1 ± 11.9	101.0 ± 4.1	95.6 ± 11.3
I/30 (TBP-301)	8.0 ± 2.9	7.0 ± 1.1	6.6 ± 1.0	7.0 ± 0.9	37.2 ± 10.4	99.6 ± 6.9
I/7 (ABB-011)	8.5 ± 2.4	7.2 ± 2.3	7.4 ± 1.4	7.5 ± 0.6	64.1 ± 8.0	97.3 ± 7.2
I/124 (TBP-333)	7.7 ± 1.8	8.0 ± 1.0	7.0 ± 0.9	7.0 ± 1.1	10.0 ± 1.4	90.2 ± 12.9
I/111 (TBP-272)	8.1 ± 1.9	7.1 ± 2.0	7.7 ± 1.3	33.8 ± 5.0	93.6 ± 6.9	97.7 ± 10.8
I/133 (TBP-400)	8.1 ± 2.6	8.0 ± 2.0	7.6 ± 1.1	45.7 ± 4.4	98.8 ± 6.7	97.1 ± 9.4
I/107 (CZT-136)	9.0 ± 2.9	8.3 ± 2.9	8.7 ± 1.9	48.7 ± 1.7	101.2 ± 6.5	100.2 ± 7.7

Table 5: Cell viability measurements on LNCaP cell line

LNCaP (prostate, carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	15.2 ± 1.7	28.1 ± 1.7	100.6 ± 4.5	101.4 ± 3.3	98.6 ± 5.7	99.4 ± 6.7
I/3 (CZT-021) (ref.)	12.7 ± 1.2	13.8 ± 1.7	24.9 ± 2.2	95.0 ± 7.4	98.7 ± 7.4	99.7 ± 8.8
I/6 (TBP-218) (ref.)	14.4 ± 0.7	15.4 ± 2.0	26.6 ± 2.3	97.9 ± 7.3	101.8 ± 5.6	99.1 ± 7.2
I/149 (TBP-353) (ref.)	13.7 ± 1.1	13.4 ± 0.8	18.0 ± 1.4	54.5 ± 6.4	98.4 ± 3.5	99.7 ± 1.9
I/30 (TBP-301)	11.3 ± 1.1	11.4 ± 0.6	11.8 ± 0.2	15.0 ± 0.4	29.6 ± 2.5	93.6 ± 5.0
I/7 (ABB-011)	13.9 ± 1.0	13.1 ± 0.8	13.8 ± 0.9	17.2 ± 1.9	45.6 ± 7.0	95.0 ± 8.5
I/124 (TBP-333)	13.0 ± 0.7	12.7 ± 0.8	12.4 ± 1.2	13.3 ± 1.6	20.8 ± 2.0	80.9 ± 12.5
I/111 (TBP-272)	11.7 ± 0.7	11.8 ± 0.7	13.6 ± 0.4	32.4 ± 2.3	97.1 ± 2.6	99.6 ± 3.9
I/133 (TBP-400)	13.3 ± 1.2	12.3 ± 1.1	14.7 ± 1.4	33.4 ± 3.1	95.9 ± 6.1	95.8 ± 5.1
I/107 (CZT-136)	13.6 ± 0.6	13.5 ± 0.7	16.3 ± 1.9	37.9 ± 3.3	95.3 ± 2.3	98.1 ± 3.4

Table 6: Cell viability measurements on PC-3 cell line

PC-3 (prostate, adenocarcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	33.3±3.3	60.4±5.2	104.5±5.2	102.4±3.7	106.0±2.8	100.9 ± 4.2
I/3 (CZT-021) (ref.)	32.3±2.6	34.7±1.0	56.5±3.3	110.2±4.1	100.8±4.0	102.9 ± 5.1
I/6 (TBP-218) (ref.)	34.1±2.0	35.3±0.5	60.0±3.3	108.7±7.7	103.6±4.4	100.8 ± 2.4
I/149 (TBP-353) (ref.)	33.1±3.3	33.6±2.2	38.4±1.0	98.6±13.6	104.6±6.5	102.7 ± 6.5
I/30 (TBP-301)	31.3±2.5	32.1±3.6	33.0±1.9	36.6±1.8	78.9±4.6	106.0 ± 2.9
I/7 (ABB-011)	34.3±3.4	34.6±3.0	32.5±3.1	38.9±2.1	82.1±1.6	104.6 ± 3.3
I/124 (TBP-333)	32.7±4.4	32.8±3.2	32.1±3.4	33.9±3.3	46.9±4.2	99.4 ± 7.1
I/111 (TBP-272)	31.3±3.0	32.0±3.2	33.2±3.5	75.5±5.9	106.8±2.4	103.7 ± 7.0
I/133 (TBP-400)	32.9±4.6	32.6±3.3	36.2±2.2	83.5±6.4	105.6±1.8	105.4 ± 2.9
I/107 (CZT-136)	32.8±4.1	32.7±2.6	37.2±1.9	82.3±7.0	107.1±5.9	100.3 ± 3.0

Table 7: Cell viability measurements on Panc-1 cell line

Panc-1 (pancreas, carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	56.9±2.4	81.8±8.1	105.8±5.8	104.6 ± 7.2	105.8±0.5	100.1±2.0
I/3 (CZT-021) (ref.)	53.9±6.5	59.9±7.3	109.4±13.5	105.7 ± 4.2	105.8±4.0	101.8±5.8
I/6 (TBP-218) (ref.)	57.2±3.2	57.1±0.1	86.8±8.7	109.1 ± 4.2	107.8±6.0	104.4±3.8
I/149 (TBP-353) (ref.)	54.0±3.1	58.3±2.5	66.7±3.6	109.1 ± 6.9	107.1±9.0	103.6±5.7
I/30 (TBP-301)	49.1±0.2	46.4±3.1	46.6±2.4	54.4 ± 3.5	100.8±3.4	103.3±3.3
I/7 (ABB-011)	53.5±0.6	60.0±3.6	57.0±2.0	61.1 ± 3.4	108.6±7.6	105.9±8.4
I/124 (TBP-333)	51.2±0.4	50.9±0.3	52.7±4.3	56.9 ± 0.2	99.7±3.0	102.6±4.0
I/111 (TBP-272)	49.7±1.2	50.1±2.0	56.1±4.9	99.6 ± 1.4	106.1±7.3	103.3±7.0
I/133 (TBP-400)	55.7±2.5	56.1±3.7	60.2±6.0	94.1 ± 4.0	108.9±10.2	107.0±14.9
I/107 (CZT-136)	55.0±3.2	54.2±0.6	60.1±9.1	93.6 ± 8.1	107.9±11.5	104.6±7.6

Table 8: Cell viability measurements on Capan-1 cell line

Capan-1 (pancreas, adenocarcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	36.8±3.4	88.1±4.2	98.9 ± 5.4	99.2 ± 3.8	100.3 ± 4.0	99.0 ± 7.1
I/3 (CZT-021) (ref.)	33.0±2.2	38.7±2.8	91.6 ± 10.6	101.5 ± 5.5	102.7 ± 6.9	104.9 ± 8.1
I/6 (TBP-218) (ref.)	37.4±3.5	40.1±3.7	90.2 ± 5.9	106.0 ± 8.0	105.9 ± 7.8	100.8 ± 11.1
I/149 (TBP-353) (ref.)	35.3±3.3	34.8±1.9	49.6 ± 5.0	99.6 ± 6.1	99.9 ± 9.6	101.0 ± 8.6
I/30 (TBP-301)	33.1±1.7	34.0±2.8	34.7 ± 2.0	38.4 ± 4.5	100.8 ± 5.3	98.5 ± 5.3
I/7 (ABB-011)	35.5±3.3	36.1±2.8	38.5 ± 5.3	46.0 ± 7.5	101.9 ± 3.8	102.9 ± 9.2
I/124 (TBP-333)	34.6±2.4	33.6±1.1	36.1 ± 1.7	35.3 ± 3.1	88.5 ± 1.8	100.8 ± 3.8
I/111 (TBP-272)	30.8±3.3	31.7±1.7	34.5 ± 3.3	95.1 ± 8.2	102.5 ± 4.0	107.5 ± 2.2
I/133 (TBP-400)	34.0±2.8	32.6±4.0	36.4 ± 3.8	98.8 ± 7.9	108.3 ± 8.3	107.0 ± 8.1
I/107 (CZT-136)	34.7±2.9	37.6±2.3	42.2 ± 3.0	101.2 ± 5.2	103.5 ± 3.0	105.0 ± 4.7

Table 9: Cell viability measurements on MIA PaCa-2 cell line

MIA PaCa-2 (pancreas, carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	17.3 ± 8.8	43.4±13.5	106.1± 8.9	108.5±11.2	105.7±8.5	105.1±4.9
I/3 (CZT-021) (ref.)	17.2±7.5	17.5±7.9	34.2± 10.0	105.2±3.4	107.1±6.4	107.4±2.5
I/6 (TBP-218) (ref.)	19.5 ± 7.5	21.6±9.1	58.7 ± 14.7	110.7±6.4	101.6±10.8	107.0±5.9
I/149 (TBP-353) (ref.)	18.2± 8.3	17.8±6.8	21.1± 10.1	95.0±11.8	104.7±9.9	106.9±7.5
I/30 (TBP-301)	17.6 ± 7.6	16.7±6.7	16.8 ± 7.1	19.1±8.0	65.7±12.6	105.3±10.4
I/7 (ABB-011)	19.8 ± 8.6	18.5±6.8	18.9 ± 8.3	23.1±6.3	87.4±12.0	107.3±4.0
I/124 (TBP-333)	18.0 ± 7.3	18.7±7.1	17.8 ± 7.0	18.9±7.1	33.5±12.1	102.7±2.6
I/111 (TBP-272)	16.2 ± 6.9	16.5±6.7	18.0 ± 5.9	90.6±9.3	100.2±8.8	106.6±5.1
I/133 (TBP-400)	17.4 ± 7.9	18.4±7.8	21.6 ± 8.9	92.1±13.2	104.0±7.3	110.2±2.5
I/107 (CZT-136)	17.4 ± 7.1	18.8±6.2	23.1 ± 7.8	91.5±6.6	102.5±5.4	106.6±8.0

Table 10: Cell viability measurements on Detroit 562 cell line

Detroit 562 (pharynx, carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	32.0±5.0	35.8±4.4	57.0±20.1	104.0±7.2	104.1±4.8	104.6±4.5
I/3 (CZT-021) (ref.)	28.7±4.1	28.9±5.3	31.8±4.9	44.3±11.0	99.5±8.4	101.7±5.1
I/6 (TBP-218) (ref.)	33.0±3.4	31.6±2.1	34.2±3.2	50.9±15.2	99.8±6.4	103.1±7.4
I/149 (TBP-353) (ref.)	34.1±4.4	29.9±4.5	29.3±4.6	33.4±5.2	76.7±9.1	99.2±5.8
I/30 (TBP-301)	28.7±2.5	24.7±2.9	26.1±3.8	27.0±5.5	31.9±6.7	61.9±14.5
I/7 (ABB-011)	33.0±4.8	28.6±3.6	27.0±4.9	28.4±4.2	35.1±5.8	85.6±7.7
I/124 (TBP-333)	31.5±3.7	28.3±3.6	28.1±3.9	27.8±3.6	29.4±4.9	40.4±6.5
I/111 (TBP-272)	28.8±3.8	26.8±2.8	27.3±2.7	32.8±3.7	48.0±21.7	102.3±5.9
I/133 (TBP-400)	31.3±4.1	28.8±3.6	28.5±3.9	31.1±6.5	47.1±16.0	102.1±2.3
I/107 (CZT-136)	34.1±6.5	31.4±2.9	32.9±3.6	36.8±5.1	86.6±5.7	101.7±3.0

Table 11: Cell viability measurements on SCC-25 cell line

SCC-25 (tongue, squamous cell carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	37.6±4.3	76.7±9.9	106.7±9.3	105.5±12.7	108.5±8.6	101.3±6.8
I/3 (CZT-021) (ref.)	33.1±1.7	34.9±3.9	64.7±4.6	95.1±5.1	101.0±4.9	103.8±2.7
I/6 (TBP-218) (ref.)	41.5±3.1	42.5±1.8	80.1±10.1	96.2±4.8	101.8±4.3	98.7±6.6
I/149 (TBP-353) (ref.)	42.3±4.7	35.4±2.0	40.2±4.0	89.0±5.5	97.5±7.7	99.4±7.0
I/30 (TBP-301)	36.3±2.6	32.9±2.1	32.6±2.0	35.0±4.5	78.7±5.0	103.4±11.6
I/7 (ABB-011)	39.7±2.7	35.1±2.2	36.5±3.7	45.1±4.7	88.7±5.6	93.6±5.7
I/124 (TBP-333)	42.3±5.2	37.5±1.4	34.8±3.6	34.3±2.0	47.2±5.8	89.3±5.4
I/111 (TBP-272)	36.4±0.9	32.5±1.2	34.4±2.7	79.0±11.0	98.3±4.0	102.1±4.0
I/133 (TBP-400)	40.9±3.7	34.7±4.9	35.5±1.7	72.9±3.0	102.3±4.2	102.3±3.4
I/107 (CZT-136)	42.4±5.7	35.4±5.7	41.7±2.1	75.6±6.8	97.3±3.8	94.2±9.5

Table 12: Cell viability measurements on FaDu cell line

FaDu (pharynx, squamous cell carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	25.6±5.3	46.9±5.6	97.7 ± 8.3	98.8±4.7	102.4 ± 7.4	101.7±2.4
I/3 (CZT-021) (ref.)	22.4±5.9	26.2±7.9	51.4 ± 25.3	100.9±5.9	101.6 ± 3.4	98.3±4.2
I/6 (TBP-218) (ref.)	25.2±4.6	27.3±5.2	50.7 ± 8.5	97.8±5.2	97.9 ± 3.9	98.8±10.4
I/149 (TBP-353) (ref.)	23.4±3.6	25.0±5.4	27.9 ± 5.1	83.1±9.5	98.2 ± 3.2	99.5±4.9
I/30 (TBP-301)	22.3±5.2	21.4±4.5	22.4 ± 5.4	24.7±3.7	69.5 ± 11.0	99.4±5.4
I/7 (ABB-011)	26.1±4.5	24.0±3.8	24.6 ± 3.5	29.1±5.6	80.1 ± 13.3	100.8±6.6
I/124 (TBP-333)	24.7±5.0	24.5±4.6	23.8 ± 4.1	26.1±6.2	45.6 ± 20.7	95.3±7.0
I/111 (TBP-272)	22.1±4.8	21.7±5.4	24.8 ± 3.6	66.8±12.0	97.7 ± 3.9	100.7±2.4
I/133 (TBP-400)	24.6±6.8	25.3±6.4	26.5 ± 6.4	70.7±13.1	100.0 ± 6.2	100.3±5.8
I/107 (CZT-136)	25.5±4.3	24.6±3.6	27.1 ± 4.0	74.3±4.2	98.6 ± 1.9	102.2±7.8

