EGFR-Targeted Pentacyclic Triterpene Analogues for Glioma Therapy

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Glioma, particularly its most malignant form, glioblastoma multiforme (GBM), is the most common and aggressive malignant central nervous system tumor. The drawbacks of the current chemotherapy for GBM have aroused curiosity in the search for targeted therapies. Aberrantly overexpressed epidermal growth factor receptor (EGFR) in GBM results in poor prognosis, low survival rates, poor responses to therapy and recurrence, and therefore EGFR-targeted therapy stands out as a promising approach for the treatment of gliomas. In this context, a series of pentacyclic triterpene analogues were subjected to in vitro and in silico assays, which were conducted to assess their potency as EGFR-targeted anti-glioma agents. In particular, compound 10 was the most potent anti-glioma agent with an IC50 value of 5.82 µM towards U251 human glioblastoma cells. Taking into account its low cytotoxicity to peripheral blood mononuclear cells (PBMCs), compound 10 exerts selective antitumor action towards Jurkat human leukemic T-cells. This compound also induced apoptosis and inhibited EGFR with an IC50 value of 9.43 µM compared to erlotinib (IC50 = 0.06 µM). Based on in vitro and in silico data, compound 10 stands out as a potential orally bioavailable EGFR-targeted anti-glioma agent endowed with the ability to cross the blood–brain barrier (BBB).

apoptosis epidermal growth factor receptor glioblastoma multiforme gliomas pentacyclic triterpenes

1. Introduction

Gliomas, which consist of a group of heterogeneous brain tumors originating from three types of glial cells, namely astrocytes, oligodendrocytes, and ependymal cells, account for almost 80% of primary malignant brain tumors in adults [1–6] [1][2][3][4][5][6][7][8]. The incidence and mortality rate associated with gliomas are expected to increase dramatically in the upcoming years, particularly in developing countries[4] . According to the World Health Organization (WHO) grading, gliomas are categorized into four grades (I-IV) [5]. Among these four grades [6], glioblastoma multiforme (GBM; grade IV) is the most common, aggressive, and malignant form of glioma [7][8]. The main features of GBM are rapid proliferation with poor differentiation, diffuse infiltration into normal brain tissues, angiogenesis, tendency to necrosis, resistance to apoptosis, widespread genomic aberrations[8][9] , and these features make GBM challenging to treat[8][9][10][11] . Current treatment protocol involves surgical resection followed by concurrent radiotherapy and temozolomide (TMZ) for 6 weeks, then adjuvant TMZ for 6 months[1][8]. Despite tremendous efforts devoted to improving the therapeutic strategies towards GBM, the prognosis for patients with GBM still remains poor [3] and the median survival rates of these patients are very low [8,9]. The risk of recurrence

is high since current therapies do not take into account the unique molecular features of different subtypes of glioma[4]

The efficacy of anti-glioma chemotherapy is limited because of poor drug delivery and inherent chemoresistance [3] or acquired chemoresistance (e.g., to TMZ) after initial treatment [12]. Due to unfavorable pharmacokinetics of chemotherapeutic drugs, poor drug delivery across the blood–brain barrier (BBB) and blood–tumor barrier (BTB) prevents them from exerting their therapeutic action properly. The inherent chemoresistance of the brain endothelium and glioma cells, expressing the drug efflux protein *p*-glycoprotein also impairs the therapeutic efficacy[2][3]. Moreover, clinical applications are limited by adverse effects, such as bone marrow suppression, genotoxic, and teratogenic effects[10].

The shortcomings in the current chemotherapy for malignant gliomas, particularly GBM have aroused great interest in the search for targeted therapies acting on specific molecular targets involved in the pathogenesis of gliomas [12]. One of the most widely studied targets is the epidermal growth factor receptor (EGFR), also referred to as ErbB-1/HER1. EGFR is a 170 kDa transmembrane glycoprotein that belongs to the ErbB/HER family of receptor tyrosine kinases (RTKs) . EGFR is involved in key signaling pathways responsible for the growth, proliferation, migration, and survival of tumor cells . The overexpression of EGFR and/or its constitutively activated variant EGFRvIII contributes to the pathogenesis of many types of cancer including GBM [13][14]. Aberrant EGFR signaling leads to poor prognosis and low survival rates for GBM patients, poor responses to therapy, and earlier recurrence after treatment [14] and therefore EGFR-targeted therapy has emerged as a promising approach for the management of gliomas [15][12][13][14][16].

Diverse applications of natural products trace back thousands of years for the treatment of severe diseases. Among these biologically active phytochemicals, pentacyclic triterpenes (PTs) have attracted a great deal of interest as the most valuable sources of pharmacological agents due to their wide range of biological activities including anticancer, antiviral, anti-inflammatory, antimicrobial, antioxidant, antimalarial, neuro-, and hepatoprotective activities [17][18][19][20]. In particular, the number of patents related to PTs endowed with potent anticancer activity is significantly increasing [17]. PTs and their synthetic analogues have been reported to show selective cytotoxic activity towards a huge diversity of cancer cells [17] including GBM cells [21][22][23]. They exert their antitumor action through multiple mechanisms such as apoptosis, cell cycle arrest, and autophagy triggered by their effects on transforming growth factor-beta (TGF-β) and HER cell surface receptors and the downstream signaling pathways, including phosphoinositide 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR), IkB kinase (IKK)/nuclear factor kappa B (NF-κB), signal transducers and activators of transcription (STAT)-3 pathway and mitogen-activated protein kinase (MAPK) cascades [24][25][26][27][28][29]. The search for antitumor PTs has mainly focused on the most abundant groups of PTs, namely the oleanane- (e.g., oleanolic acid, 18β-glycyrrhetinic acid), ursane- (e.g., ursolic acid, asiatic acid) and lupane- (e.g., betulinic acid) triterpenoids [24][25][26][27][28][29][30][31][32][33][34][35]. According to in vitro and in vivo assays, the effects of antitumor PTs on EGFR signaling are fulfilled on the receptor level. Both natural (ursolic, glycyrrhetinic, and oleanolic acids, lupeol, and dimethyl melaleucate) and semisynthetic PTs were found to inhibit tumor cell growth by decreasing phosphorylation of EGFR leading to

suppression of downstream signaling (MAPK, PI3K/Akt/NF-κB and/or STAT) pathways [24]. Based on in silico molecular modeling studies conducted to explain the decrease of EGFR-phosphorylation triggered by PTs, ursolic acid, glycyrrhetinic acid, dimethyl melaleucate, some synthetic derivatives of glycyrrhetinic acid and glycyrrhizin are capable of binding to the EGFR tyrosine kinase domain [24]. Some PTs (e.g., oleanolic acid) also decrease EGFR protein expression in some different cancer types [24].

