CARBORANYLANILINOQUINAZOLINE EGFR-INHIBITORS: TOWARDS "LEAD-TO-CANDIDATE" STAGE IN THE DRUG-DEVELOPMENT PIPELINE

- **Abstract: Background:** Carboranylanilinoquinazoline-hybrids, developed for BNCT, have demonstrated cytotoxicity against murine-glioma cells with EGFR-inhibition ability. In addition, their adequate aqueous/metabolic stabilities and ability to cross BBB make them good leads as to become anti-glioma drugs.

Aim: Analyze drug-like-properties of representative carboranylanilinoquinazolines.

Methods: To expand carboranylanilinoquinazolines therapeutic spectrum, we studied their ability to act against glioma-mammal cells, U-87 MG, and other tyrosine kinase-overexpress cells, HT-29. Additionally, we predicted theoretically and studied experimentally drug-like-properties, i.e. OECD-recommended toxicity-studies and, due to some aqueous-solubility problems, and vehicularization for oral and intravenous administrations.

Conclusion: We have identified a promising drug-candidate with broad activity spectrum, appropriate drug-like properties, adequate toxicological behavior, and able to be loaded in suitable vehicles.

- **Keywords:** carboranylanilinoquinazoline; U-87 MG cytotoxicity; HT-29 cytotoxicity; Ames test; acute oral toxicity; nanovehicles

Glioblastoma multiforme (GBM) is a malignant tumor from glial cells with the highest frequency among brain tumors, with a very low survival rate and a limited prognosis, despite the variety of modern therapies and diagnostic advances [1]. According to WHO more than 50 % of diagnosed gliomas are the most malignant form of the glioblastoma, i.e. grade IV type [2]. Despite the GBM heterogeneity some common molecular characteristics could be stated [3], like the amplification of epidermal growth factor receptor (EGFR) [3]. This receptor, EGFR, is a member of transmembrane tyrosine kinase receptor ErbB family that its activation promotes tumoral growth and progression, via angiogenesis, invasion and metastasis, and additionally resistance to drugs and inhibition of apoptosis is involved [4]. For that reason, the anilinoquinazolines erlotinib, gefitinib, vandetanib, lapatinib and CUDC-101 have been studied in GBM as small-drugs which target the tyrosine kinase receptors. Specifically, the tyrosine kinase inhibitor erlotinib (**erl**, Figure 1) prevents the intracellular autophosphorylation of the EGFR tyrosine kinase domain. Erlotinib has been used in glioblastoma for example in combination with temozolomide, a classical methylating agent for GBM, resulting well tolerated and improving patients survival [5]. Other assayed GBM-therapy has been boron neutron capture therapy (BNCT). BNCT is a non-invasive chemotherapy modality for treating locally invasive malignancies. The mode of action of BNCT is based on the production of harmful ions by nuclear reaction, $^{10}B(n,\alpha)^{7}Li$, between nonradioactive nuclide ^{10}B (20 % of natural abundance) and thermal neutrons [6]. Initially, the nuclear reaction produces excited ^{11}B that after a fast nuclear fission yields both reactive α-particle ($^{4}He^{2+}$) and ^{7}Li nucleus ($^{7}Li^{3+}$). Because of the short trajectory of these cations, near to 10 μm, the radiation damage is confined to the tumoral cells loaded with ^{10}B-containing compound [7]. Although BNCT has also been used in the treatment of melanoma, liver, and colon tumors [8,9], most studies to date have focused on the treatment of GBM. Regarding to clinical agents used in BNCT the BSH (disodium ^{10}B-mercaptoundecahydro- *closo*-dodecaborate, Figure 1) is one of the most relevant. Nevertheless, it is not selectively accumulated into tumor cells, which results in cytotoxicity and limits its application [10].

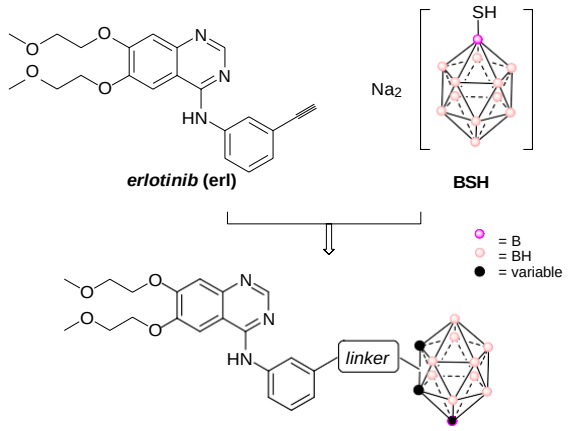
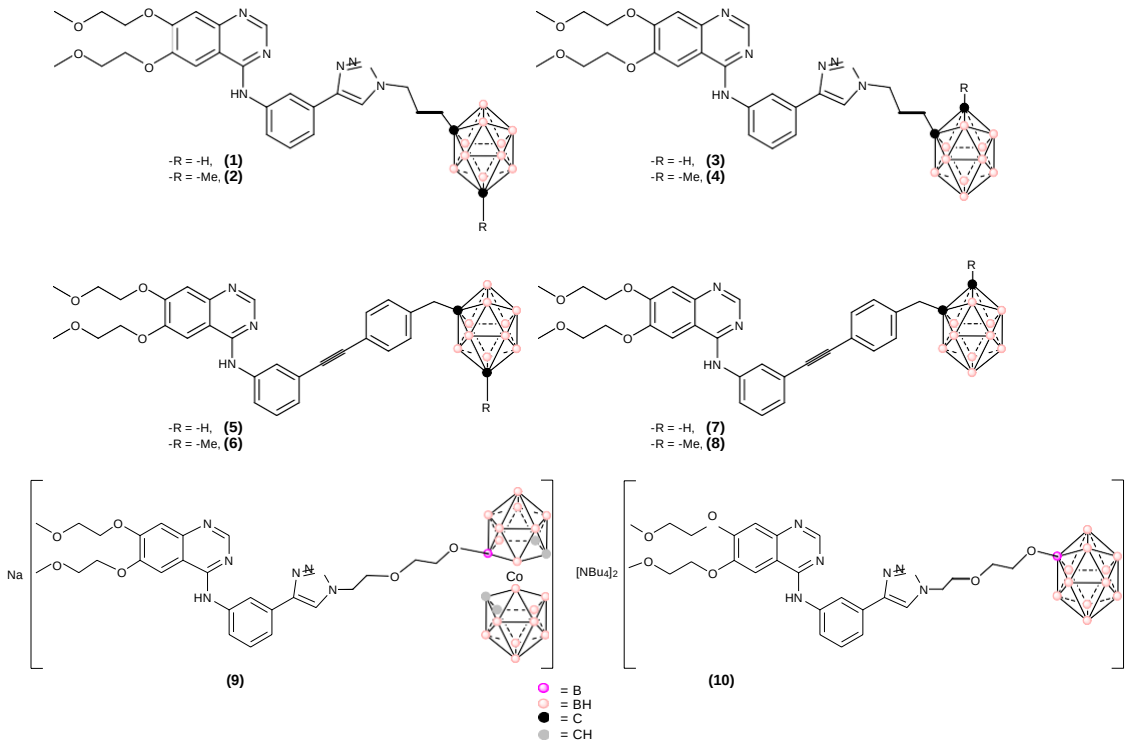


Figure 1. Tyrosine kinase receptor inhibitor erlotinib, BNCT-agent BSH and general structure of carboranylanilinoquinazoline developed as hybrids [12].