Table 13: Cell viability measurements on EBC-1 cell line

EBC-1 (lung, squamous cell carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	24.2 ± 2.1	43.4±6.8	99.2±0.9	110.1±11.9	98.8±5.8	95.3±2.2
I/3 (CZT-021) (ref.)	22.2 ± 0.1	25.1±0.2	82.6±6.4	96.3±1.4	97.8±4.0	99.7±0.8
I/6 (TBP-218) (ref.)	24.1 ± 2.4	25.1±0.3	56.6±0.5	101.7±3.3	101.0±9.5	100.2±1.5
I/149 (TBP-353) (ref.)	23.6 ± 1.3	23.2±0.7	25.8±1.0	89.0±5.6	97.7±13.2	102.7±15.3
I/30 (TBP-301)	20.1 ± 0.0	21.3±0.6	20.8±0.5	24.8±1.1	76.4±2.2	103.9±7.1
I/7 (ABB-011)	23.8 ± 0.6	23.2±0.9	23.4±0.2	25.6±0.1	79.8±4.1	101.3±0.5
I/124 (TBP-333)	23.5 ± 1.3	22.4±0.5	22.6±1.6	25.3±0.7	70.9±8.3	103.3±1.5
I/111 (TBP-272)	20.0 ± 0.0	20.6±0.6	21.4±0.5	54.0±6.5	94.1±3.0	99.7±3.8
I/133 (TBP-400)	22.2 ± 1.3	23.2±0.7	23.1±2.1	60.1±6.7	97.1±6.0	100.8±4.3
I/107 (CZT-136)	23.5 ± 2.5	23.5±1.0	23.7±1.3	70.3±0.9	99.9±2.5	100.0±5.9

Table 14: Cell viability measurements on MDA-MB-231 cell line

MDA-MB-231 (breast, adenocarcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	56.8±2.1	87.9±4.4	98.5±1.6	97.9±1.1	101.0±4.4	99.9 ± 6.2
I/3 (CZT-021) (ref.)	53.7±3.1	56.5±3.4	84.2±7.0	99.8±1.8	101.4±1.4	99.3 ± 3.4
I/6 (TBP-218) (ref.)	57.4±1.7	57.7±3.8	91.8±4.3	99.4±4.1	100.5±5.4	98.9 ± 4.7
I/149 (TBP-353) (ref.)	57.5±3.8	59.1±3.5	63.0±4.3	100.0±1.3	101.6±4.0	100.9 ± 2.2
I/30 (TBP-301)	52.6±2.5	52.1±1.8	54.6±1.1	59.9±2.1	100.0±4.5	99.8 ± 3.6
I/7 (ABB-011)	59.4±3.0	58.6±3.0	58.8±1.9	63.6±4.4	99.6±2.2	98.4 ± 2.1
I/124 (TBP-333)	54.2±2.4	55.9±5.3	56.5±2.8	59.4±4.5	82.4±7.2	100.8 ± 0.9
I/111 (TBP-272)	52.8±2.7	56.9±2.2	59.8±3.6	98.5±2.1	102.7±2.8	102.2 ± 3.2
I/133 (TBP-400)	56.4±3.5	58.2±2.5	60.8±2.4	100.4±2.9	101.4±2.0	102.1 ± 2.5
I/107 (CZT-136)	55.7±0.9	57.3±1.8	59.6±3.0	98.6±0.8	102.1±3.5	100.6 ± 5.8

Table 15: Cell viability measurements on MDA-MB-453 cell line

MDA-MB-453 (breast, carcinoma) cell viability (% of control)						
	300 nM	100 nM	33.3 nM	11.1 nM	3.7 nM	1.2 nM
I/1 (ONC-212) (ref.)	32.5±0.5	37.9±1.3	107.4±12.7	110.8 ± 2.3	111.0±7.3	104.0±3.3
I/3 (CZT-021) (ref.)	29.8±1.5	33.2±2.0	37.3±0.5	97.0 ± 13.3	110.3±7.7	109.9±5.4
I/6 (TBP-218) (ref.)	32.0±1.1	33.9±1.3	39.8±1.9	103.7 ± 10.4	113.6±9.8	113.6±8.3
I/149 (TBP-353) (ref.)	31.2±0.9	33.3±2.6	34.1±2.4	57.0 ± 18.5	108.6±13.2	110.0±3.0
I/30 (TBP-301)	28.9±1.0	31.1±1.8	32.1±2.0	34.8 ± 3.3	58.0±24.4	109.5±10.7
I/7 (ABB-011)	31.6±0.9	33.1±1.1	34.0±1.9	35.8 ± 1.3	55.8±16.5	104.7±14.5
I/124 (TBP-333)	30.9±1.4	31.2±2.2	33.0±1.0	33.5 ± 1.8	37.9±3.2	86.3±23.2
I/111 (TBP-272)	28.8±0.4	30.3±1.0	34.1±2.4	45.7 ± 5.0	107.2±5.9	115.8±7.5
I/133 (TBP-400)	31.7±0.5	31.9±1.3	35.0±1.7	50.1 ± 10.6	108.6±9.7	110.3±8.8
I/107 (CZT-136)	32.2±1.3	32.3±1.3	35.2±2.1	47.8 ± 6.2	107.2±9.1	108.1±7.7

Example 22:

This example demonstrates the cytotoxic effect of some compounds according to the invention on Panc-1 human cell line. The IC₅₀ values were determined in xCELLigence SP instrument (see Figures 52-61). The following table shows the IC₅₀ values obtained after 24 h, 48 h, 72 h and 96 h treating.

Table 16: Results of cytotoxicity measurements on Panc-1 cell line

No. of compound (code)	IC ₅₀ (24 h) [nM]	IC ₅₀ (48 h) [nM]	IC ₅₀ (72 h) [nM]	IC ₅₀ (96 h) [nM]
I/1 (ONC-212) (ref.)	ND	ND	274 ± 15.9	185 ± 44.4
I/3 (CZT-021) (ref.)	ND	ND	45.3 ± 3.34	22.6 ± 0.856
I/6 (TBP-218) (ref.)	ND	ND	126 ± 5.96	75.5 ± 6.76
I/149 (TBP-353) (ref.)	ND	ND	45.0 ± 27.3	21.8 ± 5.29
I/30 (TBP-301)	ND	ND	2.08 ± 0.267	0.517 ± 0.0232
I/7 (ABB-011)	ND	ND	27.3 ± 15.4	22.1 ± 5.98
I/124 (TBP-333)	ND	ND	1.31 ± 0.286	<0.25
I/111 (TBP-272)	ND	ND	20.1 ± 1.68	8.58 ± 0.256
I/133 (TBP-400)	ND	ND	9.05 ± 0.669	4.73 ± 0.109
I/107 (CZT-136)	ND	ND	102 ± 24.4	49.6 ± 17.0

ND: not detectable

Example 23:

This example demonstrates the antitumoral effects of compounds I/1 (ONC 212), I/7 (ABB-011), I/124 (TBP-333) and I/107 (CZT-136) on subcutaneously growing MDA-MB-231 tumor xenografts in SCID mice (immunosuprimized mice).

MDA-MB-231 human triple-negative breast cancer xenografts were formed by subcutaneous inoculation of tumor cells into the back of the immunodeficient (SCID) mice. The investigated materials were injected into the animals intraperitoneal every two-three days for 3 weeks.

The experimental results showed that all of the investigated compounds reduced the tumor volume, however, this effect was significant only in the case of I/124 (TBP-333).

Experimental design:

The tested compounds:

I/1 (ONC 212) as reference: 0.022 mg/animal/treatment; dose: 0.88 mg/kg

0.036 mg/animal/treatment; dose: 1.466 mg/kg

I/7 (ABB-011): 0.0213 mg/animal/treatment, dose: 0.85 mg/kg

0.0355 mg/animal/treatment, dose: 1.416 mg/kg

I/124 (TBP-333): 0.022 mg/animal/treatment dose: 0.89 mg/kg

0.036 mg/animal/treatment; dose: 1.466 mg/kg

I/107 (CZT-136): 0.022 mg/animal/treatment, dose: 0.89 mg/kg

0.036 mg/animal/treatment; dose: 1.466 mg/kg

and physiological saline solution with 1% DMSO as control.

Forty SCID mice were included in the experiment. Each animal group was kept in separate cage during the experiments. On each cage, there was an identification card with birth date, the date of tumor cell injection, number and gender of animals. The injection of each substance was written on the identification card. Eight animals per substance were used and identified by ear cutting.

The animals used in these studies were cared for according to the „Guiding Principles for the Care and Use of Animals” based upon the Helsinki declaration, and the studies were approved by the local ethics committee.

Animals were housed in IVC (Individually Ventilated Cages) system in sterilized cages, with a controlled cycle of 12 hours light and 12 hours dark, the light phase being between 07:00h and 19:00h. Temperature and humidity were recorded daily during the whole experiment. Sterilized rodent special quality control diet (VRF1, Special Diets Services Ltd, Witham, UK) and acidified (pH=3) sterilized distilled water were available ad libitum throughout the study periods.

Each batch of diet was delivered with an accompanying certificate of analysis detailing nutritional composition. The health status of the mice was assessed by animal house staff.

MDA-MB-231 human triple-negative breast cancer cells were grown in RPMI-1640 Medium (Sigma Chemical Co., St. Louis, MO), supplemented with 10% fetal bovine serum (Sigma) and 1% penicillin-streptomycin (Sigma) at 37°C in a humidified atmosphere of 5% CO₂. Cells from monolayer culture were detached with 0.02% EDTA (Sigma), washed twice with serum-free medium, and one-cell suspension was inoculated subcutaneously into the back of SCID mice with a number of 1.3×10⁶ cells/animal. When the tumor volumes reached the detectable size (~100-200 mm³, 12 days after tumor cell inoculation) the test products were inoculated intraperitoneal.

All of the test compounds were provided as powder. The powders were dissolved in DMSO. For the final treatment concentration, the compounds were diluted in physiological saline solution 1:100 (1% DMSO).

All of the test compounds were planned to be administered by intraperitoneal injection in a 0.3 ml volume in physiological saline solution with 1% DMSO three times per week. However, during the experiment this schedule was modified: from 07.19 intraperitoneal injection in a 0.5 ml volume every day was executed (Table 17).

Table 17: Treatment schedule

Treatment schedule				
2021.06.14	2021.06.15	2021.06.16	2021.06.17	2021.06.18
		Tumor inoculation*	Tumor growth	
2021.06.21	2021.06.22	2021.06.23	2021.06.24	2021.06.25
2021.06.28	2021.06.29	2021.06.30	2021.07.01	2021.07.02
2021.07.05	2021.07.06	2021.07.07	2021.07.08	2021.07.09
First treatment 300 µl ≈0.88 mg/kg		300µl ≈0.88 mg/kg		300µl ≈0.88 mg/kg
2021.07.12	2021.07.13	2021.07.14	2021.07.15	2021.07.16
300µl ≈0.88 mg/kg		300µl ≈0.88 mg/kg	300µl ≈0.88 mg/kg	300µl ≈0.88 mg/kg
2021.07.19	2021.07.20	2021.07.21	2021.07.22	2021.07.23
500 µl ≈1.4 mg/kg	500 µl ≈1.4 mg/kg	500 µl ≈1.4 mg/kg	500 µl ≈1.4 mg/kg	Termination

*: Number of inoculated cell: $1.3 \cdot 10^6$

During the experiment weight of the animals and size of the tumors were recorded. The investigated compounds did not cause weight loss (Figure 62).

Tumor volume was determined three times a week during the experiment. All of the investigated compounds reduced the tumor growth, but the effect was significant only in the case of TBP-333 (Figure 63).

Anti-tumor effect of compounds was evaluated also measuring tumor weight in each group after termination of experiment (Figures 64 and 65). Based on tumor weight we could obtain that

the highest, significant inhibition showed TBP-333, but all of the investigated compounds decreased the tumor weight in comparison to control.

The experimental results showed that the tested drugs have an antitumor effect, which was shown both in the volumes and in the tumor masses at the end of the experiment, however, this effect was significant only in the case of TBP-333. It can be assumed that if the solubility of the materials can be improved, or the amount delivered can be increased in any other way, even more significant effects can be achieved.

Based on the forgoing results it should be noted the following:

As for the effect of the substitution pattern of the benzyl groups attached to 4 and 7 positions of the imipridone skeleton, we have unambiguously established that [7-(3'',5''-difluorobenzyl)]-substituted compounds with identical substituent in position 4' (in benzyl group on N-4 atom) exhibit significantly stronger antiproliferative activity than their [7-(3''-fluorobenzyl)]-substituted counterparts (TBP-301 vs. CZT-021, ABB-011 vs. TBP-218 and TBP-333 vs. TBP-353) as clearly reflected from the IC₅₀ values (Table 16) and cell viability data demonstrated in Example 21 obtained by *in vitro* assays performed on human malignant cell lines by different methods. It must be emphasized that even all [7-(3''-fluorobenzyl)]-imipridones including our original azidobenzyl derivatives are far more active than ONC-212 in each comparative *in vitro* test of this invention. The original monoazido derivatives also demonstrated an exceptionally outstanding activity profile against the investigated cell lines. It is of pronounced significance and novelty in efficiency profile that [7-(3''-azidobenzyl)]imipridones TBP-272 and TBP-400 show higher degree of cytotoxicity than the corresponding [7-(3''-fluorobenzyl)]imipridones CZT-021 and TBP-353, respectively, as demonstrated by the IC₅₀ values measured on PANC-1 cell line (Table 16). This tendency was also manifested in the assays carried out by CellTiter-Glo[®] luminescent cell viability assay on a series of cell lines (see Example 21).

Finally, taking all the measured IC₅₀ and cell viability data into account it can be stated that at *in vitro* level imipridone 4-(4-chlorobenzyl)-7-(3,5-difluorobenzyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-*a*]pyrido[3,4-*e*]pyrimidin-5(1*H*)-one (TBP-333), prepared and investigated by us for the first time, is the most potent representative of the members of small molecule anticancer imipridone family physically identified so far.

INDUSTRIAL APPLICABILITY

The compounds of the invention has anticancer activity, thus these compounds are suitable for use in medicine.