Previously, we performed in vitro and in silico studies for the benzyl esters of asiatic acid (**1**), betulinic acid (**2**), glycyrrhetinic acid (**3**), hederagenin (**4**), oleanolic acid (**5**), ursolic acid (**6**), and gypsogenin (**7**), and the substituted benzyl esters of gypsogenin (**8, 9**) (**Figure 1**) to identify anti-chronic myelogenous leukemia (CML) agents targeting ABL1 kinase [35]. A batch of outstanding publications related to the potent antitumor activity of PTs through diverse mechanisms (e.g., EGFR signaling) [17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35] prompted us to identify EGFR-targeted PTs for the treatment of GBM. In this context, the synthesis of new PTs (**10–13**) was performed efficiently and in vitro and in silico studies were conducted to assess the potential of compounds **1–13** as EGFR-targeted anti-glioma agents.

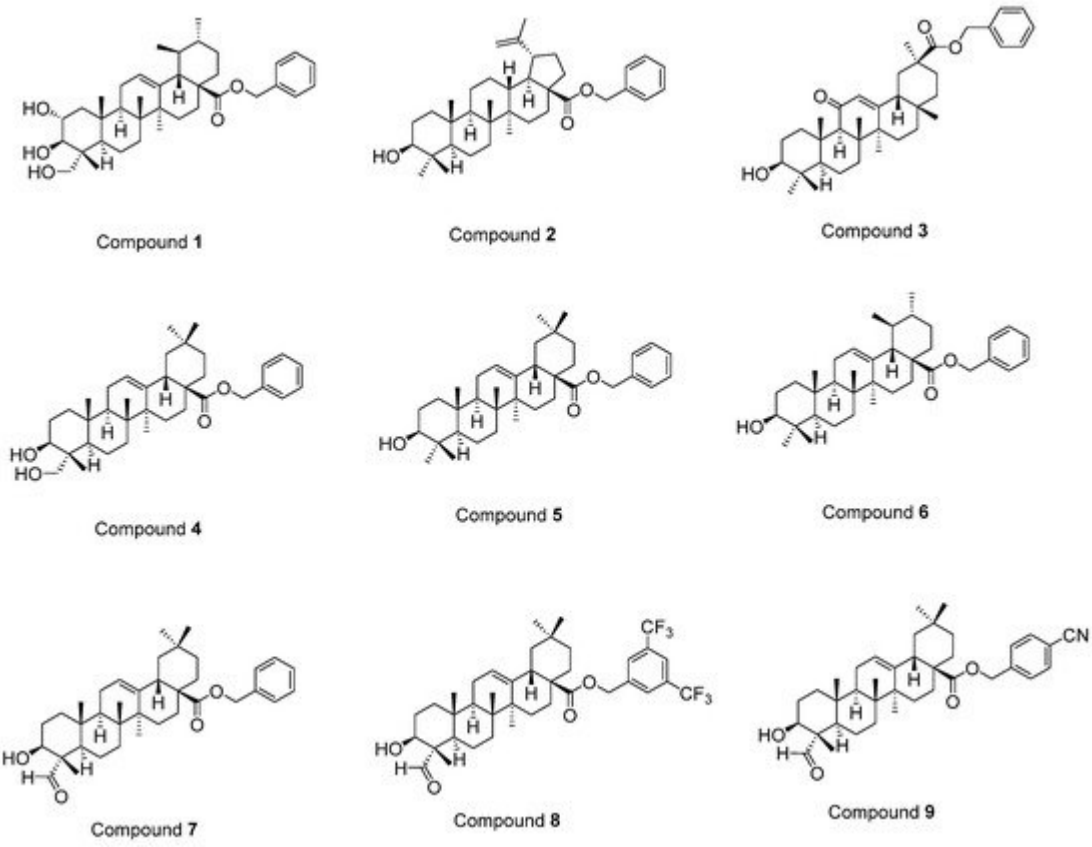


Figure 1. The structures of compounds **1–9** [35].

2. EGFR-Targeted Pentacyclic Triterpene Analogues for Glioma Therapy

Compounds **1–6**, **8,** and **9** were synthesized as described before [35], whereas compound **7** was prepared according to a previous study [36]. All spectral data were in agreement with those reported. Herein, we report the first reductive amination of a PT, gypsogenin, being endowed with a unique carbaldehyde group. The latter was aminated by four different aromatic amines in the presence of sodium triacetoxyborohydride (**Figure 2**).