Recently, combining both concepts, TK inhibitors incorporating boron-rich moieties have been exploited as bifunctional hybrid boron-containing drug targeting within glioma cells (Figure 1) [11-13]. However the developed boronic-acid derivatives [11] could have limited application in BNCT due to the low concentration of boron in the cells. But, on the other hand carboranyl derivatives [12,13] have high boron content, which is optimal for BNCT use, and also have favorable drug-like properties. In this sense, carboranyl-erlotinib derivatives, i.e. **1-10** (Table 1), have been studied as cytotoxic agents for glioma cells with potential applicability in BNCT [12]. Some of these derivatives, i.e. **1-3**, and **9**, were more cytotoxic against murine-cells C6 than **erl** and selective for the glioma-

system (Table 1). In regards to their mechanism of action, EGFR-inhibition ability could be, at least in part, responsible for their bio-activity (Table 1) [13]. Additionally, the most interesting hybrid, i.e. 1: i) was able to inhibit one of the EGFR mutated form (EGFR T790M), with an IC50 of 1.125 µM, were erlotinib was ineffective [13]; ii) displayed aqueous/metabolic stability and; iii) showed ability to cross blood-brain barrier (BBB, Table 1).



Cpd	$IC_{50,C6}$[a]	$IC_{50,EGFR}$[b]	Stabilities		BBB[e]
			aq[c]	hep[d]	
1	30[f,g]	2.3	S[h]	S	(+)[i]
2	99	312.2			
3	34[g,m]				
4	>100				

Cpd	$IC_{50,C6}$[a]
5	>100
6	>100
7	>100
8	>100

Cpd	$IC_{50,C6}$[a]	$IC_{50,EGFR}$[b]	Stabilities		BBB[e]
			aq[c]	hep[d]	
9	44[g,j]	296.3	S	NS[k]	(-)[l]
10	>100				
erl	>100[g,n]	22.9	S	PS[o]	(±)[p]

Concentrations, in µM, required to inhibit the C6 growth by 50 %. C6 cells consist on a rat glioma cell line that overexpress of EGFR21f [12]; [b] Concentrations, in nM, required to inhibit the phosphorylation of the poly(Glu:Tyr) substrate by 50 % [13]; [c] In aqueous-buffer solutions at 2.0, 7.4, and 8.6 pH [13]; [d] In hepatic cytosolic and microsomal fractions [13]; [e] Ability to cross BBB [13]; [f] Selectivity index ($IC_{50,mix\ of\ primary\ glial\ cells}$ / $IC_{50,C6\ cells}$)= 3.3; [g] From [12]; [h] S: stable over a period of 24 h incubation for aqueous solutions or over a period of 30 min for hepatic fractions, @ 37 °C; [i] Able to cross BBB; [j] Selectivity index ($IC_{50,mix\ of\ primary\ glial\ cells}$ / $IC_{50,C6\ cells}$)= 2.3; [k] NS: non-stable over a period of 30 min incubation, @ 37 °C; [l] Unable to cross BBB; [m] Selectivity index ($IC_{50,mix\ of\ primary\ glial\ cells}$ / $IC_{50,C6\ cells}$)= 2.9; [n] Higher doses than 100 µM could not be evaluated due to solubility problems; [o] SP: partial metabolism over a period of 30 min incubation, @ 37 °C; [p] Medium ability to cross BBB.

Table 1. Summary of carboranylanilinoquinozaline-hybrids that allow identifying lead anti-glioma agents.

These studies [12,13] have arisen lead-compounds for BNCT-glioma treatment, i.e. derivatives 1-3, and 9. However, in order to fully assess the safety profile so as to turn them into drug-candidates further studies are needed. In this study, we analyze some relevant features necessary to propose these compounds as drug candidates. On the one hand, we confirmed their ability to act against glioma-mammal system, using U-87 MG cells, and expanded their

their therapeutic spectrum evaluating them against another tumoral model, HT-29 colon-cells which express high contents of EGFR. Secondly, we predicted some drug-like descriptors theoretically and others experimentally studying the in vitro mutagenic potential and in vivo oral LD50 value for derivative with the best performances in the previous studies, i.e. 1. And finally, in order to avoid some aqueous-solubility and potential low-bioavailability problems, different vehicles for oral and intravenous administrations were studied.

Materials & Methods

General

Chemicals and reagents

Hybrids 1-10 were prepared according to literature [12,13] and erl was purchased from Hong Kong Guokang Bio-Technology Co. (Baoji, China); Pluronic® F127 (F127), Pluronic® L121 (L121) and Tetronic® T1307 (T1307) were gifted by BASF Corporation (Florham Park, NJ, USA). Fetal bovine serum (FBS), penicillin, streptomycin, phosphate-buffered saline (PBS), dimethylsulfoxide (DMSO), trichloroacetic acid, sulphorhodamine B (SRB), unbuffered Tris Base, sodium chloride, polysorbate 80 (Tween 80™), and cholesterol were obtained from Sigma-Aldrich Co. (St Louis, MO, USA). Dulbecco's modified Eagle's milieu (DMEM) high glucose with stable glutamine with sodium pyruvate milieu was obtained from Capricorn Scientific GmbH (Ebsdorfergrund, Germany). Phosphatidylcholine was obtained from Avanti Polar Lipids Inc. (Alabaster, Alabama, USA). Difco Bacto® agar milieu was obtained from Werner Lab (Montevideo, Uruguay). In all the experiments, MilliQ ultrapure water was used.

Cell lines and culture conditions

The cells, i.e. mammal malignant glioblastoma cell line U-87 MG (HTB-14™, ATCC®), mammal colon adenocarcinoma cell line HT-29 (HTB-38™, ATCC®), and rat astrocytoma derived cell line C6 (CCL-107™, ATCC®), were cultured in DMEM high glucose (4.5 g/L), with stable glutamine, with sodium pyruvate milieu supplemented with 10 % inactivated FBS and penicillim/streptomycin (1 %). The cells were cultured at 37 °C under a 5 % CO2 humidified environment, the mileu was changed every 2–3 days, and cells were sub-cultured once they reached 90-95 % confluence.

Animals

All protocols for animal experimentation were carried out in accordance with procedures authorized by the University's Ethical Committee for Animal Experimentation, Uruguay, to whom this project was previously submitted. The authors state that they followed the principles outlined in the Declaration of Helsinki for all animal experimental investigations. Animals were housed in wire mesh cages at 20 ± 2 oC with 12 h light-dark cycles. The animals were fed ad libitum to standard pellet diet and water and were used after a minimum of 3 days acclimation to the housing conditions.

In vitro cytotoxicity assays

Cells were seeded in 96-well plates (7000-10000 cells/well) in 100 µL final volume and were allowed to grow 24 h. After that, 125 µL of fresh culture milieu was added and the cells were allowed to grow again for additional 24 h. Then, 25 µL of a solution of desired dose of the tested compounds in the culture milieu and 1 % DMSO were added onto the cells and were further incubated for 24 h. Afterwards, culture milieu was removed and cells were washed twice with 200 µL of PBS. Then, they were fixed with trichloroacetic acid (50 µL) for 60 min at 4 °C. After that the plates were washed with distilled water (× 5) and dried in the air. Fifty µL of SRB solution was added to each well and allowed staining for 30 min at room temperature. The plates were washed quickly with acetic acid (1 % v/v) until excess dye was fully removed (at least 5 times) and dried in the air. The bound sulphorhodamine B was solubilized by adding unbuffered Tris Base (10 mM, pH 10.5) (100 µL) and the resulting solution was shook for 5 min on a shaker platform and the optical density was read at 540 nm. Cell viability percentage (CV%) was calculated according to the following equation: CV% = (A540 – B)/(C - B), where A540 is the optical density (OD) of the studied wells, B is the OD of untreated wells (without cells), and C is the OD of control wells (cells treated with 1 % of DMSO). CV% values were plotted against studied compound doses and the IC50 values were determined.

Drug-like properties prediction

Drug-like properties were calculated using admetQSAR tool kit (http://lmmd.ecust.edu.cn/admetsar1/predict/) from the molecule SMILE code generated with ChemDraw Standard 14.0 software.