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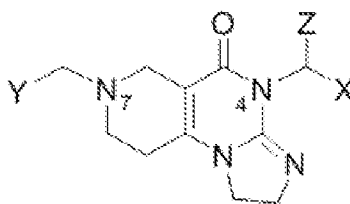
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Claims

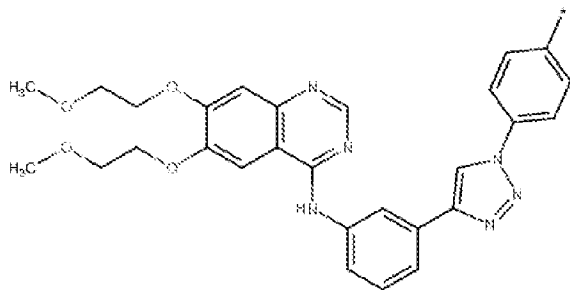
1. Compounds of formula (I)



(I)

wherein

if Y is phenyl and Z is H, then X is 3-fluorophenyl-methyl (Compound I/44), 3-(aminomethyl)phenyl (Compound I/58), 4-(aminomethyl)phenyl (Compound I/62), 3-azetidiny (Compound I/90), 4-piperidiny (Compound I/96), 3-azidophenyl (Compound I/102), 4-azidophenyl (Compound I/104), (4-ferrocenyl-1H-1,2,3-triazol-1-yl)phenyl (Compound I/121) or:



(Compound I/123); or

if Y is 3-fluorophenyl and Z is H, then X is 2-iodoferrocenyl (Compound I/52), 3-(aminomethyl)phenyl (Compound I/60), 4-(aminomethyl)phenyl (Compound I/63), 3-azidophenyl (Compound I/105) or 4-azidophenyl (Compound I/106); or

if Y is 3,5-difluorophenyl and Z is H, then X is 3-(aminomethyl)phenyl (Compound I/61), 4-(aminomethyl)phenyl (Compound I/64), 4-azidophenyl (Compound I/107), 3-azidophenyl (Compound I/127), (4-ferrocenyl-1H-1,2,3-triazol-1-yl)phenyl (Compound I/132); or

if Y is 4-(trifluoromethyl)phenyl and Z is H, then X is or 3-(aminomethyl)phenyl (Compound I/68); or

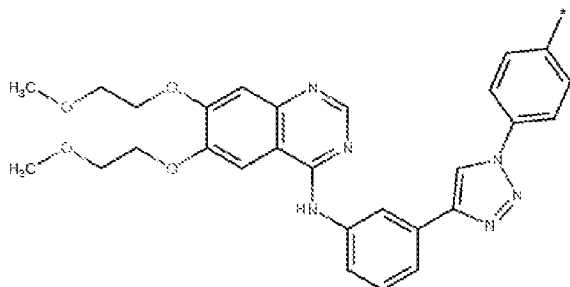
if Y is 3-fluoro-4-(trifluoromethyl)phenyl and Z is H, then X is 3-(aminomethyl)phenyl (Compound I/73); or

if Y is 3-fluorophenylmethyl and Z is H, then X is 2-methylphenyl (Compound I/45); or

if Y is 3-(aminomethyl)phenyl and Z is H, then X is 4-iodophenyl (Compound I/59), ferrocenyl (Compound I/67), 4-(trifluoromethyl)phenyl (Compound I/69) or ferrocenylmethyl (Compound I/70); or

if **Y is ferrocenyl and Z is H**, then X is 3-(aminomethyl)phenyl (Compound I/65); or
 if **Y is ferrocenylmethyl and Z is H**, then X is 3-(aminomethyl)phenyl (Compound I/66); or
 if **Y is 3-(methoxycarbonylamino)methylphenyl and Z is H**, then X is ferrocenylmethyl (Compound I/71) or ferrocenyl (Compound I/72); or
 if **Y is 4-aminophenyl and Z is H**, then X is 3-azetidiny (Compound I/91) or 4-piperidiny (Compound I/93); or
 if **Y is 3-azetidiny and Z is H**, then X is 2-methylphenyl (Compound I/92); or
 if **Y is 4-piperidiny and Z is H**, then X is 2-methylphenyl (Compound I/94) or 4-fluorophenyl (Compound I/95); or
 if **Y is 3-pyrrolidiny and Z is H**, then X is 2-methylphenyl (Compound I/97) or 4-fluorophenyl (Compound I/98); or
 if **Y is 2-pyrrolidiny and Z is H**, then X is 2-methylphenyl (Compound I/99) or 4-fluorophenyl (Compound I/100); or
 if **Y is 4-azidophenyl and Z is H**, then X is 2-methylphenyl (Compound I/101); 3-fluorophenyl (Compound I/108), 4-fluorophenyl (Compound I/109), 4-(trifluoromethyl)phenyl (Compound I/110), 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/114), 4-iodophenyl (Compound I/115), 3,4,5-trimethoxy-phenyl (Compound I/116), 4-azidophenyl (Compound I/117), 3-azetidiny (Compound I/118) or 4-piperidiny (Compound I/119); or
 if **Y is 3-azidophenyl and Z is H**, then X is 2-methylphenyl (Compound I/103), 4-(trifluoromethyl)phenyl (Compound I/111), 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/112), 4-iodophenyl (Compound I/113) or 4-chlorophenyl (Compound I/133); or
 if **Y is (4-ferrocenyl-1H-1,2,3-triazol-1-yl)phenyl and Z is H**, then X is 2-methylphenyl (Compound I/120); or

if **Y is**



then X is 2-methylphenyl (Compound I/122); or

if **Y is 3,5-diazidophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/138) or 4-(trifluoromethyl)phenyl (Compound I/139); or

if **Y is 3-thiocyanatophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/142) or 4-(trifluoromethyl)phenyl (Compound I/143); or

if **Y is 3-selenocyanatophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/145) or 4-(trifluoromethyl)phenyl (Compound I/146);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

2. The compounds according to Claim 1, wherein

if **Y is 3-fluorophenyl and Z is H**, then X is 4-azidophenyl (Compound I/106); or

if **Y is 3,5-difluorophenyl and Z is H**, then X is 4-azidophenyl (Compound I/107); or

if **Y is 3-azidophenyl and Z is H**, then X is 4-(trifluoromethyl)phenyl (Compound I/111) or 4-chlorophenyl (Compound I/133); or

if **Y is 3,5-diazidophenyl and Z is H**, then X is 4-chlorophenyl (Compound I/138) or 4-(trifluoromethyl)phenyl (Compound I/139);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

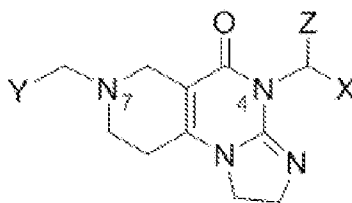
3. The compounds according to Claim 1, wherein

if **Y is 3,5-difluorophenyl and Z is H**, then X is 4-azidophenyl (Compound I/107); or

if **Y is 3-azidophenyl and Z is H**, then X is 4-(trifluoromethyl)phenyl (Compound I/111) or 4-chlorophenyl (Compound I/133);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

4. The compounds of formula (I)



(I)

wherein

if **Y is phenyl and Z is H**, then X is 4-fluorophenyl (Compound I/5), 3-fluorophenyl (Compound I/8), 3,4,5-trifluorophenyl (Compound I/9), 2,3,4-trifluorophenyl (Compound I/29), 2-fluoro-4-nitrophenyl (Compound I/48) or 3-aminophenyl (Compound I/75); or

if Y is 3-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/31), 4-iodophenyl (Compound I/46), 2-fluoro-4-nitrophenyl (Compound I/49), 2-methylphenyl (Compound I/53) or 4-aminophenyl (Compound I/84); or

if Y is 3,5-difluorophenyl and Z is H, then X is 4-fluorophenyl (Compound I/7), 4-(trifluoromethyl)phenyl (Compound I/30), 4-iodophenyl (Compound I/38), 4-bromophenyl (Compound I/39), 2-fluoro-4-nitrophenyl (Compound I/50), 4-chlorophenyl (Compound I/124) or 3-aminophenyl (Compound I/126) or 2-methylphenyl (Compound I/37); or

if Y is 2-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/26) or 4-iodophenyl (Compound I/54); or

if Y is 4-(trifluoromethyl)phenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/27); or

if Y is 4-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/28) or 4-iodophenyl (Compound I/55); or

if Y is 4-aminophenyl and Z is H, then X is 4-(trifluoromethyl)phenyl (Compound I/81), 4-fluorophenyl (Compound I/86), 4-iodophenyl (Compound I/87) or 3,4,5-trimethoxyphenyl (Compound I/89); or

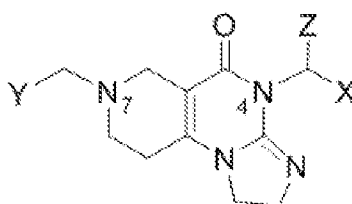
if Y is 3,5-difluorophenyl and Z is hydroxymethyl, then X is 4-chlorophenyl (racemic) (Compound I/128), 4-fluorophenyl (R-enantiomer) (Compound I/129 (R)) or 4-fluorophenyl (S-enantiomer) (Compound I/129 (S));

if Y is 3,5-dicyanophenyl and Z is H, then X is 4-chlorophenyl (Compound I/136) or 4-(trifluoromethyl)phenyl (Compound I/137); or

if Y is phenyl and Z is H, then X is 2-iodophenyl (Compound I/11);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof for use as a medicament.

5. The compounds of formula (I)



(I)

wherein

if Y is phenyl and Z is H, then X is 4-fluorophenyl (Compound I/5), 3-fluorophenyl (Compound I/8), 3,4,5-trifluorophenyl (Compound I/9), 2,3,4-trifluorophenyl (Compound I/29), 2-fluoro-4-nitrophenyl (Compound I/48) or 3-aminophenyl (Compound I/75); or

if Y is 3-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/31), 4-iodophenyl (Compound I/46), 2-fluoro-4-nitrophenyl (Compound I/49), 2-methylphenyl (Compound I/53) or 4-aminophenyl (Compound I/84); or

if Y is 3,5-difluorophenyl and Z is H, then X is 4-fluorophenyl (Compound I/7), 4-(trifluoromethyl)phenyl (Compound I/30), 4-iodophenyl (Compound I/38), 4-bromophenyl (Compound I/39), 2-fluoro-4-nitrophenyl (Compound I/50), 4-chlorophenyl (Compound I/124) or 3-aminophenyl (Compound I/126) or 2-methylphenyl (Compound I/37); or

if Y is 2-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/26) or 4-iodophenyl (Compound I/54); or

if Y is 4-(trifluoromethyl)phenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/27); or

if Y is 4-fluorophenyl and Z is H, then X is 3-fluoro-4-(trifluoromethyl)phenyl (Compound I/28) or 4-iodophenyl (Compound I/55); or

if Y is 4-aminophenyl and Z is H, then X is 4-(trifluoromethyl)phenyl (Compound I/81), 4-fluorophenyl (Compound I/86), 4-iodophenyl (Compound I/87) or 3,4,5-trimethoxyphenyl (Compound I/89); or

if Y is 3,5-difluorophenyl and Z is hydroxymethyl, then X is 4-chlorophenyl (racemic) (Compound I/128), 4-fluorophenyl (R-enantiomer) (Compound I/129 (R)) or 4-fluorophenyl (S-enantiomer) (Compound I/129 (S));

if Y is 3,5-dicyanophenyl and Z is H, then X is 4-chlorophenyl (Compound I/136) or 4-(trifluoromethyl)phenyl (Compound I/137); or

if Y is phenyl and Z is H, then X is 2-iodophenyl (Compound I/11);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof for use in the treatment of cancer.

6. The compounds for use according to the Claim 5, wherein the cancer is selected from the group consisting of prostate carcinoma, pancreatic cancer, lung carcinoma, breast cancer, glioma, cancers of head and neck, colon cancer, skin cancer.

7. The compounds for use according to Claims 4 to 6, wherein

if Y is 3-fluorophenyl and Z is H, then X is 2-methylphenyl (Compound I/53); or

if Y is 3,5-difluorophenyl and Z is H, then X is 4-fluorophenyl (Compound I/7), 4-(trifluoromethyl)phenyl (Compound I/30), 4-bromophenyl (Compound I/39), 4-chlorophenyl (Compound I/124) or 2-methylphenyl (Compound I/37); or

if Y is 3,5-dicyanophenyl and Z is H, then X is 4-chlorophenyl (Compound I/136) or 4-(trifluoromethyl)phenyl (Compound I/137); or

if Y is phenyl and Z is H, then X is 2-iodophenyl (Compound I/11);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

8. The compounds for use according to Claims 4 to 6, wherein

if Y is 3,5-difluorophenyl and Z is H, then X is 4-fluorophenyl (Compound I/7), 4-(trifluoromethyl)phenyl (Compound I/30) or 4-chlorophenyl (Compound I/124);

or a stereoisomer, enantiomer, mixture of enantiomers, mixture of diastereomers or a pharmaceutically acceptable salt thereof.

9. The compounds according to the Claims 1-3 for use as a medicament.

10. The compounds according to the Claims 1-3 for use in the treatment of cancer.

11. The compounds for use according to the Claim 10, wherein the cancer is selected from the group consisting of prostate carcinoma, pancreatic cancer, lung carcinoma, breast cancer, glioma, cancers of head and neck, colon cancer, skin cancer.