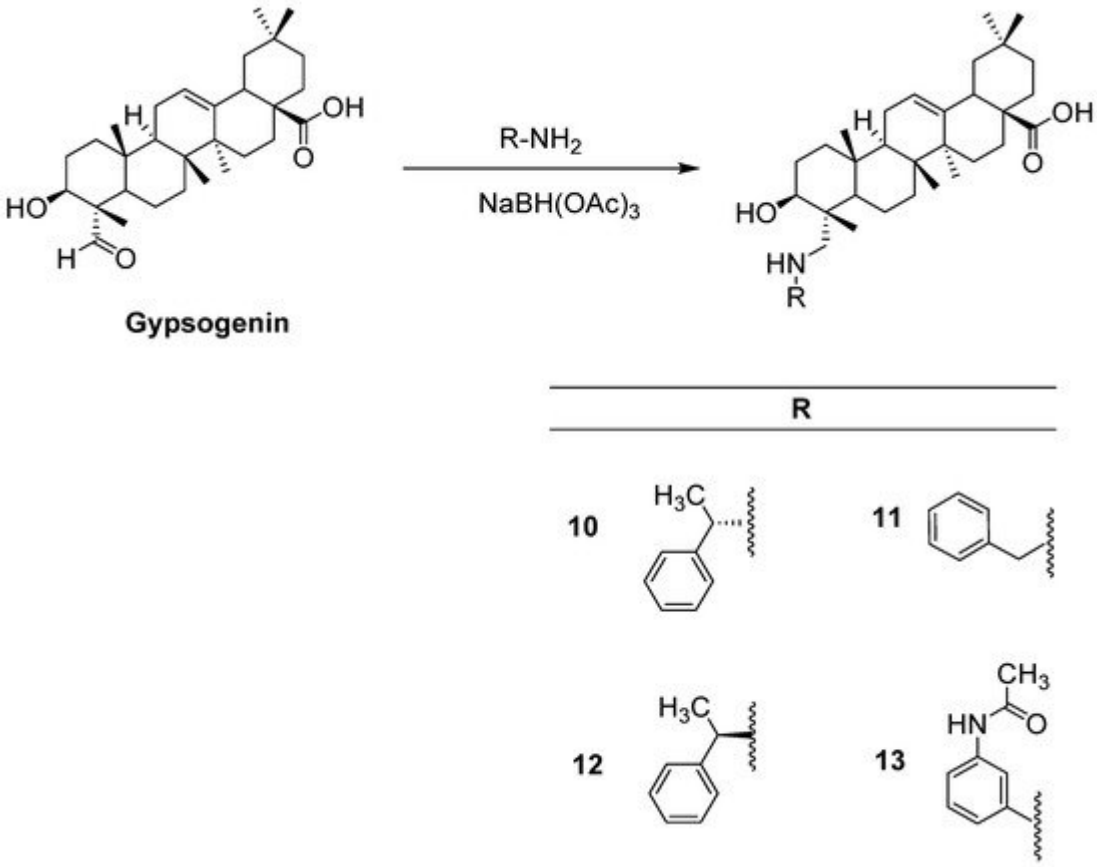


Figure 2. The synthetic route for the preparation of new compounds (**10–13**).

MTT assay, the most widely used colorimetric assay for in vitro drug screening [37], was conducted to assess the cytotoxic effects of compounds **1–13** and cisplatin (positive control) on U251, T98G, and U87 human glioblastoma cell lines. As indicated in **Table 1**, compound **10** was identified as the most effective anti-glioma agent in this series. This compound exerted antitumor action towards U251, T98G, and U87 cells with IC_{50} values of 5.82 µM, 8.19 µM, and 17.04 µM, respectively superior to cisplatin (IC_{50} = 7.70 µM, 16.92 µM, and 20.90 µM, respectively). The potency order of cytotoxic effects of the other compounds on U251 cells was determined as compound **4** > compound **13** > compound **1** > compound **9** > compound **6** > compound **7** > compound **2**. In accordance with the data recorded for U251 cells, compounds **4**, **13,** and **1** followed the same order for T98G and U87 cell lines. The IC_{50} values of compounds **4**, **13,** and **1** for U251 cells were detected as 8.06 µM, 9.95 µM, and 13.18 µM, whereas their IC_{50} values for T98G cells were found as 9.86 µM, 20.19 µM, and 20.54 µM, respectively. Compounds **4**, **13,** and **1** exhibited anticancer activity against U87 cells with IC_{50} values of 19.54 µM, 21.71 µM, and 22.64 µM, respectively. As depicted in **Figure 3**, compounds **4**, **10,** and **13** showed pronounced antiproliferative effects on U251, T98G, and U87 cells compared to cisplatin at varying concentrations. On the other hand, compounds **3**, **5**, **8**, **11,** and **12** displayed no significant anticancer activity against all tested GBM cell lines at 100 µM concentration.