***In vitro* genetic-toxicity**

S. typhimurium TA 98 strain was incubated in agar minimum glucose milieu solution (Difco Bacto® agar) and aqueous glucose solution (40 %). First at all, the direct toxicity of the hybrid 1 against S. typhimurium TA 98 strain was studied. From these data the mutagenic assay was performed. Briefly, 1 in phosphate buffer (0.1 M, pH 7.4) and DMSO (10 % v/v) at seven doses, 1000.0, 333.0 166.0, 111.0, 83.0, 66.7, and 55.0 µg/plate, starting at the highest doses without toxic effects (1000.0 µg/plate), were studied in triplicate. When the influence of metabolic activation was analyzed, 1, at the same seven doses, was mixture with S9 fraction of mouse liver (Moltox, Inc., Annapolis, MD, USA) (500 µL) instead of phosphate buffer. Controls: i) positive control in the assay without S9 activation: 4-nitro-o-phenylendiamine (NPD, 20.0 µg/plate); ii) positive control in the assay with S9 activation: 2-aminofluorene (2AF, 10.0 µg/plate); iii) negative control: phosphate buffer and DMSO (10 % v/v) (no effect on cells growth was observed by this mixture of solvents). The revertant numbers were counted and the studied system was considered mutagenic if the colonies number was at least doubled the natural revertants (negative control) for two or more consecutive doses.

***In vivo* oral acute-toxicity**

Formulation of **1** for *in vivo* trial: Compound **1** was suspended in a mixture of sterile physiological saline/Tween 80 (4:1) solution (96 %) and DMSO (4 %) (vehicle solution), immediately prior to oral administration. This preparation was made under aseptic conditions.

Animals: The experiments were carried out on 7-8 weak-old Balb/c female mice (17.6 to 18.0 g of body weight, bw) bred under specific pathogen-free conditions. At the end of experiments they were anaesthetized with isoflurane and sacrificed by cervical dislocation.

"Limit test" treatment: Each animal, n = 3, was dosed with **1**, at 2000 mg/kg bw (0.5 mL of suspension), orally using an intragastric syringe. Animals were observed individually, submitting them to Irwin test, after dosing during the first 0.5, 1.5, 3, 5, 12, 24, 48 and 72 hours, and daily until day 14. The observations were recorded individually for each animal. According to O.E.C.D. recommendations changes in skin and fur, changes in eyes and mucous membranes, respiratory-, circulatory-, autonomic-, central nervous systems-, and somatomotor activity-changes and behavior pattern, presence of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma, were recorded. Daily individual weight of each animal was determined. Weight changes were systematically recorded. At day 14 surviving animals were weighed and humanely killed. Then each animal was subjected to gross necropsy. All gross pathological changes were recorded for each animal. A new cycle with 3 animals was repeated using 2000 mg/kg bw of **1** orally administered. The observations were repeated for this group of animals during 14 days.

Formulations

Preparation of compounds-loaded polymeric micelles (PM)

PM encapsulated compounds systems were fabricated by direct hydration method. For 1 mg of compound 1 were used 270 mg of mixture composed of Pluronics F127 and L121 or 150 mg of mixture composed of Pluronic F127 and Tetronic T1307. The hydration of all the copolymers was performed by the addition of water (2.5 mL). The preparation was left overnight at 4 ^{o}C. Then, water was added up to 3.0 mL, final volume, at room temperature. Finally, compound **1** was added (1 mg/mL) and the preparation was stirred for about 3 h to obtain a solid compound/copolymer matrix.

Preparation of compounds-loaded liposome

Compounds-containing liposomes were fabricated by thin-film hydration method. Three mg of compound **1**, 165.4 mg of phosphatidylcholine and 50 mg of cholesterol were dissolved in 12.5 mL of chloroform in a round-bottom flask. The solvent was evaporated by rotary evaporation at 50 ^{o}C for about 1 h to obtain a thin film that was hydrated with 3 mL of PBS (50 mM, pH 7.01). The mixture was stirred at 700 rpm for 30min and at 50 ^{o}C to obtain a micelle solution. Immediately, the liposomes were formed by pressure extrusion using an Avanti Mini Extruder.

The resulting multilamellar liposomes were subjected to 11 passages through Whatman polycarbonate filters having pore diameters of 400, 200 and 100 nm.

Determination of particle size and zeta potential

The average size of the formulations and their polydispersity index (PDI) (size distribution) were determined by dynamic light scattering method (Zetasizer Nano-ZS, Malvern Instruments, UK) with a dispersion instrument equipped with a laser He-Ne (633 nm) and a digital correlator, model ZEN3600. Measurements, after adequate dilution with water at 25 oC, were made in triplicate at an angle of dispersion of 173 o and a fixed laser light of 4.65mm. The measurements were taken immediately prior to cytotoxicity tests. The same conditions and equipment were used to measure zeta potential. The data are represented as mean ± standard deviation.

Results and Discussion

***In vitro* activity against other overexpressing EGFR-cells**

To confirm the potential use as drug of hybrid carboranylanilinoquinazolines (Table 1), cytotoxicity assays were performed using different kind of human cells. On the one hand, U-87 MG (HTB-14™, ATCC®) cell line was employed, which are cells derived from *Homo sapiens* malignant gliomas that overexpresses EGFR [14]. On the other hand, HT-29 (HTB-38™, ATCC®), derived cells from *Homo sapiens* colorectal adenocarcinoma EGFR-overexpressing [15], was also included as manner to potentially expand BNCT-strategy in other tumoral-pathology [8]. The studied hybrids **1-10** were active, displaying different level of cytotoxicities against both mammal-cells (Table 2). For both cellular systems, the hybrids with an ethynylbenzyl linker, **5-8**, were the least active while the hybrids with a [1,2,3]triazolylpropyl linker, **1-4** and **9**, were among the most active. This effect is in concordance with those shown in C6 cell line [12]. Additionally we observed that carboranyl isomer type or carbon cluster pattern substitution plays a key role in the bioactivity. Against HT-29 cells, compounds **1** and **2**, *meta*-carboranyl-isomers, were more active than the corresponding *ortho*-isomers **3** and **4** (Table 2). This could be partially explained by the difference of the dipole moments and hydrophobicity of *closo*-carboranyl moiety, which is strongly dependent on the position of the carbon atoms in the carboranyl cage. The hydrophobicity of *closo*-carboranyl isomers increases in the following order: *ortho* < *meta* < *para*. On the other hand, the methyl-substitution, in the *ortho* or *meta* position, i.e. hybrids **2** and **4**, produced a decrease of the cytotoxicity on colon tumoral cells compared to the un-substituted analogues, i.e. **1** and **3**. These effects of the structural-features that decorate the anilinoquinazoline system were also observed previously in C6-glioma cell line [12].

Among the ionic derivatives, compound **9** was the most active in both EGFR-overexpressing cells. However, in spite of the activity displayed and the fact that it could deliver two times more boron atoms per molecule, no further assays were performed due to the unfavorable stability and the BBB parameters observed. Some aqueous solubility problems, mainly with the parent EGFR-inhibitor **erl** (see Supplementary material), was evidenced in these *in vitro* assays.

Hybrids **1-3**, and **9** displayed similar or better biological activities against U-87 MG and/or HT-29 than **erl**.

Cpd	$IC_{50,U\text{-}87\ MG}$[a]	$I_{C50,HT\text{-}29}$[a]
1	70.0	25.0
2	NS	45.0
3	49.0	100.0
4	NS	>100.0

Cpd	$IC_{50,U\text{-}87\ MG}$[a]	$I_{C50,HT\text{-}29}$[a]
5	NS[b]	100.0
6	NS	100.0
7	NS	100.0
8	>100.0[c]	100.0

erl	63	>100.0[c]
Cpd	$IC_{50,U\text{-}87\ MG}$[a]	$I_{C50,HT\text{-}29}$[a]
9	23.0	25.0
10	NS	100.0

[a] Concentrations, in μM, required to inhibit the cellular growth by 50 %. They were determined from dose-response curves, and represent the mean ± s.d. All experiments were repeated at least three times; [b] Not studied; [c] Higher doses than 100 μM could not be evaluated due to solubility problems.