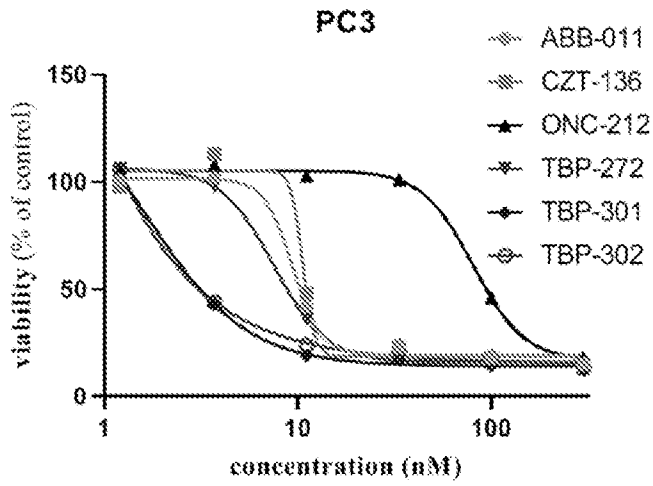


Figure 1

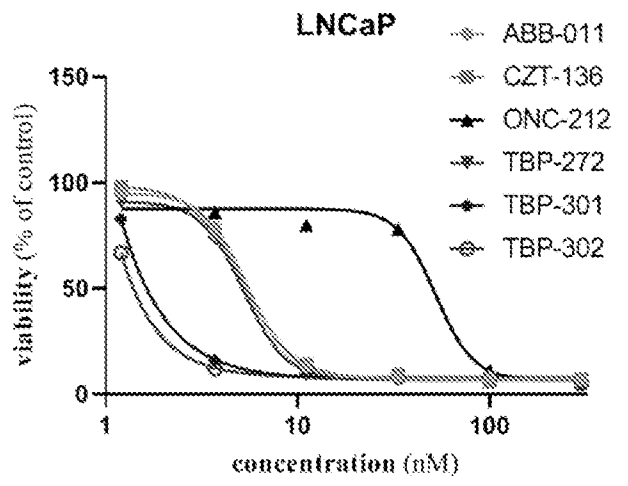


Figure 2

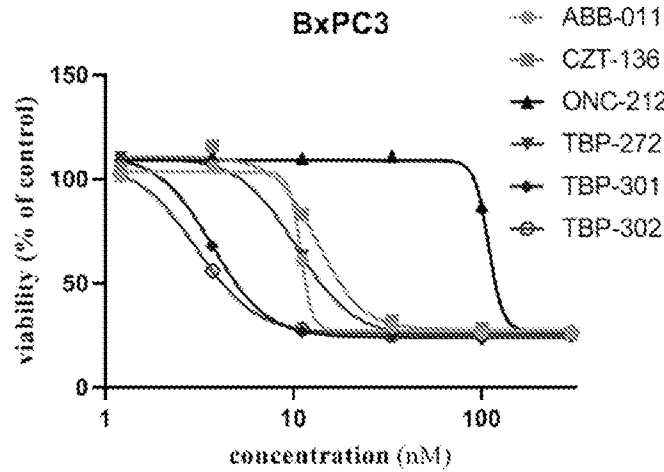


Figure 3

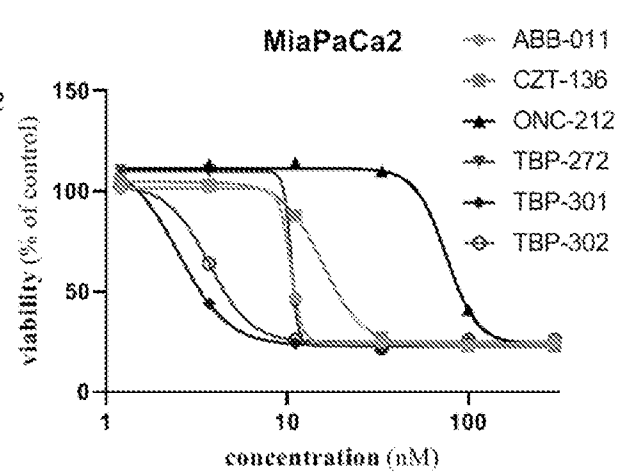


Figure 4

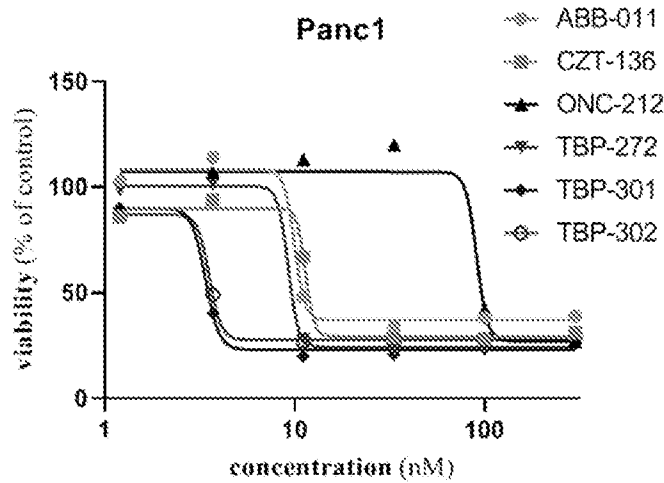


Figure 5

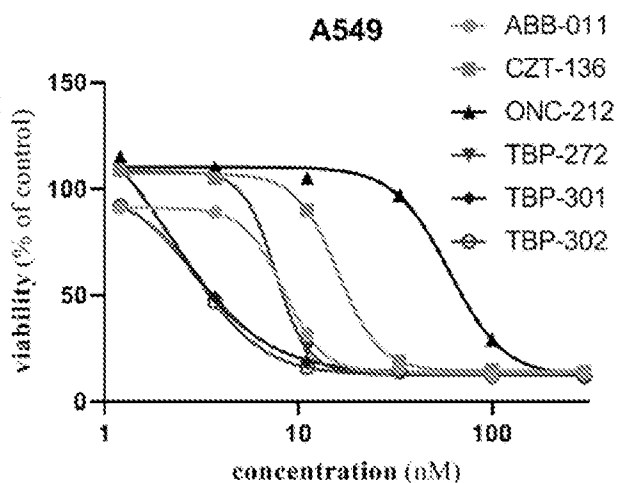


Figure 6

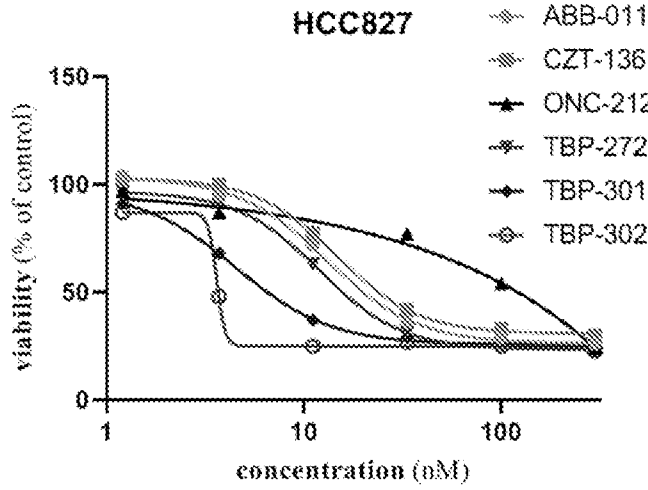


Figure 7

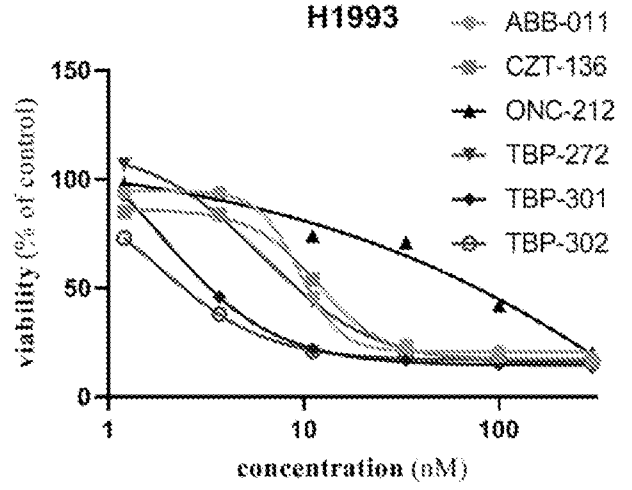


Figure 8

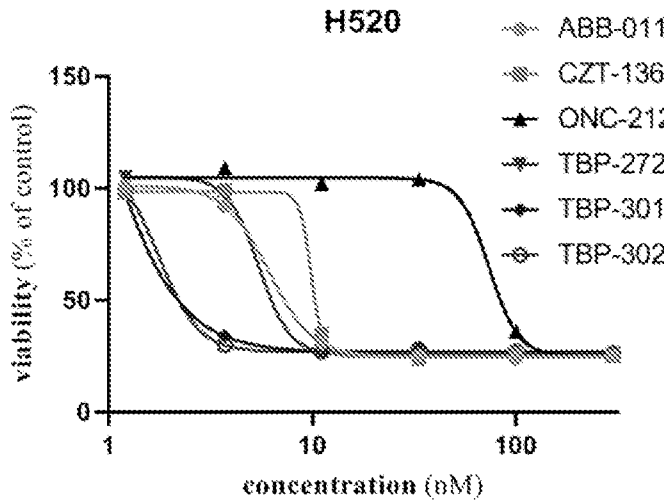


Figure 9

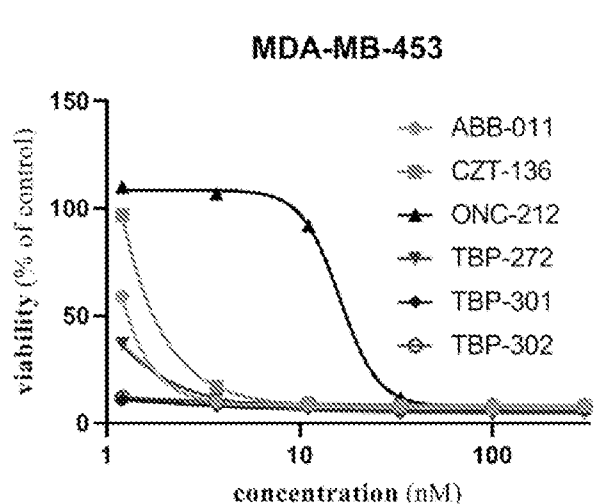


Figure 10

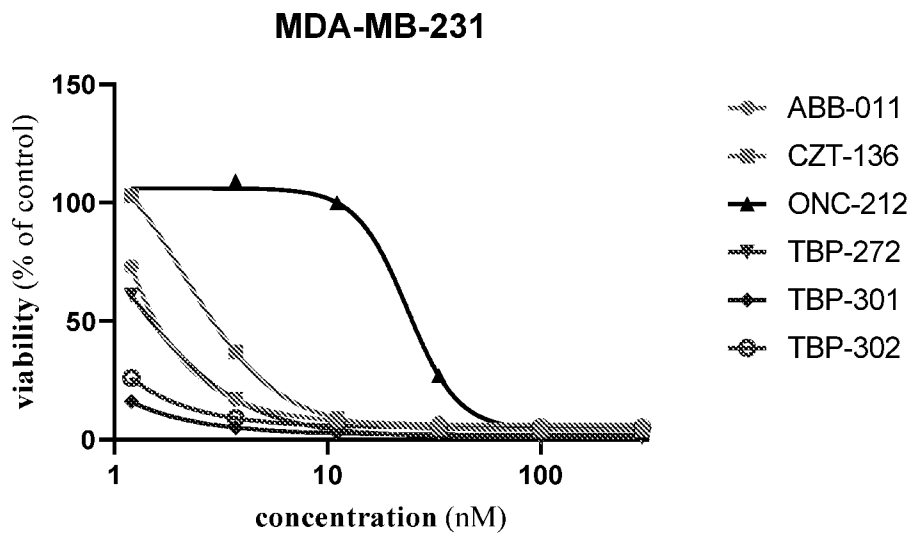


Figure 11

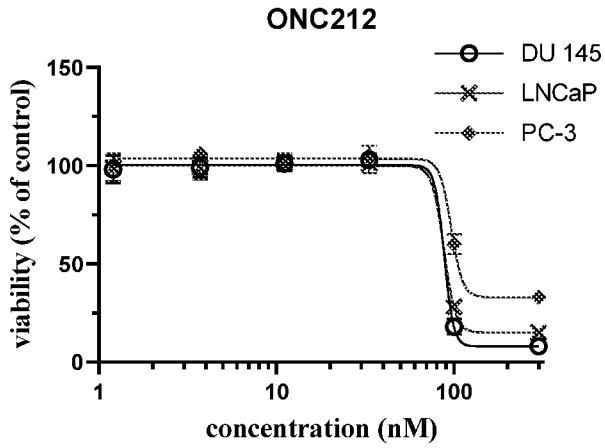


Figure 12

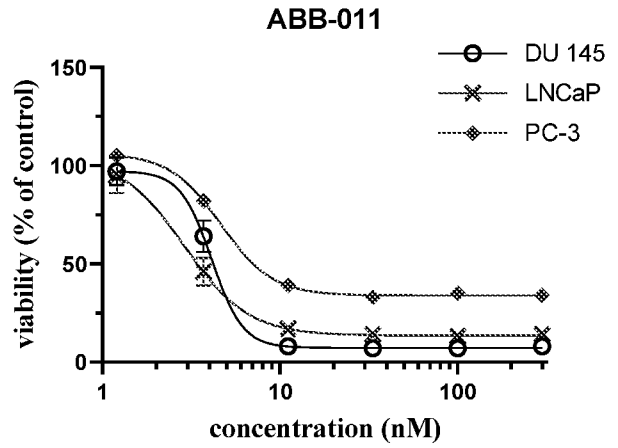


Figure 13

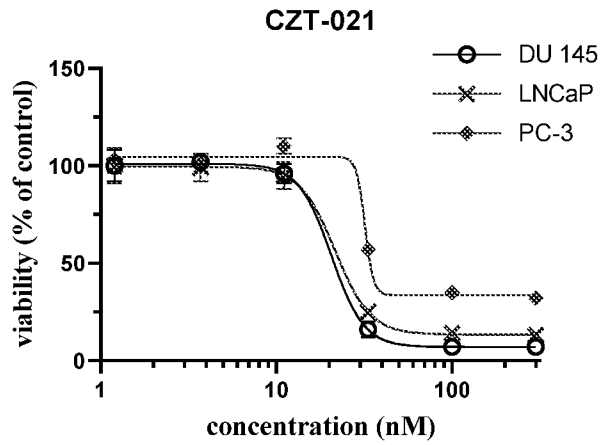


Figure 14

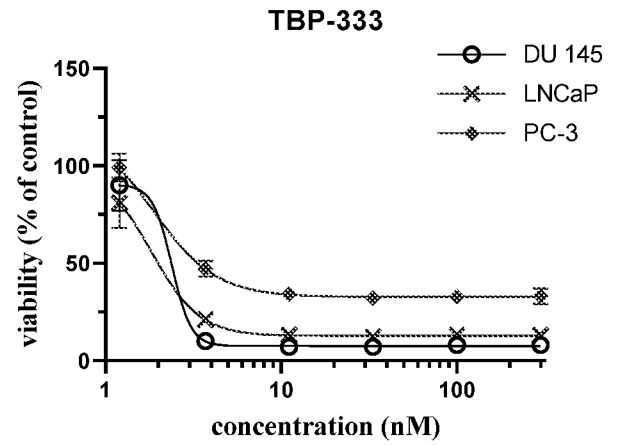


Figure 15