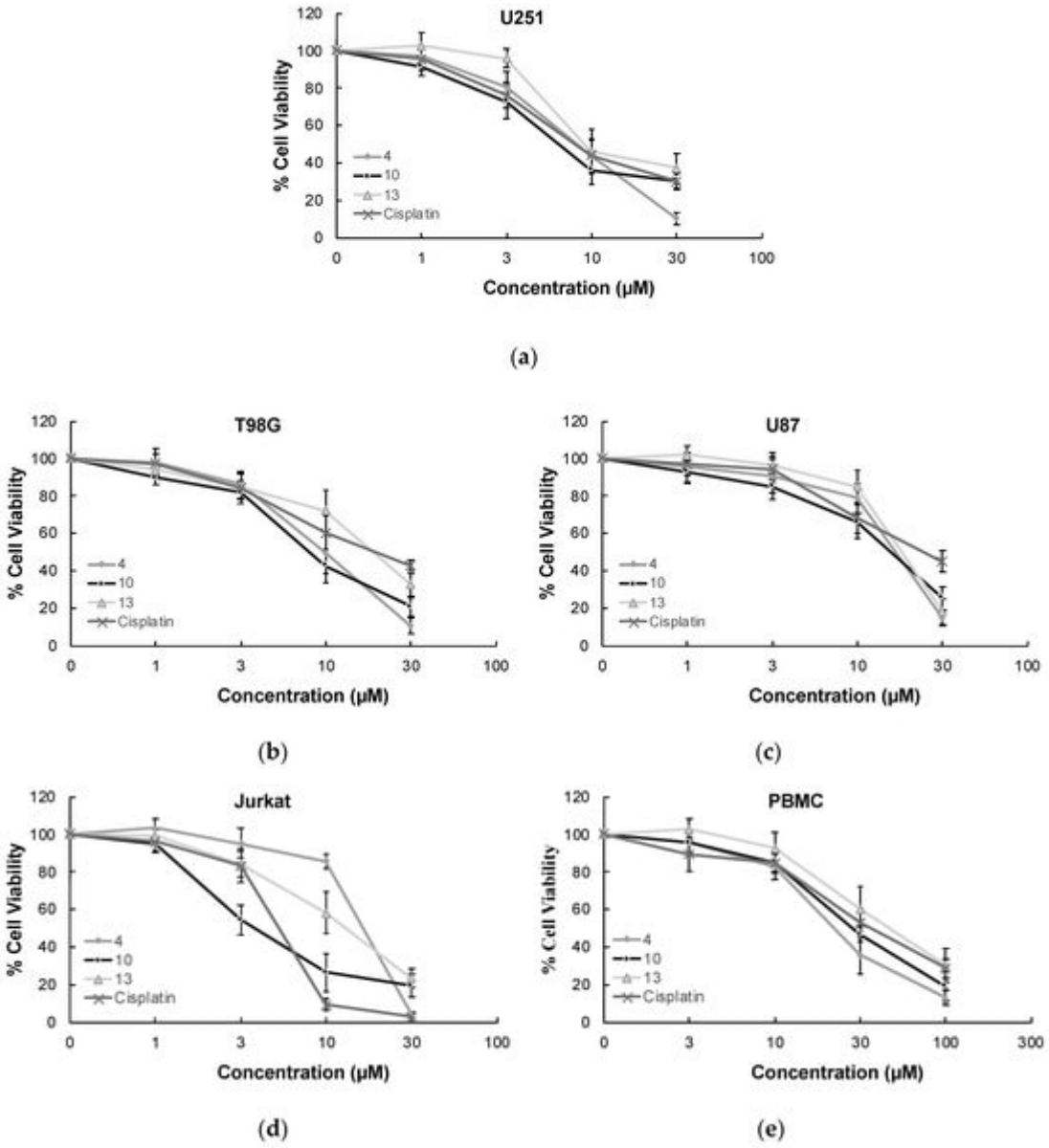


Figure 3. The anticancer effects of compounds **4, 10, 13,** and cisplatin at varying concentrations on U251 cells (**a**), T98G cells (**b**), U87 cells (**c**), Jurkat cells (**d**), and PBMCs (**e**). All descriptive data were expressed as the mean ± standard deviation (SD). All experiments were repeated three times.

Table 1. The cytotoxic effects of the compounds on U251, T98G, U87, and Jurkat cells, PBMCs.

Compound	IC_{50} value (μM)					SI[1]
	U251 cells	T98G cells	U87 cells	Jurkat cells	PBMCs	
1	13.18 ± 3.19	20.54 ± 4.34	22.64 ± 6.75			
2	24.00 ± 4.98	>100	>100			

3	>100	>100	>100			
4	8.06 ± 2.04	9.86 ± 2.21	19.54 ± 4.52	9.97 ± 3.24	21.91 ± 5.13	2.20
5	>100	>100	>100			
6	16.68 ± 3.17	64.12 ± 7.36	79.70 ± 10.08			
7	17.98 ± 2.23	61.11 ± 5.13	60.93 ± 8.87			
8	>100	>100	>100			
9	14.13 ± 3.41	56.55 ± 6.08	>100			
10	5.82 ± 1.66	8.19 ± 2.42	17.04 ± 4.92	3.56 ± 1.45	28.12 ± 5.05	7.90
11	>100	>100	>100			
12	>100	>100	>100			
13	9.95 ± 2.04	20.19 ± 5.47	21.71 ± 6.09	12.08 ± 1.64	43.15 ± 8.32	3.57
Cisplatin	7.70 ± 2.81	16.92 ± 3.95	20.90 ± 5.16	4.87 ± 2.00	34.67 ± 7.11	7.12

[1] SI = IC_{50} for PBMCs/IC_{50} for Jurkat cells.

In order to determine the selectivity of the mode of anti-glioma action, the most effective anticancer agents (compounds **4, 10,** and **13**) were further screened for their cytotoxic effects on Jurkat human leukemic T-cells and human peripheral blood mononuclear cells (PBMCs). The selectivity of compound **10** was found as the most promising between Jurkat cells and PBMCs with a selectivity index (SI) value of 7.90. The anticancer effects of compounds **4**, **10,** and **13** compared to cisplatin at varying concentrations on Jurkat cells and PBMCs also supported this outcome (**Figure 3**).

As compound **10** was designated as the most potent and selective anti-glioma agent according to MTT results, its further apoptotic and EGFR inhibitory effects were also investigated to provide mechanistic insight. Using the annexin V/ethidium homodimer III staining method, the apoptotic effects of compound **10** on U251 cells were evaluated. This method indicates apoptosis and necrosis based on staining green and red, respectively. The results indicated that compound **10** boosted apoptosis in U251 cells with 10.29% similar to cisplatin (13.83%) (**Figure 4**).