Table 2. *In vitro* activity of carboranylanilinoquinozaline-hybrids and erl against *H. sapiens* glioma- and *H. sapiens* colorectal adenocarcinoma-cells.

Drug-like properties

Predictions

In early stage of drug development the knowledge of absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties are relevant for the selection of potential drug candidates. In addition, the theoretical predictions could provide clear and compelling advantages in decision-making for drug candidates. In this study, we determined some drug-like predictors for selected carboranylanilinoquinazoline derivatives using the admetQSAR tool kit [16] (Table 3). Specifically we focused in human intestinal absorption (HIA), Caco-2 permeability, P-glycoprotein (P-gp) inhibition, renal organic cation transporter (OCT2) inhibition, ability to be substrate of CYP450s, human ether-a-go-go-related gene (hERG) inhibition, and carcinogen effects. Compounds **1-4**, **8**, and **9**, were compared against the parent compound **erl**. On the other hand, we predicted the aqueous solubility (LogS) for **8** and **erl** due to the solubility problems observed in the *in vitro* assays. Finally, using the previously described LogD [13] we analyzed the fitting on Lipinski´s "rule of five" and the binding affinity to EGFR through the lipophilic ligand efficiency descriptor (LLE = pIC_{50} - LogD) for the most relevant compound i.e. **1**, **9**, and **erl**.

One of the most important challenges in the oral process is the absorption of the drug across the intestinal epithelial barrier. Herein, the predicted HIA parameter showed that most of the derivatives were positive, indicating that they could be absorbed to the bloodstream. However, Caco-2 permeability [17,18] was less than 8×10^{-6} cm/s for all the analyzed hybrids, no finding differences according to the type of substructures. This aspect should be considered at the moment to select the administration route of the drug. Transport of drugs by P-gp is another widely studied parameter in the early stage of the drug design process. P-gp, a member of the ATP-binding cassette family extensively distributed and expressed in many human organs working as an efflux pumps in different tissues, i.e. intestinal epithelium, hepatocytes, renal proximal tubular cells, adrenal gland and endothelial capillaries of the brain [19,20].

	Absorption				Metabolism	Toxicity		LogS[h]	$LogD_{7.4}$[i]	Rule 5	LLE[k]
Cpd	HIA[a]	Caco-2[b]	P-gp inh[c]	OCT2 inh[d]	CYP-subs[e]	hERG[f]	Carc[g]			n viol[j]	
1	(+)	(-)	(-)	(-)	CYP450 3A4	w-inh [23] inh [24]	(-)	-3.13	3.10	1[l]	5.54
2	(+)	(-)	(+) [19] (-) [20]	(-)	CYP450 3A4	w-inh [23] inh [24]	(-)	-3.23	-	1[l]	-
3	(+)	(-)	(-)	(-)	CYP450 3A4	w-inh [23] inh [24]	(-)	-3.13	-	1[l]	-
4	(+)	(-)	(+) [19] (-) [20]	(-)	CYP450 3A4	w-inh [23] inh [24]	(-)	-3.23	-	1[l]	-
8	(-)	(-)	(-)	(-)	CYP450 3A4	w-inh [23] inh [24]	(-)	-3.29	-	1[l]	-
9	(+)	(-)	(-)	(-)	CYP450 3A4	w-inh [23] inh [24]	(-)	-3.20	2.51	2[m]	4.02
erl	(+)	(+)	(+) [19] (-) [20]	(-)	CYP450 3A4	w-inh [23] non [24]	(-)	-3.16	2.45	0	5.19

a Human intestinal absorption: If the compound has the HIA% less than 30 %, it is labeled as (-), otherwise it is labeled as (+) [17]; b Caco-2 permeability. If the compound has the Caco-2 permeability value ≥ 8×10-6 cm/s, it is labeled as high Caco-2 permeability (+), otherwise it is labeled as moderate-poor permeability (-) [18]; c P-glycoprotein inhibition [19,20]. Non-inhibition is labeled as (-), otherwise it is labeled as (+); d Renal organic cation transporter (OCT2) inhibition [21]. Non-inhibition is labeled as (-), otherwise it is labeled as (+); e Ability to be substrate of three Cytochrome P450 isoenzymes (CYP450 2C9, CYP450 2D6, and CYP450 3A4) [22]. It is shown the predicted isoform-CYP that the compound potentially acts as substrate; f Human ether-a-go-go-related gene (hERG) inhibition [23,24]. Non-inhibition is labeled as "non", weak-inhibition is labeled as "w-inh" and inhibition is labeled as "inh"; g Potential as carcinogens [25]. Non-carcinogen is labeled as (-); h Aqueous solubility [26]; I Distribution coefficient at pH 7.4, from [13]; j number of violations of Lipinski "Rule of five"; k Lipophilic ligand efficiency (pIC50 – LogD7.4) [27]; l MW higher than 500 Da; m MW higher than 500 Da and hydrogen bond acceptors higher than 10.

Table 3. Drug-like properties of the carboranylanilinoquinazolines studied herein and erl.

Additionally, overexpression of P-gp has been demonstrated to generate resistant phenotypes in various tumors [19,20]. According to prediction all the studied hybrids were potential substrate of P-glycoprotein (data not shown) and non P-gp-inhibitors. However, derivatives **2** and **4**, methyl-substituted belonging to [1,2,3]triazolylpropyl derivatives, like **erl,** were predicted as inhibitors with one of the methods and could be taken into account, in further synthetic developments, as an efficient way of sensitizing drug resistance in cancer chemotherapy. In reference to the drug elimination processes, kidneys have a key role in the elimination of cationic drugs among others [28]. The organic cation transporter 2 (OCT2) is involved in the initial step of renal secretion at the proximal tubule in the kidney [21]. Several drugs have been classified as substrate or inhibitor of OCT2 leading to potential drug-drug interactions. Due to the presence of a system susceptible to protonation, such as the amino-quinazoline feature in the studied compounds, we evaluated the potential inhibition of OCT2. All the studied hybrids and erl were predicted as non inhibitor of OCT2 indicating the potential absence of renal drug-drug interactions problems. Regarding to metabolism predictors, we analyzed the hybrids as substrate of Cytochrome P450 isoenzymes (CYPs). Nowadays, metabolization by CYP superfamily, together with P-gp, are the main cause of adverse drug reactions that lead to withdraw products from the market [29]. CYP3A4, one of the analyzed CYPs, is located in small bowel and liver and is the responsible for the oral drug administered metabolism [30]. Hybrids 1-4, 8 and 9, like erl were classified to be substrate of CYP450 3A4 consequently this aspect should be considered when the administration route is selected. In order to inquire about cytotoxicity, we estimated the ability of hybrids to inhibit hERG and to act as carcinogens. In reference to hERG inhibition, the hybrids were classified as weak inhibitors or inhibitors, according to the prediction-methods, while erl was predicted as non-inhibitor in the method described by Wang et al. [24]. However, experimentally erl was identified as hERG-weak inhibitor [31] compared to other tyrosine kinase inhibitors. On the other hand, carcinogen effects were discarded according to Lagunin et al. classification [25].

Absence of mutagenicity, against Salmonella typhimurium [32], and LD50, rat acute toxicity by oral exposure [33], higher than 2000 mg/kg of body weight were also predicted for all the hybrids, and erl, (data not shown). Additionally, both properties were also experimentally studied for hybrid 1 and the classifications confirmed, leading this derivative as a potential candidate for further studies (see next Section).