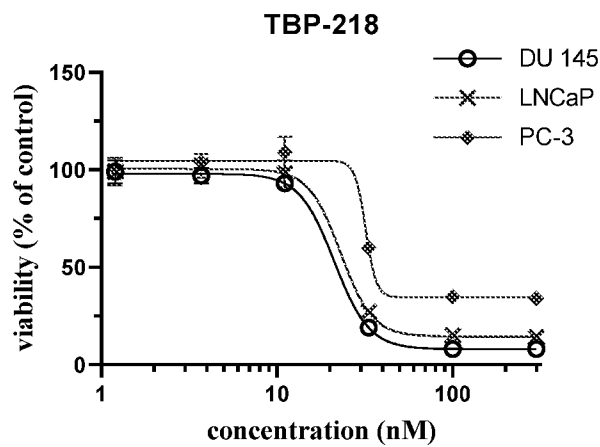


Figure 16

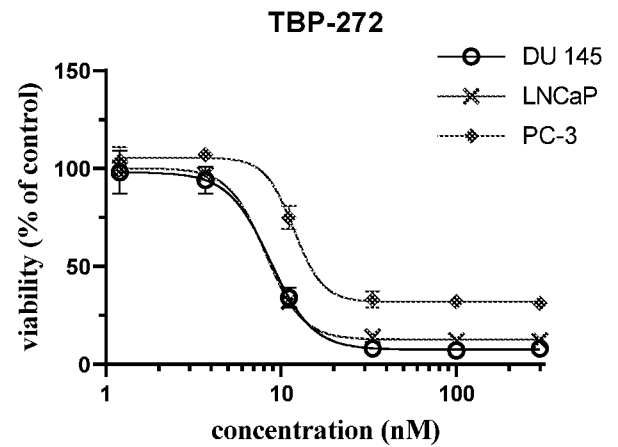


Figure 17

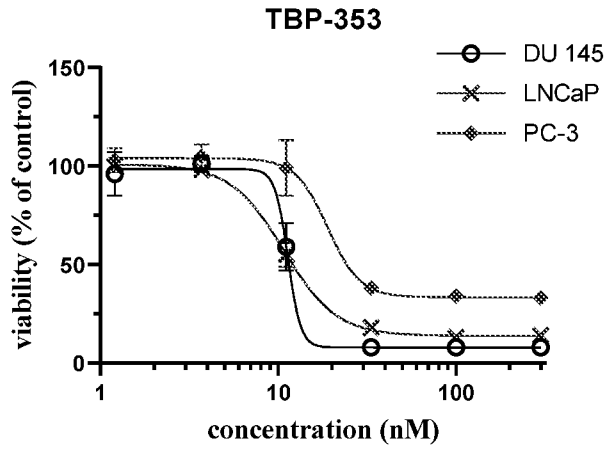


Figure 18

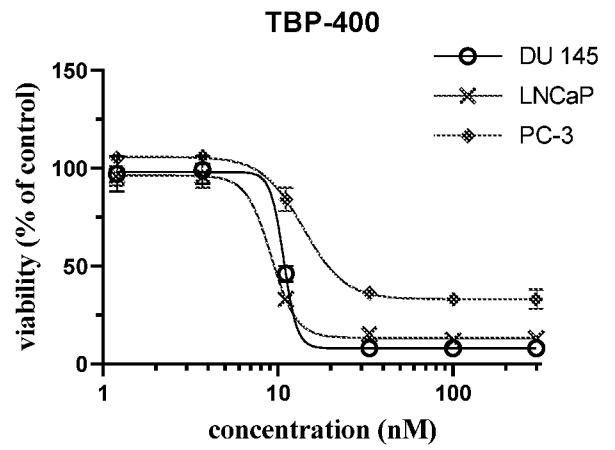


Figure 19

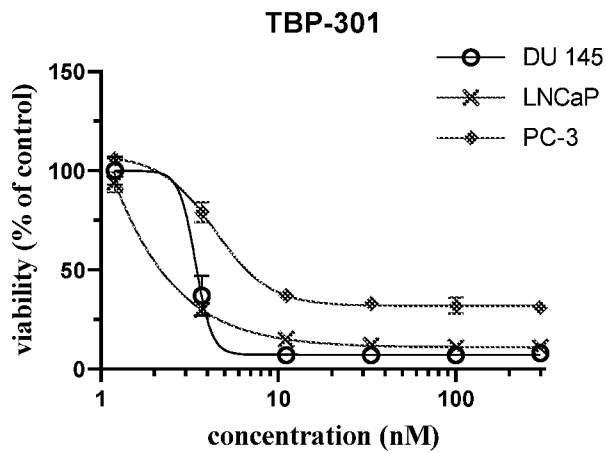


Figure 20

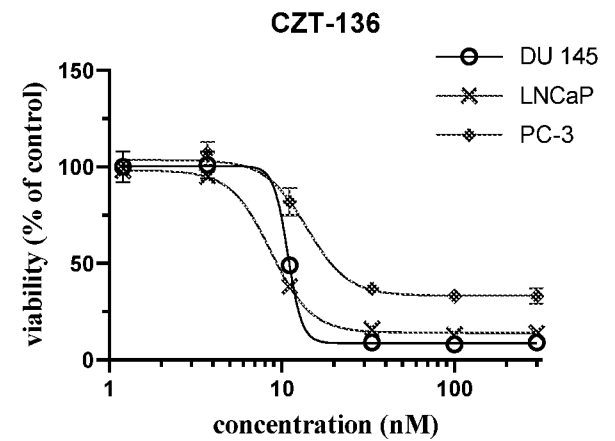


Figure 21

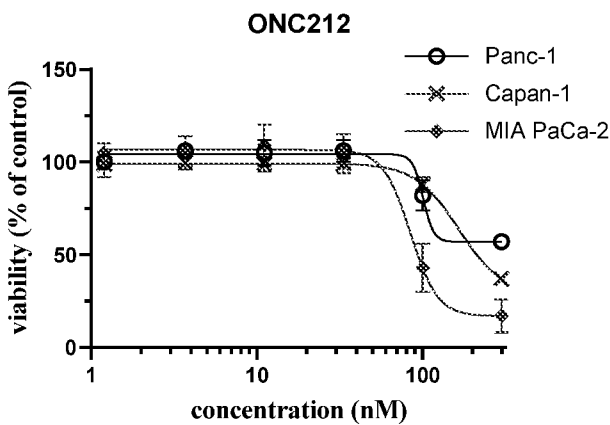


Figure 22

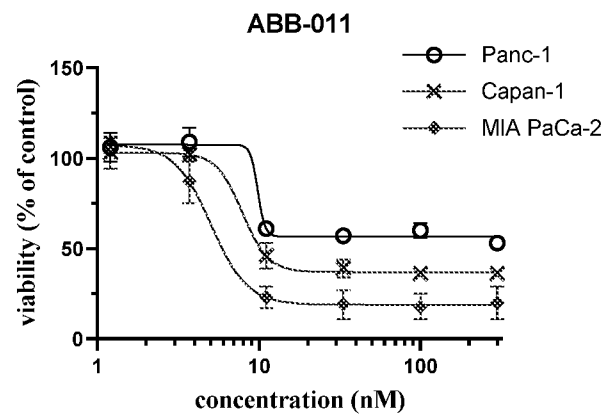


Figure 23

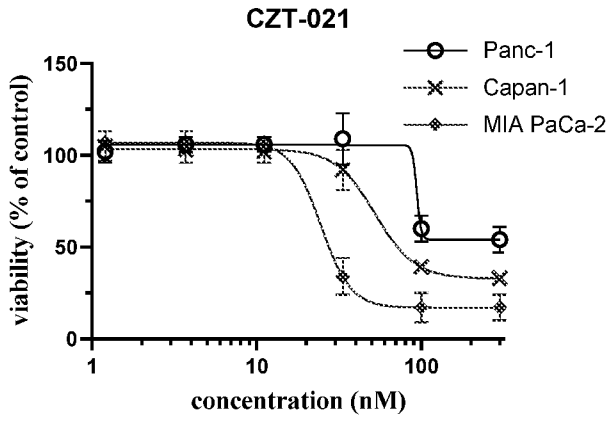


Figure 24

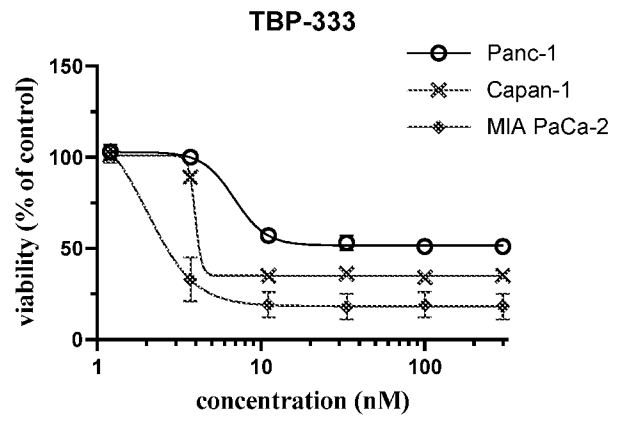


Figure 25

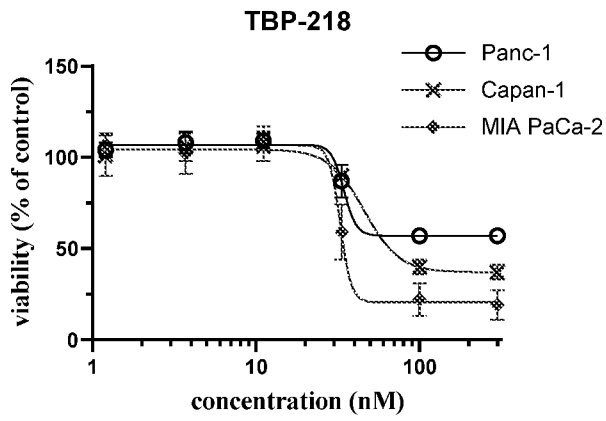


Figure 26

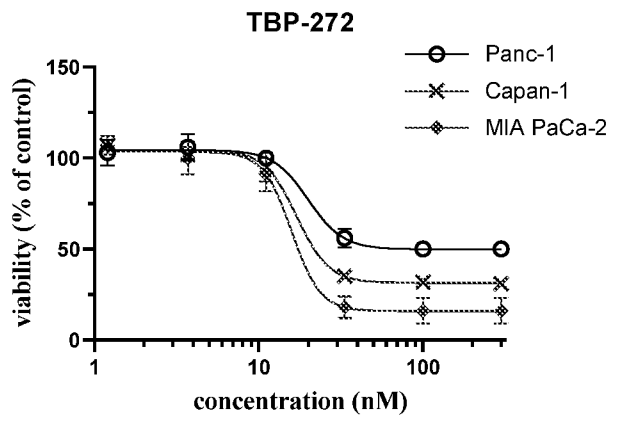


Figure 27

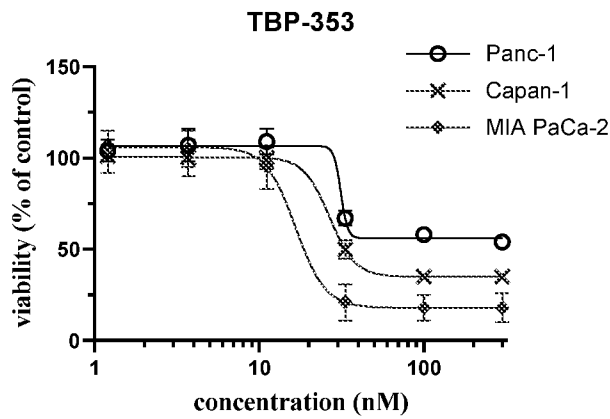


Figure 28

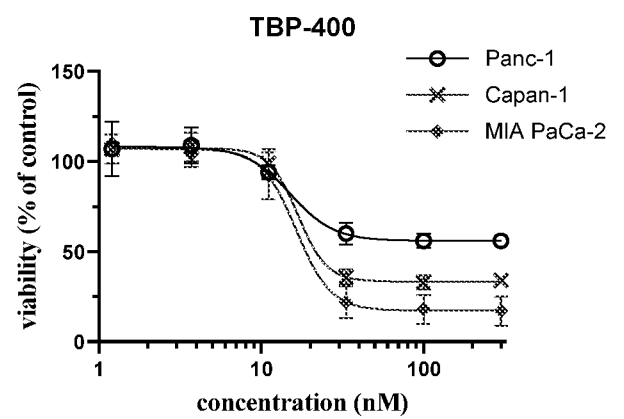


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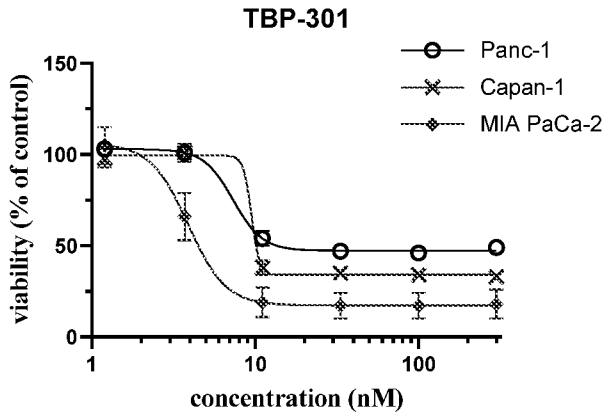