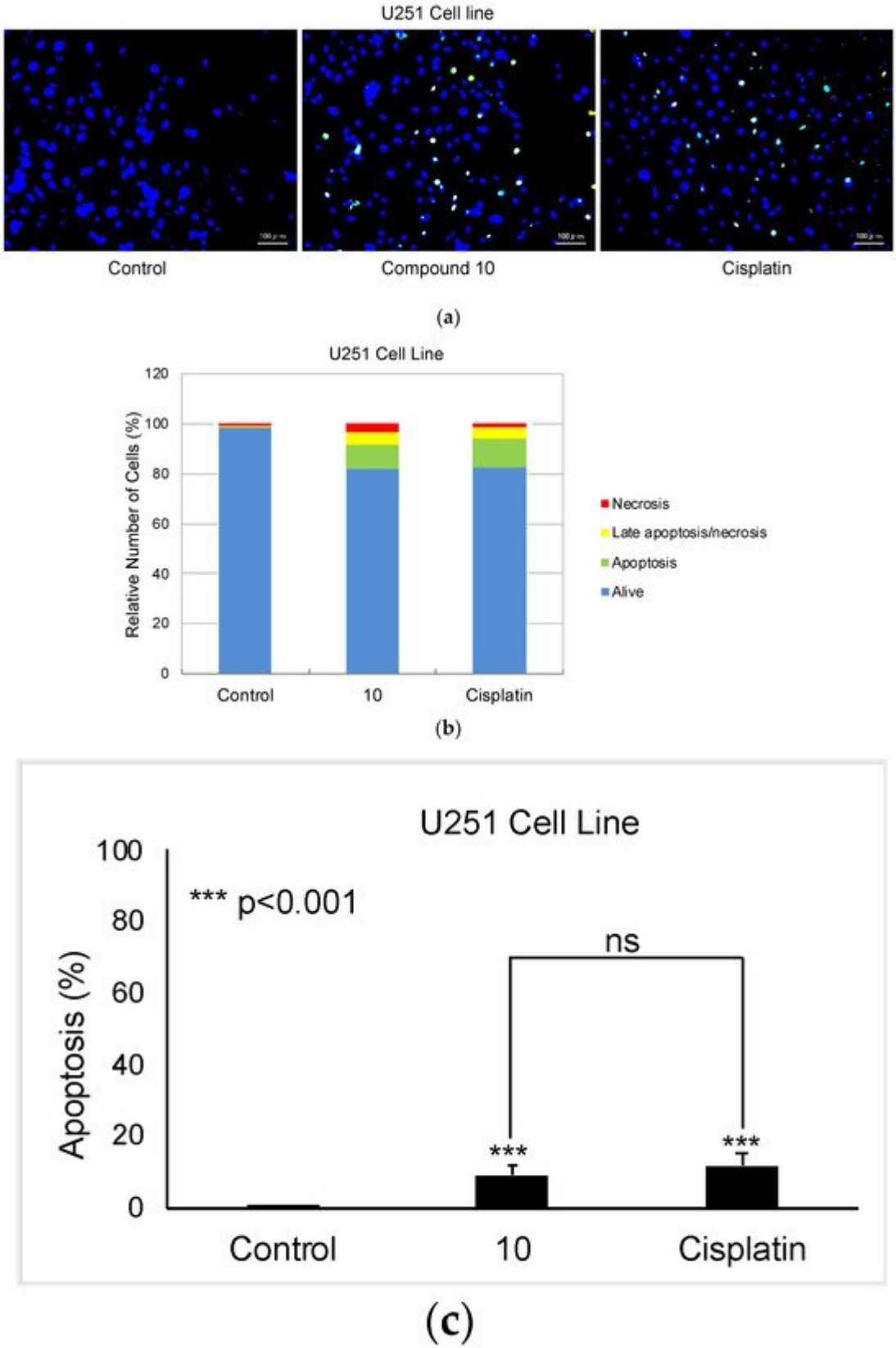


Figure 4. Alteration of U251 cells following exposure to IC_{50} concentration of the control (DMSO), compound **10,** and cisplatin (**a**) for 24 h. The percentage of alive (blue), apoptotic (green), necrotic or late apoptotic (both green and red), and necrotic (red) cells (**b**) was determined by analyzing 100 randomly chosen stained cells in each

experiment. Quantification of apoptotic effects of compound **10** and cisplatin (**c**). Data from three independent experiments were expressed as mean ± standard deviation and *p* values were determined using the student's test.

In continuation of our mechanistic research, the inhibitory effects of compound **10** on EGFR were analyzed due to the correlation between diminished EGFR signaling with increased anti-glioma activity. It was observed that compound **10** significantly inhibited EGFR with an IC_{50} value of 9.43 µM compared to erlotinib (IC_{50} = 0.06 µM), a first-generation EGFR tyrosine kinase inhibitor (**Figure 5**). Moreover, **Figure 6** also highlighted the significant EGFR activity of compound **10** at 30 µM concentration compared to erlotinib. This outcome also pointed out that newly synthesized compounds displayed higher EGFR inhibition than our previously synthesized compounds. In order to explore the kinase selectivity profiling of compound **10**, the inhibition of compound **10** at 30 µM concentration was examined on a large panel of tyrosine kinase enzymes including TK-1 (HER2, HER4, IGF1R, InsR, KDR, PDGFR-α, and PDGFR-β) and TK-2 (ABL1, BRK, BTK, CSK, FYN A, LCK, LYN B, and SRC) compared to erlotinib. Compound **10** showed the most potent inhibitory activity on the InsR followed by KDR, PDGFR-α, LCK, ABL1, HER2, CSK, PDGFR-β, and FYN A. This compound displayed no significant inhibition against HER4, IGF1R, BTK, BRK, LYN B, and SRC. Compound **10** and erlotinib exhibited similar and moderate HER2, ABL1, and FYN A inhibition, whereas compound **10** showed inhibitory effects on InsR, CSK, and LCK stronger than erlotinib. According to the results, compound **10** revealed a different kinase inhibitory profile than erlotinib as depicted in **Figure 7**. It can be concluded that compound **10** at 30 µM concentration exhibited the most selective inhibition against EGFR (approximately 2-fold stronger inhibition than InsR, the second promising tyrosine kinase target of compound **10**) among all the tested tyrosine kinases.