Aqueous solubility and Lipinski´s "rule of five" are among the most common drug-properties filters. For that reason, LogS was predicted for all derivatives finding values in an adequate range for a drug candidate (LogS higher than -5 [26]). Specifically, hybrids 1 and 3 are more soluble in water than erl suggesting that they could be administrated orally or intravenously. As expected, LogS variations were observed due to different structural features, such as methyl-substituent or ethynylbenzyl linker decreasing both the water solubility of compounds bearing these moieties (Table 2) and it could be taken into account in further synthetic efforts. Surprisingly, the ionic hybrid 9 was predicted with lower solubility than erl. In reference to the "rule of five", unlike erl the hybrids 1-4, 8 and 9 possess molecular weight greater than 500 Da. Additionally, hybrid 9 have a number of hydrogen bond acceptor, according to Lipinski definition, higher than 10. Finally, the LLEs were determined using LogD7.4 values reported previously for derivatives 1, 9 and erl [13]. LLE is a simple but relevant index (LLE = pIC50 – LogD7.4) that combines in vitro potency and lipophilicity estimating the effectiveness of a drug-biomolecule interaction with respect to the physicochemical properties of the drug. The LLE ideal value for an drug candidate is ranged between 5 and 7 units [34], showing near to 106-fold higher affinity to the bio-target compared to n-octanol. Derivative 1 stood out among the studied compounds having better behavior than erl and hybrid 9. Estimating the efficiency of a binding interaction with respect to the magnitude of the physical properties of the ligand. In these predictions hybrid 1 displayed similar or better drug-like properties than the parent EGFR-inhibitor erl.

***In vitro* genetic- and *in vivo* oral acute-toxicity**

In order to gain further information about the potentiality of hybrid **1** as drug candidate, supplementary studies related to toxicity were experimentally conducted. We centered our attention in some of the O.E.C.D. recommendations, specifically *in vitro* genetic toxicity, i.e. mutagenicity, and *in vivo* median lethal oral dose, i.e. LD_{50}, were explored [35,36].

In the *in vitro* genetic toxicity, i.e. Ames test, we classified hybrid **1** as non-mutagenic, without and with metabolic activation (Figure 2), as no dose-response was observed, working with a wide range of dose of **1**, and in all the cases the number of revertants were lower than twice-values of vehicle control (cut-off lines, Figure 2).

In reference to the *in vivo* oral toxicity, we applied the "limit test" recommended by O.E.C.D. [36] due to the admetQSAR prediction of **1**-no toxicity (see previous section). Table 4 summarizes the results of the analyses performed showing the safety profile of hybrid **1**.

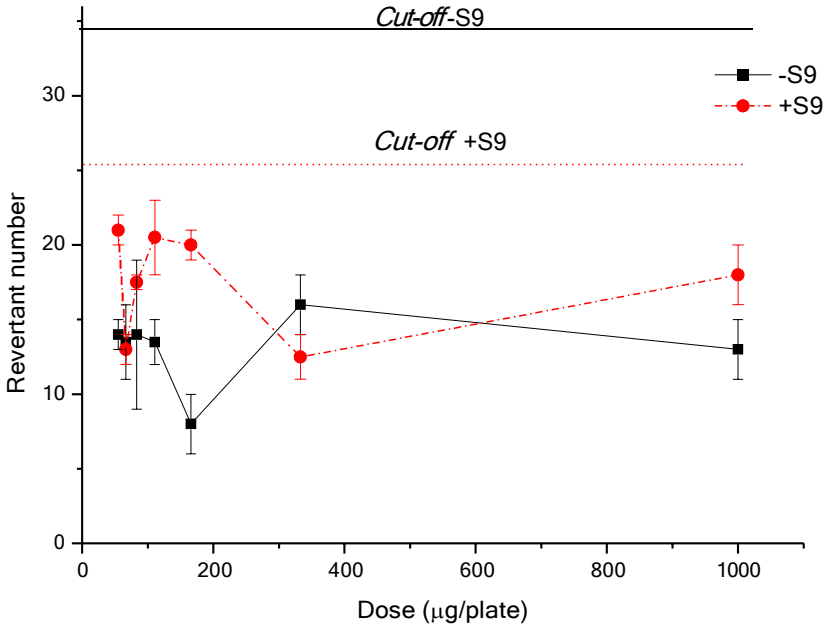


Figure 2. Number of revertant of *Salmonella typhimurium* strain TA98, in the absence (-S9) and presence (+S9) of metabolic activation, incubated with hybrid 1 at different doses. Cut-off is defined as twice of the natural revertants produced by the vehicle control (DMSO) treatments. Number of revertants for NPD (positive control, -S9): 317; Number of revertants for 2AF (positive control, +S9): 80.

administration via	vehicle	LD_{50} (mg/kg bw)	Irwin test[a,b]	Necropsies observation[c]
oral	saline:Tween 80 (4:1)[d]	> 2000	(+)	(-)

[a] According to [37]; [b] The test was performed at 0.5, 1.5, 3, 5, 12, 24, 48, and 72 h post-oral administration. If all the analyzed parameters were similar than saline treatment it is labeled as (+), otherwise it is labeled as (-); [c] (-) denotes absence of organs alterations or organs adherences; [d] DMSO (4 %) was added to allow the complete solubilization of **1**.

Table 4. Summary of *in vivo* oral acute-toxicity for hybrid 1.

Formulations

Due to the aqueous solubility problems observed in some hybrids, i.e. derivative **8**, and **erl** (see Table 2 and Table 4 footnotes), we analyzed different alternative formulations for solubilization, including polymeric micelles and liposomes. As first formulation we used PMs systems, combining Pluronic® block copolymers F127 (hydrophilic) and L121 (hydrophobic) or the hydrophilics Pluronic® F127 and Tetronic® T1307 [38,39]. Firstly, to study these PMs we selected the insoluble hybrid **8** and it was loaded in the F127/L121 system generating a formulation with homogeneous and adequate particle size (Table 5). However, similarly to what it was described for other cells [40], the observed C6-glioma cytotoxicity of unloaded F127/L121 micelles (data not shown) prevent further assays with this system. Therefore, hybrid 8 was loaded in PMs F127/T1307 system. The result confirmed that the lack of

cytotoxicity of hybrid 8 is not related to aqueous-solubility (Table 6). For hybrid 1 and erl we directly assayed the the mixture of F127 and T1307 as vehicle. This formulation did not improve erl activity (Table 6). The nanovehiculization of 1 produced two populations of particle resulting in some degree of polydispersion (Table 5) with zeta potentials slightly negatives (data not shown). The formulation of 1 in the mixture of F127/T1307 promoted lack of bio-activity (Table 6).

nanovehicle	loaded with[a]	T (K)	Peak 1		Peak 2		PDI[c]
			D_h (nm)[b]	%	D_h (nm)[b]	%	
F127/L121	-	298	442.3±18.7	100	-	-	0.252±0.029
		310	340.3±6.3		-	-	0.172±0.012
	8	298	359.5±14.2		-	-	0.234±0.020
		310	322.1±4.0		-	-	0.207±0.006
F127/T1307	-	298	57.38±1.83	70.3±0.9	1537±263	29.7±2.3	0.530±0.028
	1		57.10±1.83	14.8±2.6	1346±253	86.2±9.3	0.865±0.292
liposome	-	298	261.8±14.1	100	-	-	0.490±0.034
	1	298	321.6±0.3		-	-	0.280±0.009
		310	323.4±4.5		-	-	0.269±0.002

[a] In a concentration of 1 mg/mL; [b] Particle size; [c] Polydispersity index

Table 5. Example of characterization of some hybrids-formulations developed herein.