Figure 30

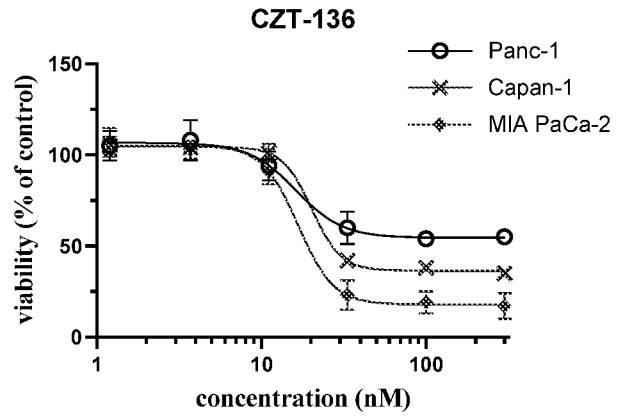


Figure 31

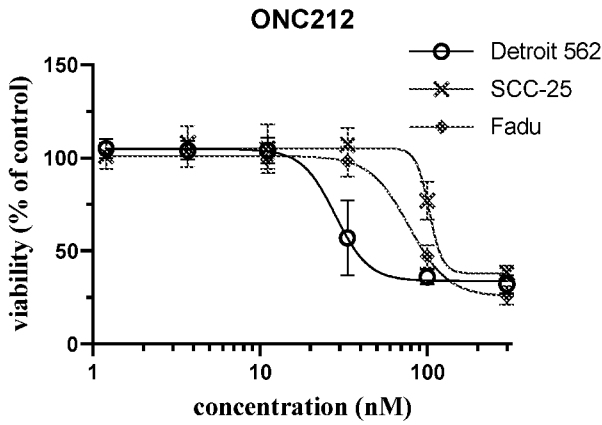


Figure 32

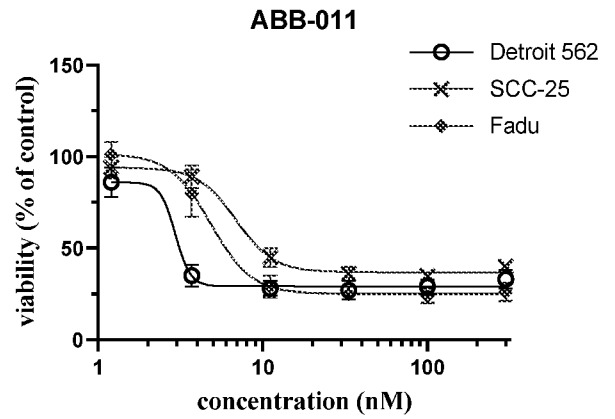


Figure 33

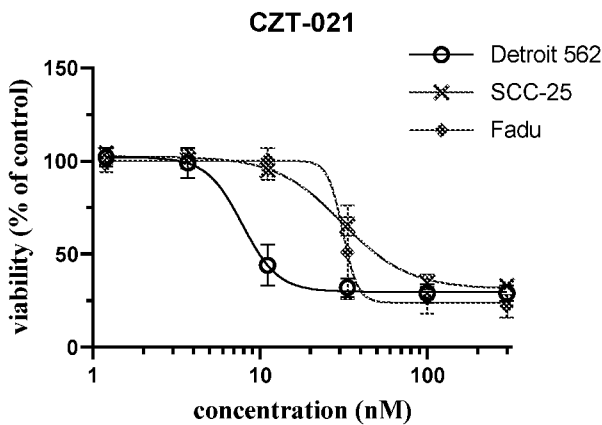


Figure 34

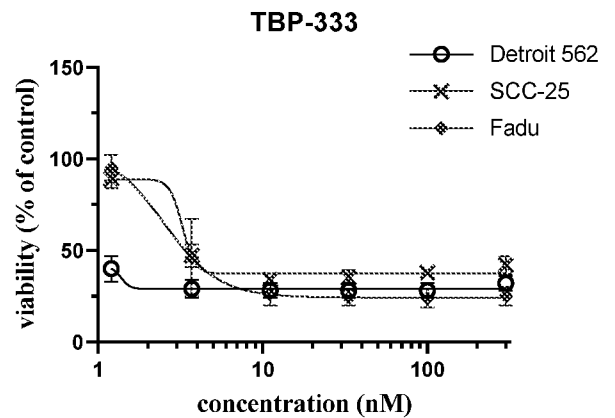


Figure 35

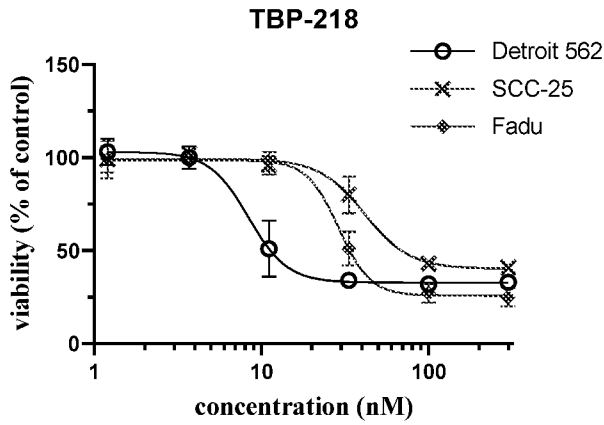


Figure 36

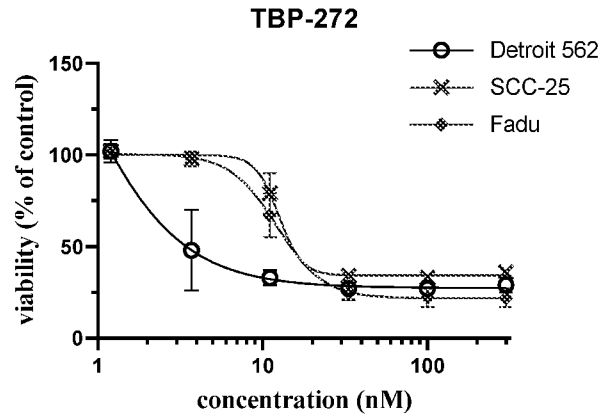


Figure 37

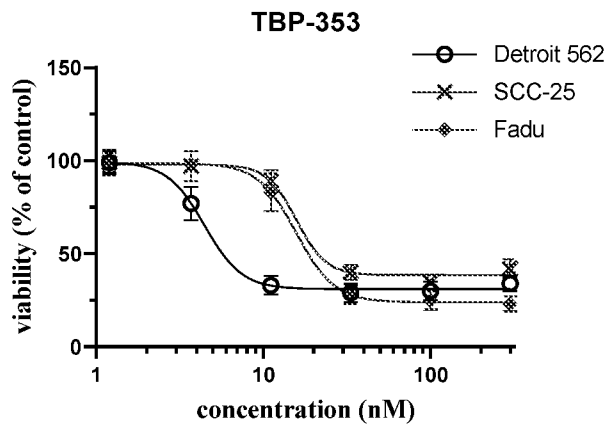


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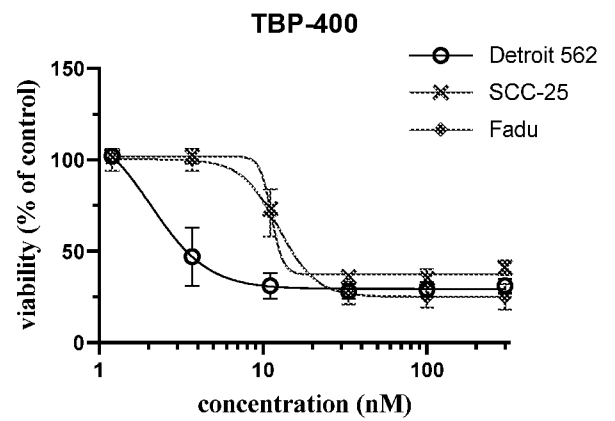


Figure 39

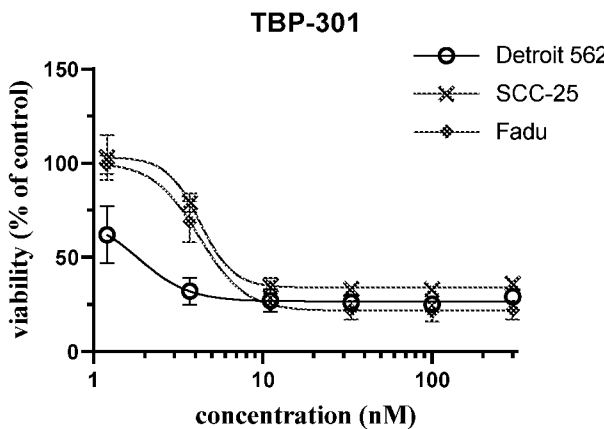


Figure 40

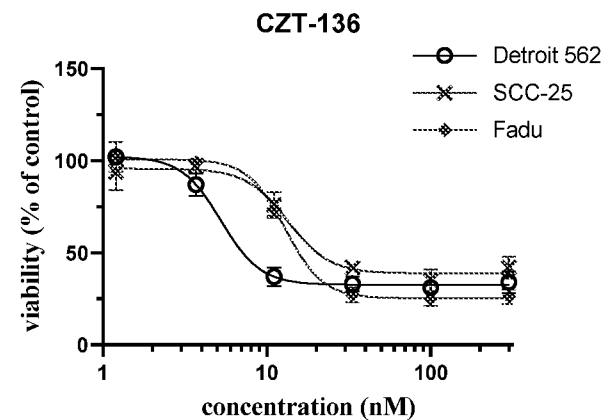


Figure 41

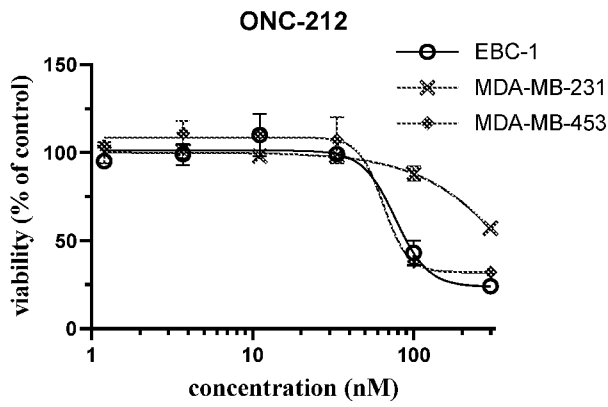


Figure 42

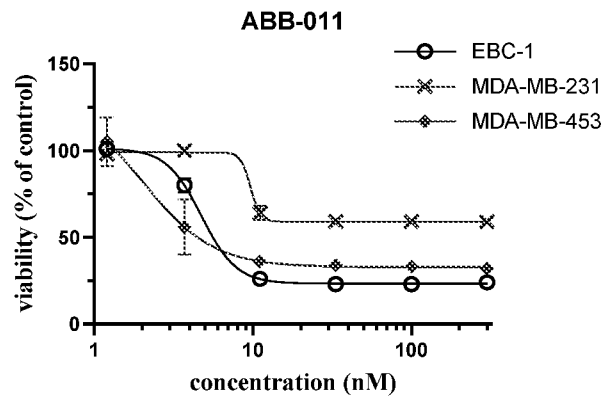


Figure 43

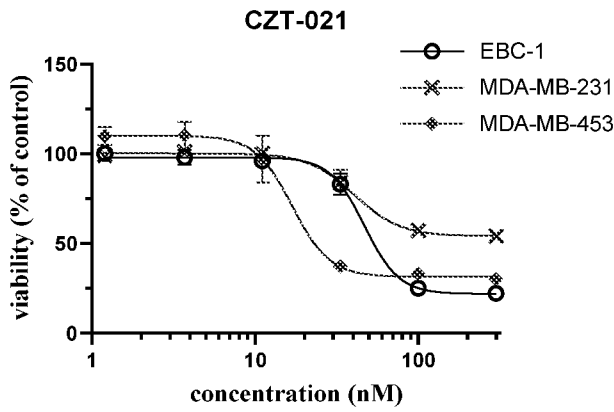


Figure 44

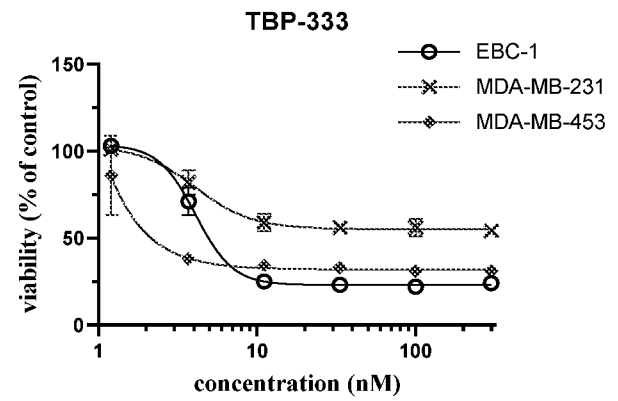


Figure 45

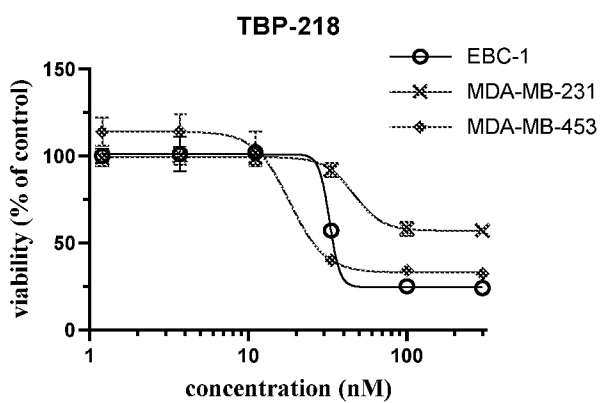


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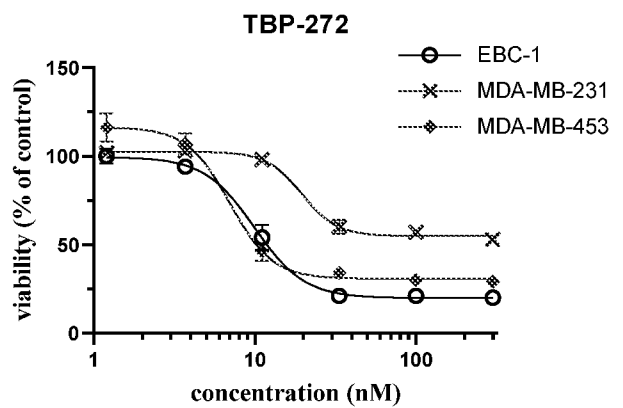