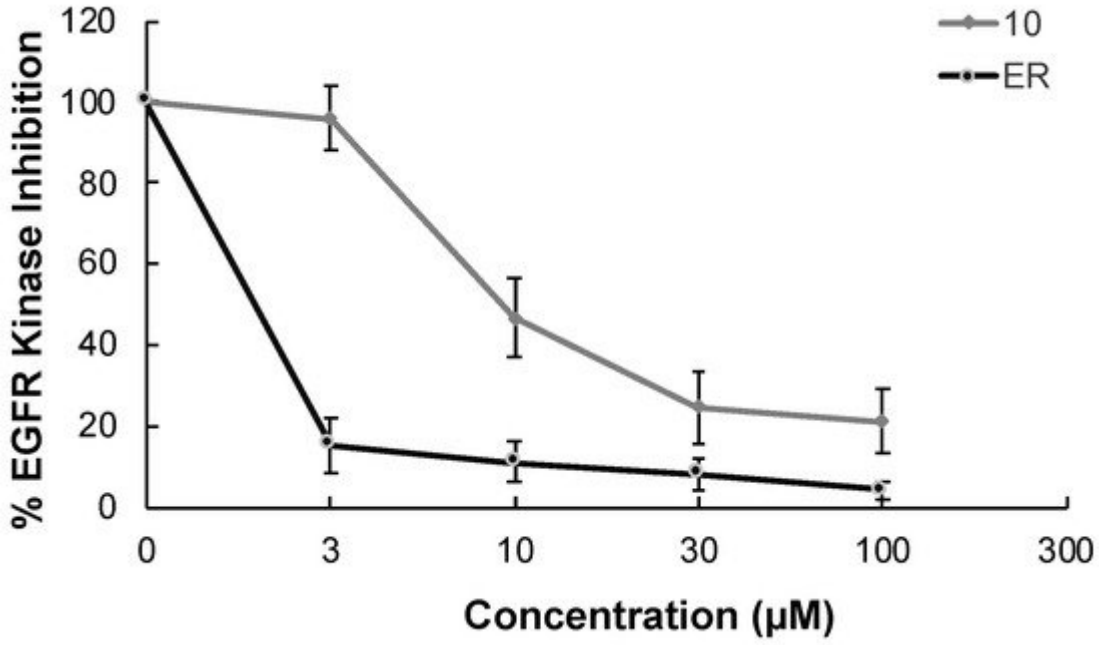


Figure 5. The EGFR kinase inhibition of compound **10** and erlotinib at different concentrations. All descriptive data were expressed as the mean ± SD. All experiments were repeated three times.

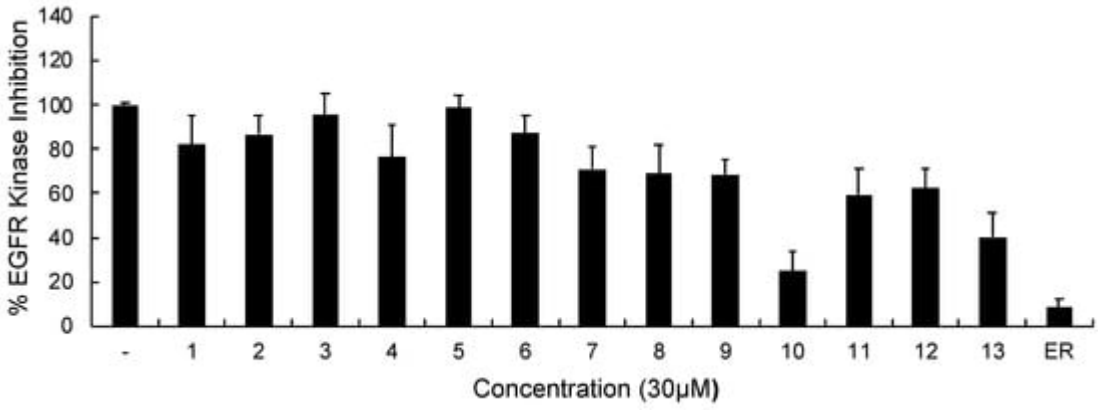


Figure 6. The EGFR kinase inhibition of compounds **1–13** and erlotinib at 30 μM concentration. All descriptive data were expressed as the mean ± SD. All experiments were repeated three times.

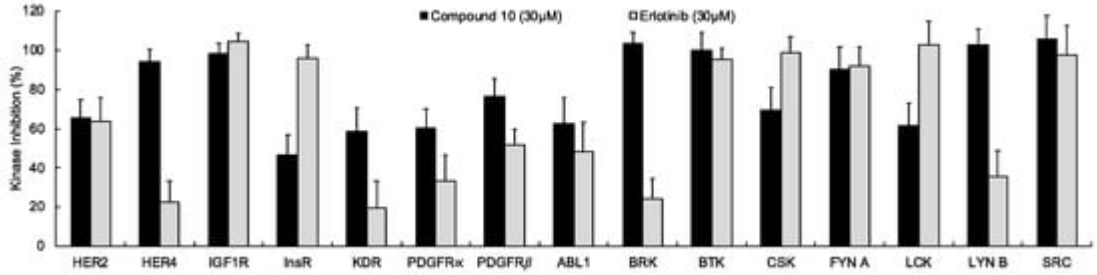
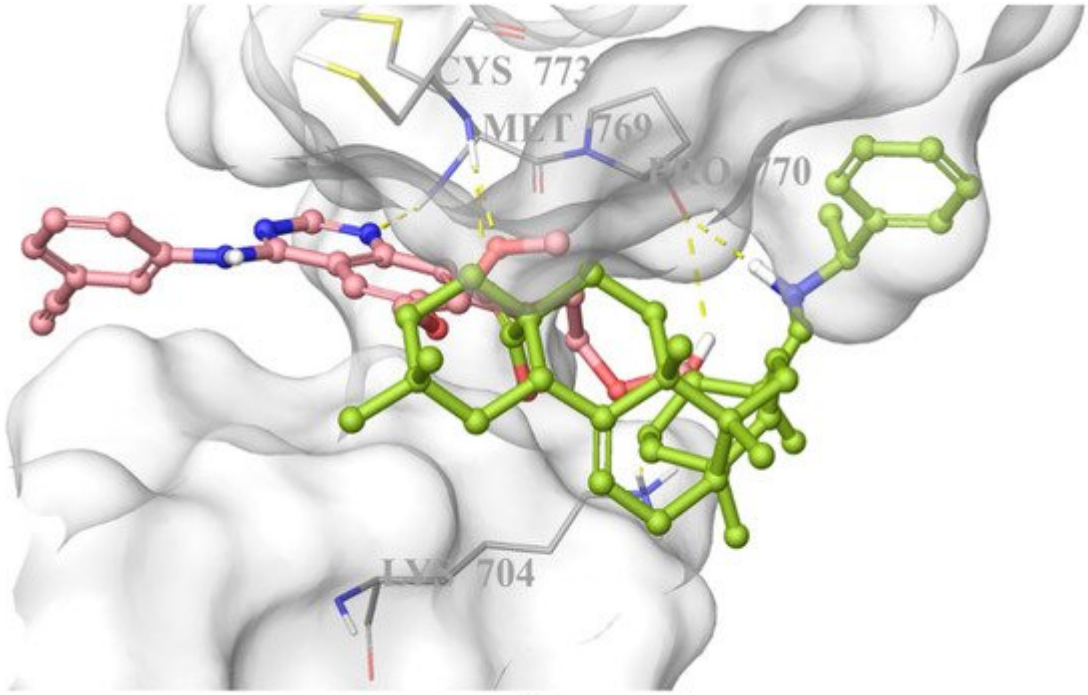
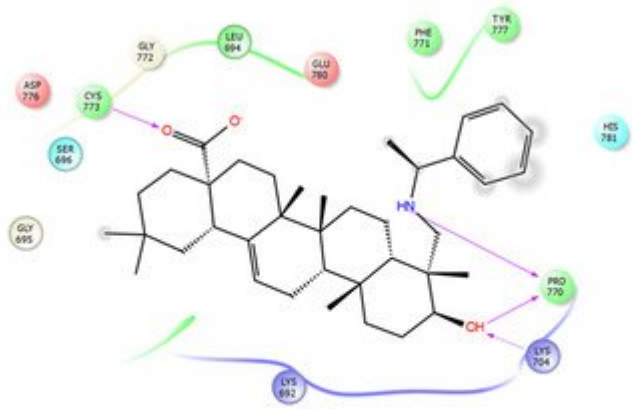