When liposomes were used as vehicle, formulation obtained for hybrid **1** and **erl** presented low polydispersion (Table 5). This formulation improved the activity of **1** in both cell lines and, as in the case of the assayed PMs, the activity of **erl** against U-87 MG glioblastoma cells increased (Table 6).

	without vehicularization		F127/T1307		Liposome	
Cpd	$IC_{50,C6}$[a,b]	$IC_{50,U\text{-}87\ MG}$[a,c]	$IC_{50,C6}$[a]	$IC_{50,U\text{-}87\ MG}$[a]	$IC_{50,C6}$[a]	$IC_{50,U\text{-}87\ MG}$[a]
1	30.0	70.0	>100.0[d]	>100.0[d]	14.0	6.25
8	>100.0[d]	>100.0[e]	>100.0[d]	NS[f]	NS	NS
erl	>100.0[e]	63	>100.0[d]	>100.0[d]	>100.0[d]	17.0

[a] Concentrations, in µM, required to inhibit the cellular growth by 50 %. They were determined from dose-response curves, and represent the mean ± s.d. All experiments were repeated at least three times; [b] From Table 1 [12]; [c] From Table 2; [d] Maximum evaluated doses; [e] Higher doses than 100 µM could not be evaluated due to solubility problems; [f] NS: not studied.

Table 6. *In vitro* activity of 1, 8, and erl alone and vehiculizated with F127/T1307 or liposome.

Interestingly, for all the vehicled compounds the formulations resulted less active when the concentration of loaded compound increases. This relevant aspect is currently studied.

Conclusions

The carboranylanilinoquinazoline **1**, with ability to inhibit EGFR in the low nanomolar range [12,13], exhibits desirable *in vitro* antitumor activities against EGFR-overexpressing mammal cells opening the possibility of new targets for BNCT applications. Moreover hybrid **1** meets many drug-like properties highlighting the absence of toxicity, adequate water-solubility and lipophilic ligand efficiency, being among the studied compounds and other EGFR-inhibitors described in the literature [41], the most efficient ligand. Nevertheless, certain absorption problems can occur when **1** is administered orally, due to the modest Caco-2 permeability predicted values. Besides eventual multidrug resistance could be expected because of the lack of P-gp inhibition, something that could be minimized with the assayed formulations [42,43]. Additionally, hybrid **1** fulfills the initial requirements of the OECD in reference to mutagenicity and acute oral toxicity. Currently, we are working in a murine glioblastoma model with hybrid 1 as a way of completing this drug-discovery stage.

Future Perspective

Great efforts are being made nowadays towards the development of multifunctional hybrid compounds which could be applied in multi-modal treatments to obtain more efficient drugs with diminishing secondary effects. BNCT, which exhibits great potential for the treatment of various cancers, has still limited clinical application. To improve this, efforts should be devoted to the developments of new multi-modal drugs that accumulate selectively in the tumoral tissues for diagnosis and therapy. It is expected that this treatment would speed up the effects while diminishing secondary effects. On the one hand, EGFR results an attractive target due to its overexpression in a variety of cancers. On the other hand, the stability of the 3D rigid carborane scaffold might be amalgamated with the capacity to functionalize the different vertexes with different moieties with ending branches (fluorescent dyes, radiopharmaceuticals, anionic or neutral boron clusters, etc.) to provide multi-modal treatments besides performing as drug delivery nanocarriers. Therefore, in the future, the following aspects should be considered in the design and synthesis of BNCT drugs: i) high tumor targeting; ii) high boron-accumulation; iii) desirable drug-like properties to satisfy the requirements in further clinical application.

Summary points

- Drug-like properties of a series of hybrid carboranylanilinoquinazoline EGFR-inhibitors were analyzed.
- Some of these compounds were active against other tyrosine kinase-overexpress mammal cellular systems.
- Predicted theoretic drug-like properties allowed to identify 4-{3-{1-[3-(1,7-dicarba-*closo*dodecaboran-1-yl)propyl]-1*H*-[1,2,3]triazol-4-yl}phenyl}amino-6,7-bis(2-methyloxyethyloxy)quinazoline as one of the best derivatives among the studied.
- 4-{3-{1-[3-(1,7-Dicarba-*closo*dodecaboran-1-yl)propyl]-1*H*-[1,2,3]triazol-4-yl}phenyl}amino-6,7-bis(2-methyloxyethyloxy)quinazoline was non-mutagenic, without and with metabolic activation, in the Ames test.
- 4-{3-{1-[3-(1,7-Dicarba-*closo*dodecaboran-1-yl)propyl]-1*H*-[1,2,3]triazol-4-yl}phenyl}amino-6,7-bis(2-methyloxyethyloxy)quinazoline has a LD_{50} in the oral administration higher than 2000 mg/kg bw.
- 4-{3-{1-[3-(1,7-Dicarba-*closo*dodecaboran-1-yl)propyl]-1*H*-[1,2,3]triazol-4-yl}phenyl}amino-6,7-bis(2-methyloxyethyloxy)quinazoline could be formulated using liposomes.

Figure legends

Figure 1. Tyrosine kinase receptor inhibitor erlotinib, BNCT-agent BSH and general structure of carboranylanilinoquinazoline developed as hybrids (X: variable) [12].
Figure 2. Number of revertant of *Salmonella typhimurium* strain TA98, in the absence (-S9) and presence (+S9) of metabolic activation, incubated with hybrid 1 at different doses. Cut-off is defined as twice of the natural revertants produced by the vehicle control (DMSO) treatments. Number of revertants for NPD (positive control, -S9): 317; Number of revertants for 2AF (positive control, +S9): 80.

Table Legends

Table 1. Summary of carboranylanilinoquinozaline-hybrids that allow identifying lead anti-glioma agents.
Footnotes: [a] Concentrations, in μM, required to inhibit the C6 growth by 50 %. C6 cells from rat glioma that overexpress of EGFR21f [12]; [b] Concentrations, in nM, required to inhibit the phosphorylation of the poly(Glu:Tyr) substrate by 50 % [13]; [c] In aqueous-buffer solutions at 2.0, 7.4, and 8.6 pH [13]; [d] In hepatic cytosolic and microsomal fractions [13]; [e] Ability to cross BBB [13]; [f] Selectivity index ($IC_{50,mix\ of\ primary\ glial\ cells}$ / $IC_{50,C6\ cells}$)= 3.3; [g] From [12]; [h] S: stable over a period of 24 h incubation for aqueous solutions or over a period of 30 min for hepatic fractions, @ 37 ºC; [i] Able to cross BBB; [j] Selectivity index ($IC_{50,mix\ of\ primary\ glial\ cells}$ / $IC_{50,C6\ cells}$)= 2.3; [k] NS: non-stable over a period of 30 min incubation, @ 37 ºC; [l] Unable to cross BBB; [m] Selectivity index ($IC_{50,mix\ of\ primary\ glial\ cells}$ / $IC_{50,C6\ cells}$)=2.9; [n] Higher doses than 100 μM could not be evaluated due to solubility problems; [o] SP: partial metabolism over a period of 30 min incubation, @ 37 ºC; [p] Medium ability to cross BBB.

Table 2. *In vitro* activity of carboranylanilinoquinozaline-hybrids and erl against *H. sapiens* glioma- and *H. sapiens* colorectal adenocarcinoma-cells.
Footnotes: [a] Concentrations, in μM, required to inhibit the cellular growth by 50 %. They were determined from dose-response curves, and represent the mean ± s.d. All experiments were repeated at least three times; [b] Not studied; [c] Higher doses than 100 μM could not be evaluated due to solubility problems.