Figure 47

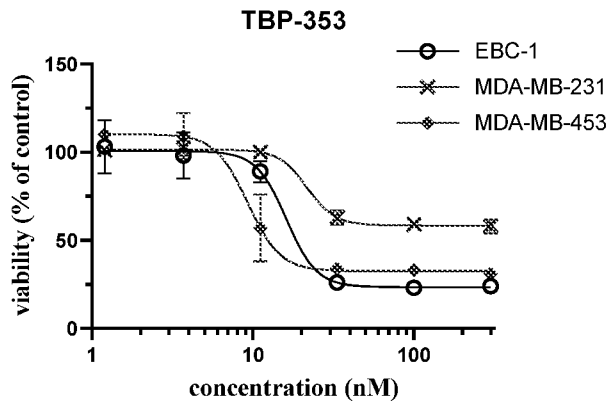


Figure 48

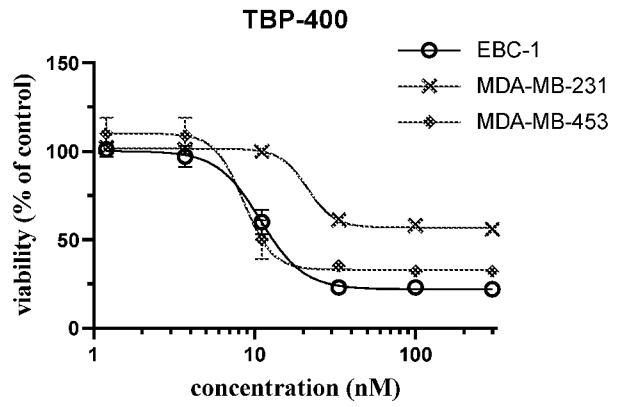


Figure 49

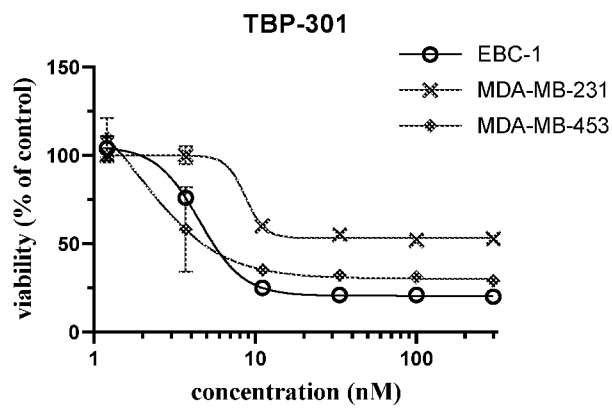


Figure 50

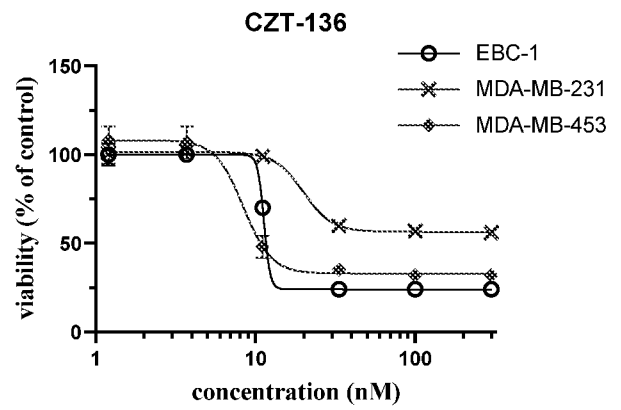


Figure 51

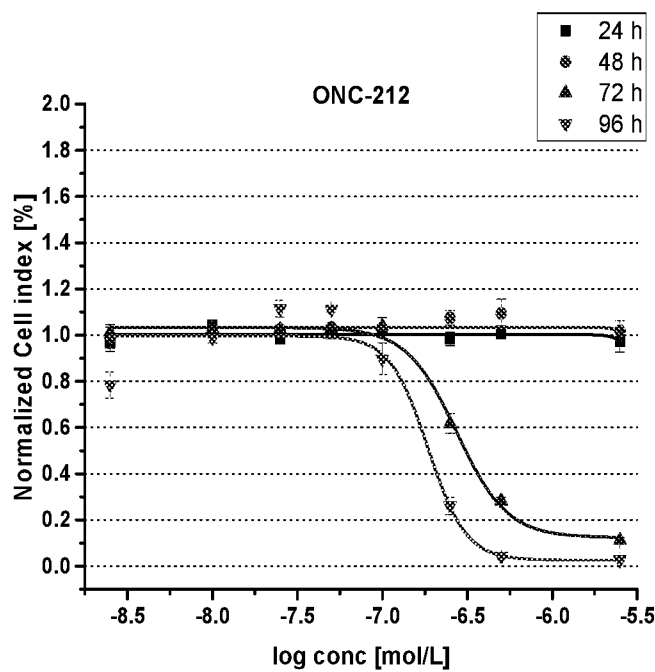


Figure 52

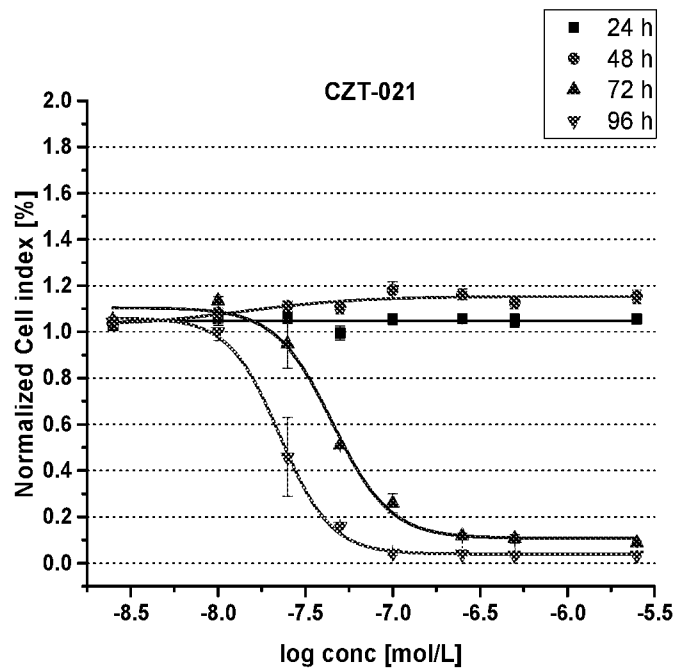


Figure 53

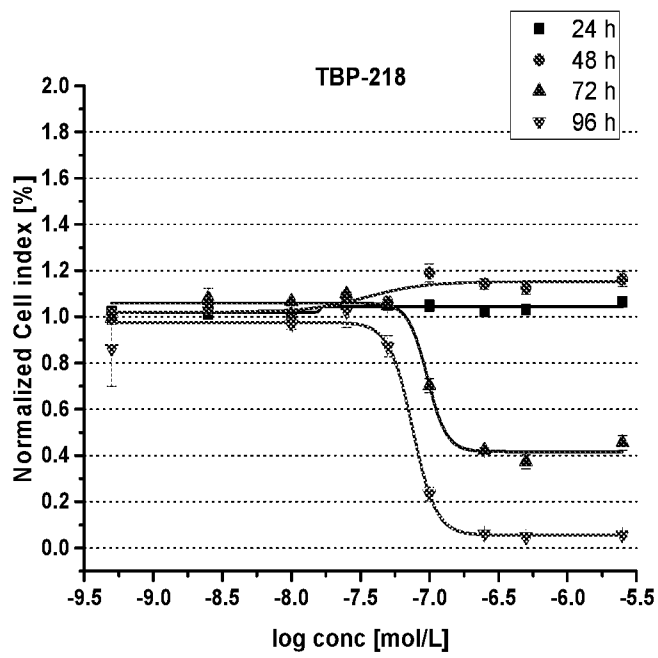


Figure 54

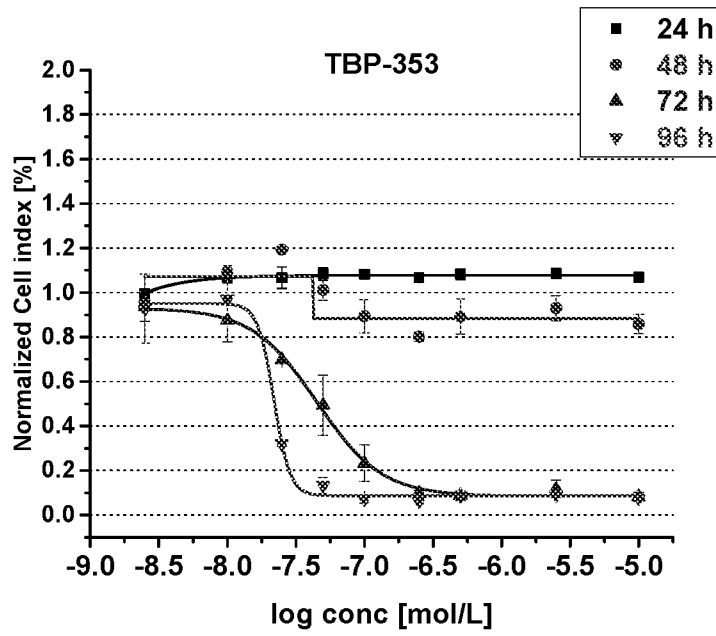


Figure 55

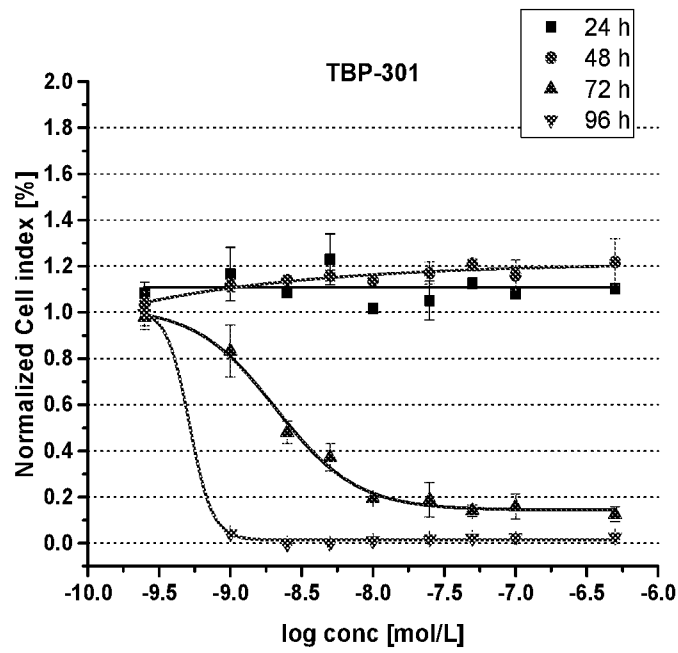


Figure 56

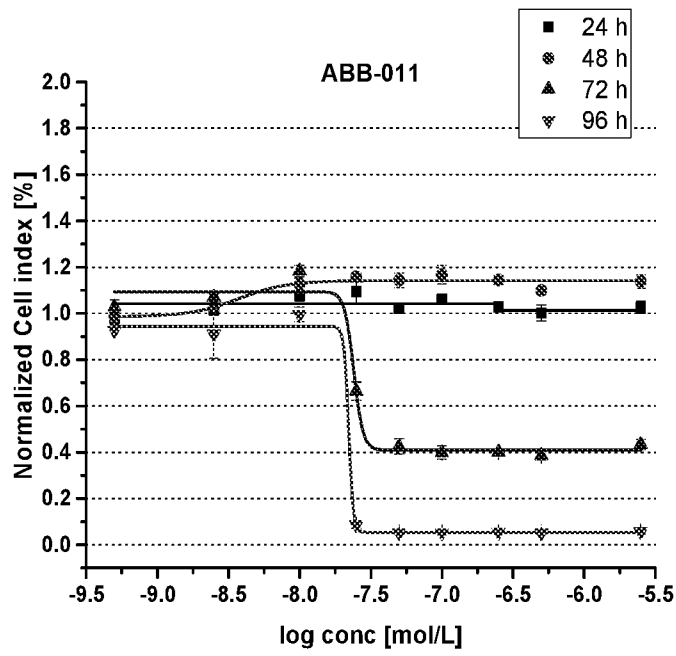


Figure 57

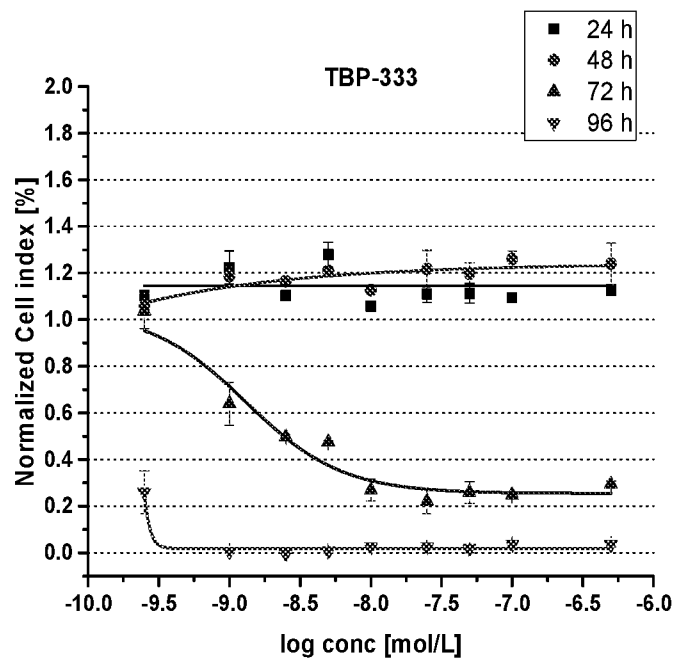


Figure 58

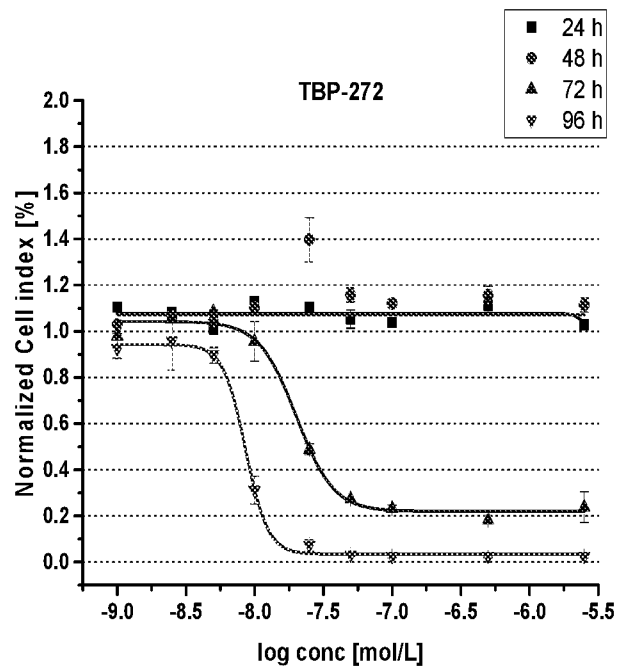


Figure 59

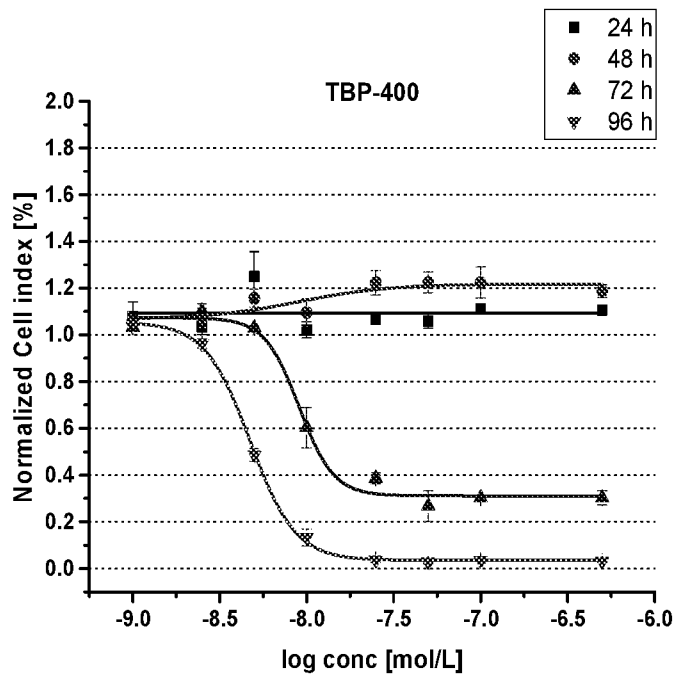


Figure 60

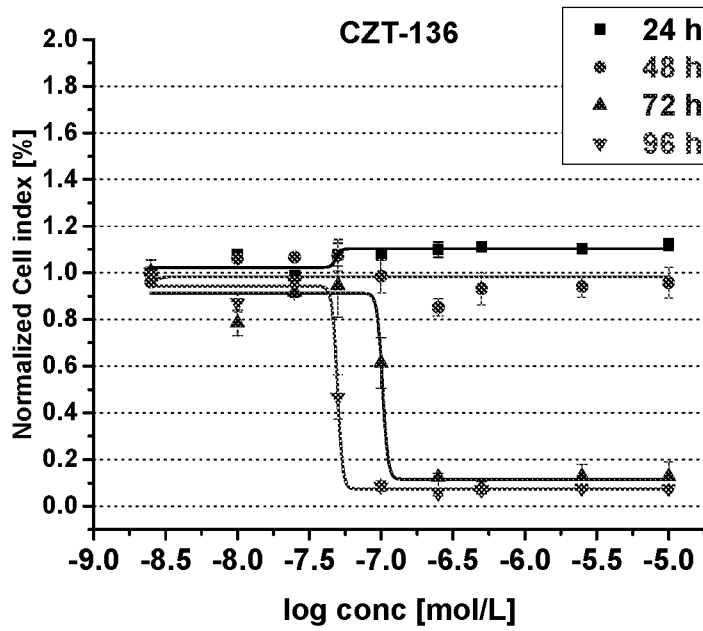


Figure 61

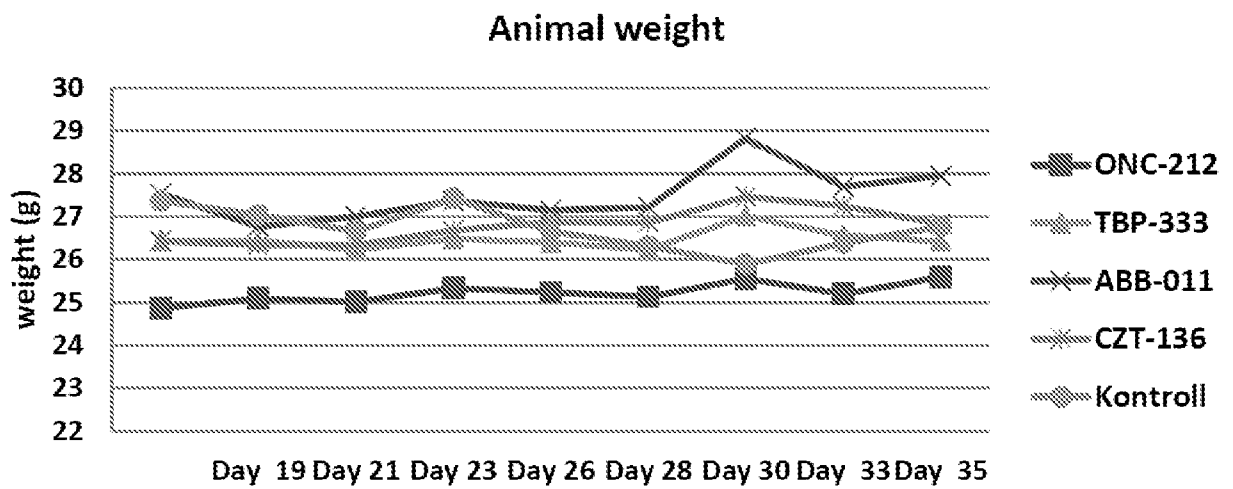


Figure 62

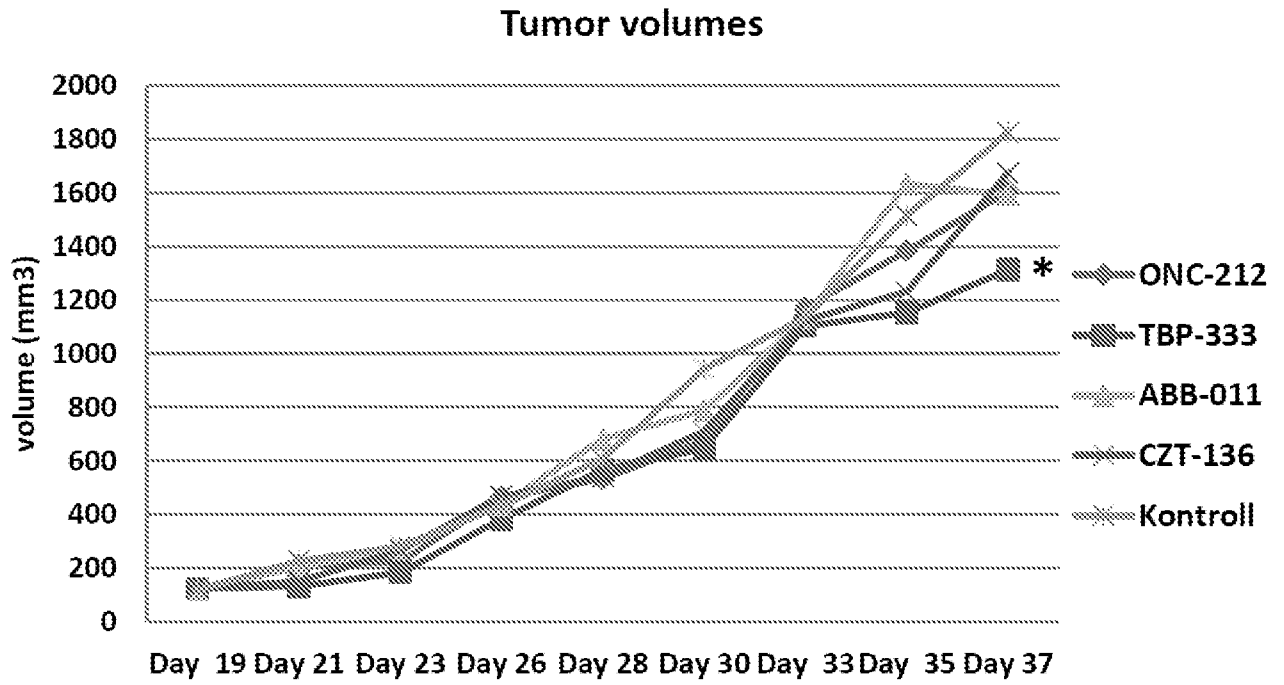


Figure 63

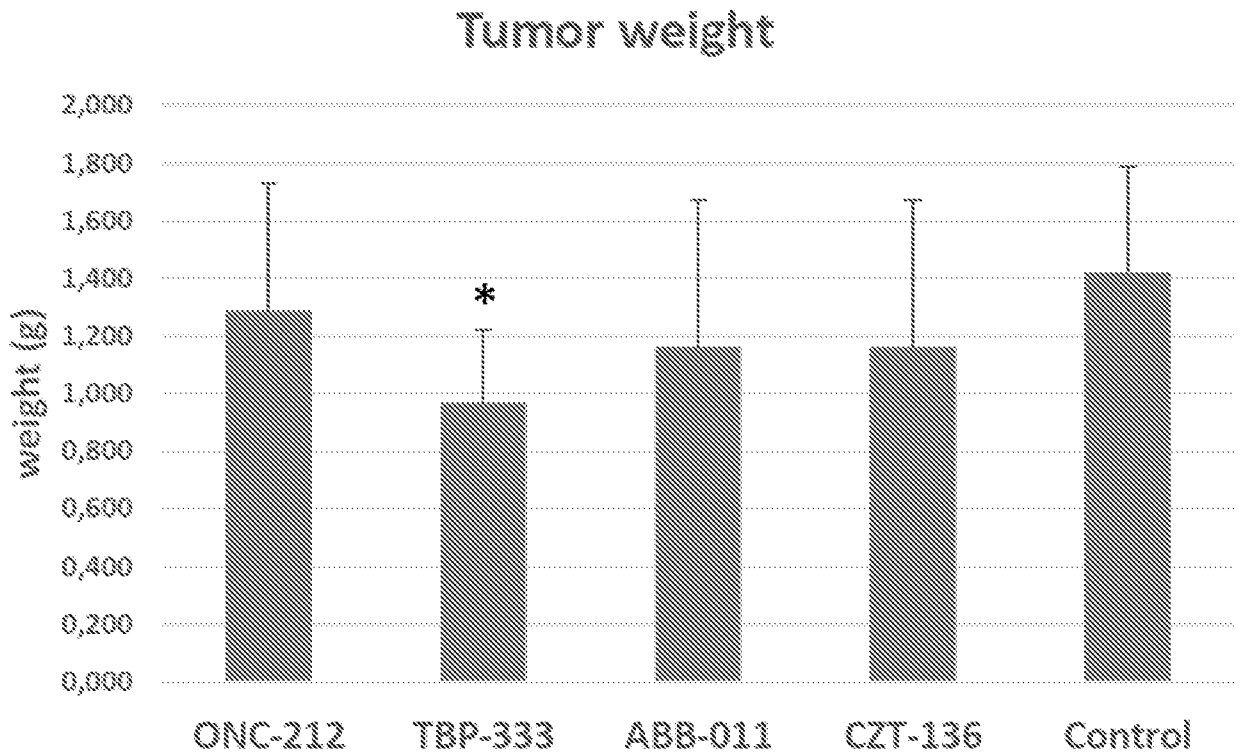


Figure 64

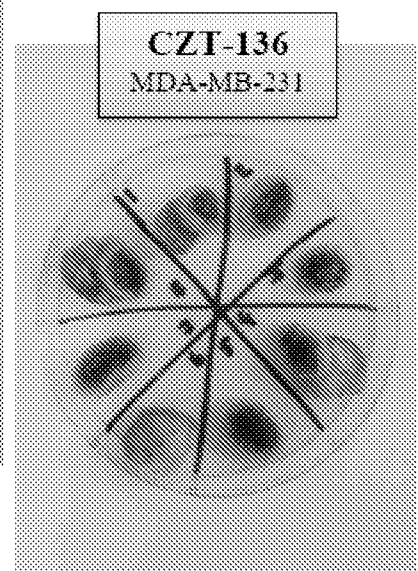
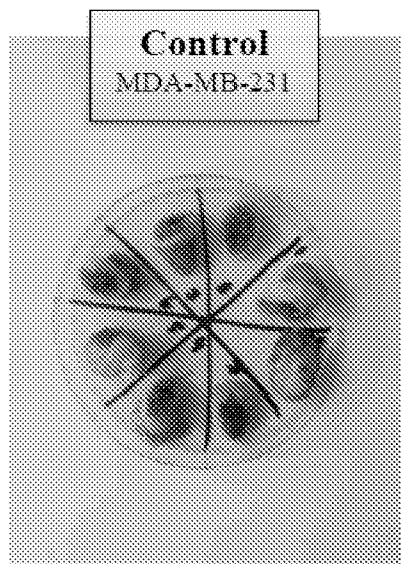
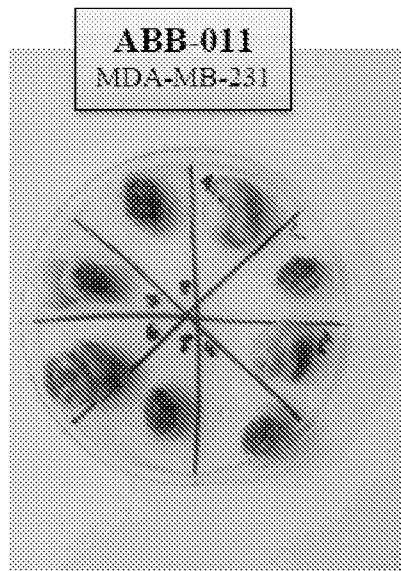
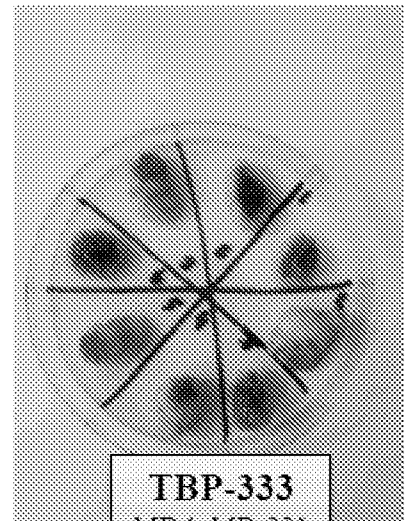
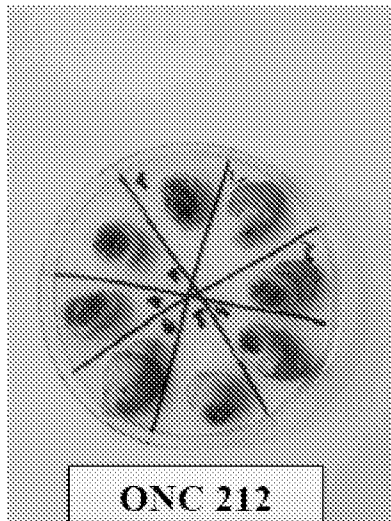


Figure 65

INTERNATIONAL SEARCH REPORT

International application No
PCT/HU2021/050047

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/519 A61K31/555 A61P35/00 C07D401/14 C07F17/02
C07D471/14

ADD.
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
C07D C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/123571 A1 (ONCOCEUTICS INC [US]; PROVID PHARMACEUTICALS INC [US]) 4 August 2016 (2016-08-04) cited in the application claims; examples -----	1, 4-7, 9-11
X	EP 3 315 498 A1 (NANJING GATOR MEDITECH COMPANY LTD [CN]) 2 May 2018 (2018-05-02) cited in the application claims; examples -----	1, 4-7, 9-11
X	WO 2018/031987 A1 (NANJING GATOR MEDITECH COMPANY LTD [CN]; IWANOWICZ EDWIN J [US]) 15 February 2018 (2018-02-15) cited in the application claims; examples -----	1, 4-7, 9-11

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier application or patent but published on or after the international filing date
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 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search 9 December 2021	Date of mailing of the international search report 23/12/2021
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Fax: (+31-70) 340-3016	Authorized officer Bedel, Christian
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/HU2021/050047

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