Figure 7. The inhibition of a panel of tyrosine kinases by compound **10** and erlotinib at 30 μM concentration. All descriptive data were expressed as the mean ± SD. All experiments were repeated three times.

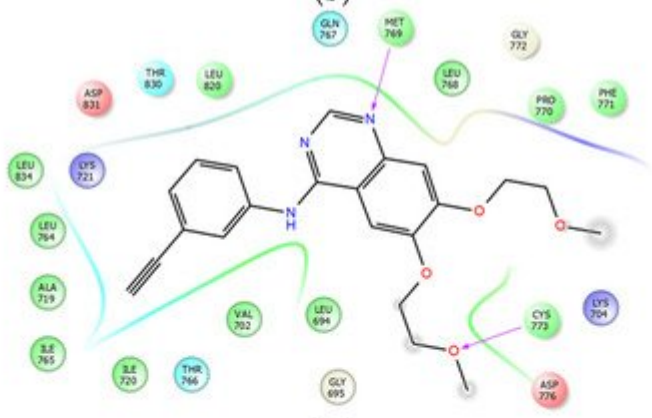
On the basis of its significant in vitro EGFR inhibitory potency, molecular docking studies were also carried out to understand the affinity of compound **10** to the adenosine triphosphate (ATP) binding site of the EGFR, which was acquired from the Protein Data Bank (PDB) server (PDB ID: 4HJO) [38]. Molecular docking data demonstrated that compound **10** presented strong affinity at ionized state with favorable hydrogen bonding with Cys773, Pro770, and Lys704 by means of carboxylic acid, (phenylethyl)amino group, and hydroxyl substituent, respectively. This strong affinity could also be attributed to its interaction with Cys773 similar to erlotinib. On the other hand, its less in vitro EGFR inhibitory activity compared to that of erlotinib could be explained by the lack of key interaction with Met769 (**Figure 8**).


CYS 773
MET 769
PRO 770
LYS 704

(a)


GLY 772
LEU 694
GLU 780
PHE 771
TYR 777
ASP 776
CYS 773
SER 696
HIS 781
GLY 695
HN
PRO 770
OH
LYS 704
LYS 692

(b)


GLN 767
MET 769
GLY 772
THR 830
LEU 820
LEU 768
ASP 831
PRO 770
PHE 771
LEU 834
LYS 721
LEU 764
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LEU 694
CYS 773
LYS 704
ILE 765
ILE 720
THR 766
GLY 695
ASP 776

(c)

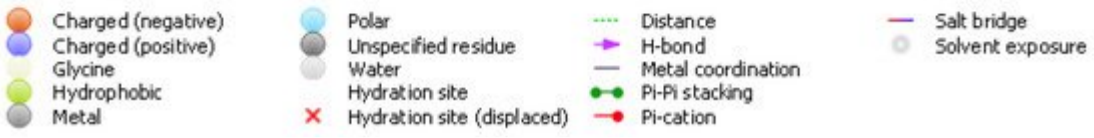
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

Figure 8. Docking poses of compound **10** and erlotinib (**a**) and docking interactions of compound **10** (**b**) and erlotinib (**c**) in the ATP binding site of EGFR (PDB code: 4HJO). Yellow dashes: hydrogen bonding. Compound **10** and erlotinib were colored in yellow green, and pink, respectively.

As compared to costly and time-consuming absorption, distribution, metabolism, excretion (ADME) experimental procedures [39], computational models are advantageous approaches to provide access to a set of rapid, yet robust predictive models for physicochemical, and pharmacokinetic properties [40]. In this direction, we performed in silico predictions of some pharmacokinetic parameters of the compounds by the QikProp, a predictive ADME module within the Maestro suite produced by Schrödinger. As depicted in **Table 2**, the brain/blood partition coefficient (QPlogBB) values of compounds **1–13** ranging from −1.175 to −0.029 were found within the specified limits (−3 to 1.2). The central nervous system (CNS) activity values of compounds **1–13** (−2 to 0) were in agreement within the range (−2 to 2). The QPlogPo/w value, which is a crucial parameter for membrane permeability, metabolism, bioavailability, the toxicity of molecules, and a ligand binding to the receptor [41], was determined as 5.744, 5.453, and 5.745 for compounds **10**, **11,** and **12**, respectively within the specified range (−2 to 6.5). The QPlogPo/w values of other compounds were detected out of limit. Taking into account the importance of hydrogen bonding with pivotal residues in the ATP binding site of EGFR, the number of donor (nHBD) and acceptor (nHBA) sites for hydrogen bonds were calculated. Appropriate nHBD (0 to 4) and nHBA (3.7 to 7.2) values of all compounds within the limits (0 to 6 and 2 to 20, respectively) also supported the outcomes of the molecular docking study. Solvent accessible surface area (SASA) is defined as the accessibility of the residue to the solvent; either it is between lipid or water accessibility and it is also essential to BBB permeability [42]. The SASA values of all compounds were in an optimal range of the specified values (300 to 1000). Compounds **1–13** violated two parameters of Lipinski's rule of five (maximum is four) and one parameter of Jorgensen's rule of three (maximum is three).

Table 2. Predicted ADME properties of compounds **1–13**.