Table 3. Drug-like properties of the carboranylanilinoquinazolines studied herein and erl.
Footnotes: [a] Human intestinal absorption: If the compound has the HIA% less than 30 %, it is labeled as (-), otherwise it is labeled as (+) [17]; [b] Caco-2 permeability. If the compound has the Caco-2 permeability value ≥ 8×10^{-6} cm/s, it is labeled as high Caco-2 permeability (+), otherwise it is labeled as moderate-poor permeability (-) [18]; [c] P-glycoprotein inhibition [19,20]. Non-inhibition is labeled as (-), otherwise it is labeled as (+); [d] Renal organic cation transporter (OCT2) inhibition [21]. Non-inhibition is labeled as (-), otherwise it is labeled as (+); [e] Ability to be substrate of three Cytochrome P450 isoenzymes (CYP450 2C9, CYP450 2D6, and CYP450 3A4) [22]. It is shown the predicted isoform-CYP that the compound potentially acts as substrate; [f] Human ether-a-go-go-related gene (hERG) inhibition [23,24]. Non-inhibition is labeled as "non", weak-inhibition is labeled as "w-inh" and inhibition is labeled as "inh"; [g] Potential as carcinogens [25]. Non-carcinogen is labeled as (-); [h] Aqueous solubility [26]; [l] Distribution coefficient at pH 7.4, from [13]; [j] number of violations of Lipinski "Rule of five"; [k] Lipophilic ligand efficiency (pIC_{50} – $LogD_{7.4}$) [27]; [l] MW higher than 500 Da; [m] MW higher than 500 Da and hydrogen bond acceptors higher than 10.

Table 4. Summary of *in vivo* oral acute-toxicity for hybrid 1.
Footnotes: [a] According to [37]; [b] The test was performed at 0.5, 1.5, 3, 5, 12, 24, 48, and 72 h post-oral administration. If all the analyzed parameters were similar that saline-treatment it is labeled as (+), otherwise it is labeled as (-); [c] (-) denotes absence of organs alterations or organs adherences; [d] DMSO (4 %) was added to allow the complete **1**-solubilization.

Table 5. Example of characterization of some hybrids-formulations developed herein.
Footnotes: [a] In a concentration of 1 mg/mL; [b] Particle size; [c] Polydispersity index.

Table 6. *In vitro* activity of 1, 8, and erl alone and vehiculizated with F127/T1307 or liposome.
Footnotes: [a] Concentrations, in μM, required to inhibit the cellular growth by 50 %. They were determined from dose-response curves, and represent the mean ± s.d. All experiments were repeated at least three times; [b] From Table 1 [12]; [c] From Table 2; [d] Maximum evaluated doses; [e] Higher doses than 100 μM could not be evaluated due to solubility problems; [f] NS: not studied.

References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

1. Alphandéry E. Glioblastoma treatments: An account of recent industrial developments. *Front. Pharmacol.* 9, 879 (2018).
2. Messaoudi K, Clavreul A, Lagarce F. Toward an effective strategy in glioblastoma treatment. Part I: resistance mechanisms and strategies to overcome resistance of glioblastoma to temozolomide. *Drug Discov. Today* 20, 899-905 (2015).
3. Crespo I, Vital AL, Gonzalez-Tablas M *et al*. Molecular and genomic alterations in glioblastoma multiforme. *Am. J. Pathol.* 185, 1820-1833 (2015).
4. Tuncel G, Kalkan R. Receptor tyrosine kinase-Ras-PI 3 kinase-Akt signaling network in glioblastoma multiforme. *Med. Oncol.* 35, 122 (2018).
5. Prados MD, Chang SM, Butowski N *et al*. Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma. *J. Clin. Oncol.* 27, 579–84 (2009).

● A typical example of clinic study with combination of drugs for glioblastoma multiforme treatment.

6. Conger AD, Giles NH. The cytogenetic effect of slow neutrons. *Genetics* 35, 397-419 (1950).
7. Calabrese G, Daou A, Barbu E, Tsibouklis J. Towards carborane-functionalised structures for the treatment of brain cancer. *Drug Discov. Today* 23, 63-75 (2018).

●● An excellent review that describes the carborane potential use in brain cancer.

8. Hiratsuka J, Kamitani N, Tanaka R *et al*. Boron neutron capture therapy for vulvar melanoma and genital extramammary Paget's disease with curative responses. *Cancer Commun. (Lond).* 38, 38 (2018).
9. Trivillin VA, Pozzi ECC, Colombo LL *et al*. Abscopal effect of boron neutron capture therapy (BNCT): proof of principle in an experimental model of colon cancer. *Radiat. Environ. Biophys.* 56, 365-375 (2017).

● An important investigation on the use of BNCT in colon cancer.

10. Hey-Hawkins E, Viñas C. Boron-Based Compounds. Potential and Emerging Applications in Medicine. John Wiley & Sons Incorporated, Newark (2018).

●● The book offers a summary of the present status and highlights the range and capacity of new boron-containing compounds in medical applications.

11. Nakamura H, Horikoshi R, Usui T, Ban HS. Selective inhibition of EGFR and VEGFR2 tyrosine kinases controlled by a boronic acid substituent on 4-anilinoquinazolines. *MedChemComm* 1, 282-286 (2010).
12. Couto M, Mastandrea I, Cabrera M *et al*. Small-molecule kinase-inhibitors-loaded boron cluster as hybrid agents for glioma-cell-targeting therapy. *Chem. Eur. J.* 23, 9233-9238 (2017).

●● An important investigation on the development of novel boron-cluster-containing kinase-inhibitors for glioma treatment.

13. Couto M, García MF, Alamón C *et al*. Discovery of potent EGFR inhibitors through the incorporation of a 3D-aromatic-boron-rich-cluster into the 4-anilinoquinazoline scaffold: Potential drugs for glioma treatment. *Chem. Eur. J.* 24, 3122-3126 (2018).

●● An important investigation on the mechanism of action confirmation of novel anti-glioma boron-cluster-containing agents.

14. Mamot C, Drummond DC, Greiser U *et al*. Epidermal growth factor receptor (EGFR)-targeted immunoliposomes mediate specific and efficient drug delivery to EGFR- and EGFRvIII-overexpressing tumor cells. *Cancer Res.* 63, 3154-3161 (2003).
15. Cheng CC, Liao PN, Ho AS *et al*. STAT3 exacerbates survival of cancer stem-like tumorspheres in EGFR-positive colorectal cancers: RNAseq analysis and therapeutic screening. *J. Biomed. Sci.* 25, 60 (2018).
16. Cheng F, Li W, Zhou Y *et al*. admetSAR: A comprehensive source and free tool for evaluating chemical ADMET properties. *J. Chem. Inf. Model.* 52, 3099-3105 (2012). http://lmmd.ecust.edu.cn/admetsar1/predict/.
17. Shen J, Cheng F, Xu Y, Li W, Tang Y. Estimation of ADME properties with substructure pattern recognition. *J. Chem. Inf. Model* 50, 1034-1041 (2010).
18. Pham-The H, González-Álvarez I, Bermejo M *et al*. In Silico prediction of Caco-2 cell permeability by a classification QSAR approach. *Mol. Inf.* 30, 376-385 (2011).

19. Chen L, Li Y, Zhao Q, Peng H, Hou T. ADME evaluation in drug discovery. 10. Predictions of P-glycoprotein inhibitors using recursive partitioning and naive Bayesian classification techniques. *Mol. Pharm.* 8, 889-900 (2011).
20. Broccatelli F, Carosati E, Neri A *et al*. A novel approach for predicting P-glycoprotein (ABCB1) inhibition using molecular interaction fields. *J. Med. Chem.* 54, 1740-1751 (2011).
21. Kido Y, Matsson P, Giacomini KM. Profiling of a prescription drug library for potential renal drug-drug interactions mediated by the organic cation transporter 2. *J. Med. Chem.* 54, 4548-4558 (2011).
22. Carbon-Mangels M, Hutter MC. Selecting relevant descriptors for classification by Bayesian estimates: A comparison with decision trees and support vector machines approaches for disparate data sets. *Mol. Inf.* 30, 885-895 (2011).
23. Marchese Robinson RL, Glen RC, Mitchell JBO. Development and comparison of hERG blocker classifiers: assessment on different datasets yields markedly different results. *Mol. Inf.* 30, 443-458 (2011).
24. Wang S, Li Y, Wang J *et al*. ADMET evaluation in drug discovery. 12. Development of binary classification models for prediction of hERG potassium channel blockage. *Mol. Pharm.* 9, 996-1010 (2012).
25. Lagunin A, Filimonov D, Zakharov A *et al*. Computer-aided prediction of rodent carcinogenicity by PASS and CISOC-PSCT. *QSAR Comb. Sci.* 28, 806-810 (2009).
26. Wang J, Krudy G, Hou T, Zhang W, Holland G, Xu X. Development of reliable aqueous solubility models and their application in druglike analysis. *J. Chem. Inf. Model.* 47, 1395-1404 (2007).
27. Murray CW, Erlanson DA, Hopkins AL *et al*. Validity of ligand efficiency metrics. *ACS Med. Chem. Lett.* 5, 616-618 (2014).
28. Varma MV, Feng B, Obach RS *et al*. Physicochemical determinants of human renal clearance. *J. Med. Chem.* 52, 4844-4852 (2009).
29. Schuster D, Laggner C, Langer T. Antitargets: Prediction and prevention of drug side effects. Vaz RJ, Klabunde T, Eds. Wiley-VCH, Weinheim (2008).
30. Dresser GK, Spence JD, Bailey DG. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet.* 38, 41-57 (2000).
31. Doherty KR, Wappel RL, Talbert DR *et al*. Multi-parameter *in vitro* toxicity testing of crizotinib, sunitinib, erlotinib, and nilotinib in human cardiomyocytes. *Toxicol Appl Pharmacol.* 272, 245-55 (2013).
32. Hansen K, Mika S, Schroeter T *et al.* Benchmark data set for *in silico* prediction of Ames mutagenicity. *J. Chem. Inf. Model.* 49, 2077-2081 (2009).
33. Zhu H, Martin TM, Ye L, Sedykh A, Young DM, Tropsha A. Quantitative structure-activity relationship modeling of rat acute toxicity by oral exposure. *Chem. Res. Toxicol.* 22, 1913-1921 (2009).
34. Leeson PD, Springthorpe B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* 6, 881-890 (2007).
- A review that describes and discusses the drug-like concepts on the medicinal chemistry decision-making.
35. OECD 471, 1997. Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals; Guideline No. 471: Genetic Toxicology: Bacterial Reverse Mutation Test. (adopted July 21, 1997).
36. OECD 423, 2001. Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for the Testing of Chemicals, Guideline No. 423: Acute Oral Toxicity – Acute Toxic Class Method. (adopted December 17, 2001).
37. Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacology (Berl.)* 13, 222-257 (1968).
38. Oh KT, Bronich TK, Kabanov AV. Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic Pluronic® block copolymers. *J. Control. Release* 94, 411-422 (2004).
39. Wei Z, Hao J, Yuan S *et al.* Paclitaxel-loaded Pluronic P123/F127 mixed polymeric micelles: Formulation, optimization and *in vitro* characterization. *Int. J. Pharm.* 376, 176-185 (2009).
40. Bruce L, Bruce I. Polymer-based anti-cancer agents. WO 2007/123468 A1 (2007).
- A description about the ability to reduce cancer cells proliferation rates of some amphiphilic block copolymers.
41. Hu J, Han Y, Wang J *et al*. Discovery of selective EGFR modulator to inhibit L858R/T790M double mutants bearing a *N*-9-diphenyl-9*H*-purin-2-amine scaffold. *Bioorg. Med. Chem.* 26, 1810-1822 (2018).

42. Guan Y, Huang J, Zuo L, *et al*. Effect of pluronic P123 and F127 block copolymer on P-glycoprotein transport and CYP3 A metabolism. *Arch. Pharmacol. Res.* 34, 1719-1728 (2011).
43. Patra A, Satpathy S, Shenoy AK, Bush JA, Kazi M, Hussain MD. Formulation and evaluation of mixed polymeric micelles of quercetin for treatment of breast, ovarian, and multidrug resistant cancers. *Int. J. Nanomed.* 13, 2869-2881 (2018).

SUPPLEMENTARY MATERIAL

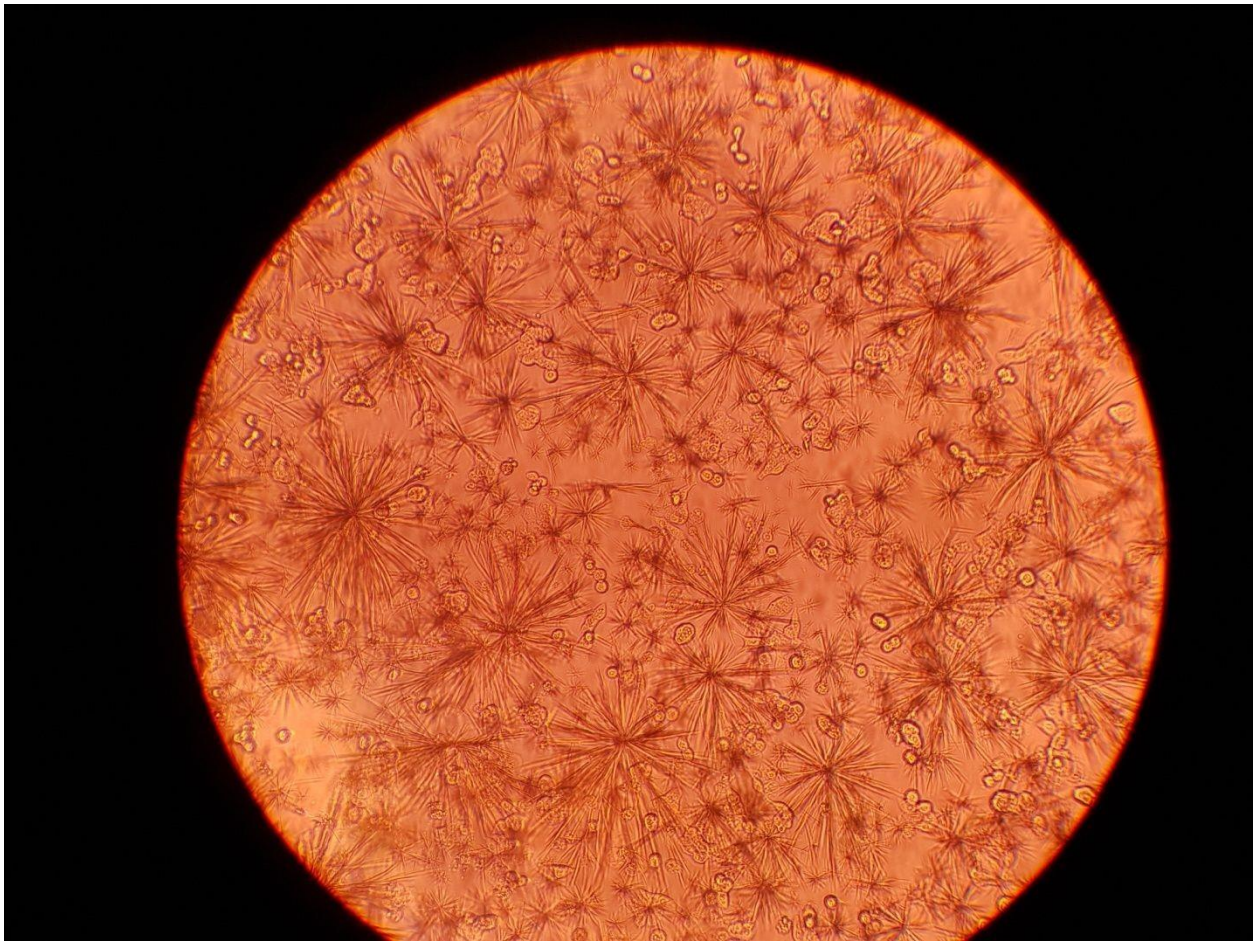


Figure S1. Microscopy of HT-29 treated with erl (100 µM). The crystallization of **erl** was clearly evidenced as conglomerate of needles.