Compound	QPlogBB * (−3 to 1.2)	CNS * (−2 to 2)	QPlogPo/w * (−2 to 6.5)	nHBD * (0 to 6)	nHBA * (2 to 20)	SASA * (300–1000)	Rule of Five **	Rule of Three ***
1	−0.595	−1	5.877	3	7.1	743.017	2	1
2	−0.388	0	8.081	1	3.7	813.580	2	1
3	−0.529	0	7.171	1	5.7	827.146	2	1
4	−0.574	0	6.781	2	5.4	756.322	2	1
5	−0.120	0	7.783	1	3.7	746.935	2	1
6	−0.105	0	7.829	1	3.7	741.337	2	1
7	−0.569	0	6.968	0	4.7	811.227	2	1
8	−0.035	0	8.795	0	4.7	799.503	2	1

Compound	QPlogBB * (−3 to 1.2)	CNS * (−2 to 2)	QPlogPo/w * (−2 to 6.5)	nHBD * (0 to 6)	nHBA * (2 to 20)	SASA * (300–1000)	Rule of Five **	Rule of Three ***
9	−1.175	−2	6.275	0	6.2	780.099	2	1
10	−0.061	−1	5.744	3	5.2	863.864	2	1
11	−0.038	−1	5.453	3	5.2	848.674	2	1
12	−0.029	−1	5.745	3	5.2	863.850	2	1
13	−1.057	−2	6.866	4	7.2	890.067	2	1

* QPlogBB: Brain/blood partition coefficient; CNS: Predicted central nervous system activity; QPlogPo/w: Predicted octanol/water partition coefficient; nHBD and nHBA: Estimated number of hydrogen bonds that would be donated and accepted, respectively, by the solute to water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer. SASA: Total solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius. ** Rule of Five: Number of violations of Lipinski's rule of five. *** Rule of Three: Number of violations of Jorgensen's rule of three.

References

1. Jacob Fisher, David Adamson. Current FDA-Approved Therapies for High-Grade Malignant Gliomas. *Biomedicines* **2021**, *9*, 324. 10.3390/biomedicines9030324.
2. Benmelouka, A.Y.; Munir, M.; Sayed, A.; Attia, M.S.; Ali, M.M.; Negida, A.; Alghamdi, B.S.; Kamal, M.A.; Barreto, G.E.; Ashraf, G.M.; et al. Neural stem cell-based therapies and glioblastoma management: Current evidence and clinical challenges. Int. J. Mol. Sci. 2021, 22, 2258. [Google Scholar] [CrossRef] [PubMed]
3. Patil, S.A.; Hosni-Ahmed, A.; Jones, T.S.; Patil, R.; Pfeffer, L.M.; Miller, D.D. Novel approaches to glioma drug design and drug screening. Expert Opin. Drug Discov. 2013, 8, 1135–1151. [Google Scholar] [CrossRef] [PubMed]
4. Ghotme, K.A.; Barreto, G.E.; Echeverria, V.; Gonzalez, J.; Bustos, R.H.; Sanchez, M.; Leszek, J.; Yarla, N.S.; Gomez, R.M.; Tarasov, V.V.; et al. Gliomas: New perspectives in diagnosis, treatment and prognosis. Curr. Top. Med. Chem. 2017, 17, 1438–1447. [Google Scholar] [CrossRef] [PubMed]
5. Choppavarapu, L.; Kandi, S.M. Circulating microRNAs as potential biomarkers in glioma: A mini-review. Endocr. Metab. Immune Disord. Drug Targets 2021, 21, 195–202. [Google Scholar] [CrossRef] [PubMed]
6. Chen, X.; Guo, G.; Lu, Y.; Wang, S.; Zhang, Y.; Huang, Q. Mechanisms and functions of long non-coding RNAs in glioma (Review). Oncol. Rep. 2021, 45, 9. [Google Scholar] [CrossRef] [PubMed]
7. Chistiakov, D.A.; Chekhonin, I.V.; Chekhonin, V.P. The EGFR variant III mutant as a target for immunotherapy of glioblastoma multiforme. Eur. J. Pharmacol. 2017, 810, 70–82. [Google Scholar] [CrossRef]
8. de Menezes, M.R.; Acioli, M.E.A.; da Trindade, A.C.L.; da Silva, S.P.; de Lima, R.E.; da Silva Teixeira, V.G.; Vasconcelos, L.R.S. Potential role of microRNAs as biomarkers in human glioblastoma: A mini systematic review from 2015 to 2020. Mol. Biol. Rep. 2021, 48, 4647–4658. [Google Scholar] [CrossRef] [PubMed]

9. Cristina Zahonero; Pilar Sánchez-Gómez; EGFR-dependent mechanisms in glioblastoma: towards a better therapeutic strategy. *Cellular and Molecular Life Sciences* **2014**, *71*, 3465-3488, 10.1007/s00018-014-1608-1.
10. Alves, A.; Costa, P.; Pinto, M.; Ferreira, D.; Correia-da-Silva, M. Small molecules of marine origin as potential anti-glioma agents. Molecules 2021, 26, 2707. [Google Scholar] [CrossRef]
11. Giamanco, K.A.; Matthews, R.T. The role of BEHAB/Brevican in the tumor microenvironment: Mediating glioma cell invasion and motility. Adv. Exp. Med. Biol. 2020, 1272, 117–132. [Google Scholar]
12. Colardo, M.; Segatto, M.; Di Bartolomeo, S. Targeting RTK-PI3K-mTOR axis in gliomas: An update. Int. J. Mol. Sci. 2021, 22, 4899.
13. Patel, M.; Vogelbaum, M.A.; Barnett, G.H.; Jalali, R.; Ahluwalia, M.S. Molecular targeted therapy in recurrent glioblastoma: Current challenges and future directions. Expert Opin. Investig. Drugs 2012, 21, 1247–1266.
14. Huang, T.T.; Sarkaria, S.M.; Cloughesy, T.F.; Mischel, P.S. Targeted therapy for malignant glioma patients: Lessons learned and the road ahead. Neurotherapeutics 2009, 6, 500–512.
15. Liang, Y.; Zhang, T.; Zhang, J. Natural tyrosine kinase inhibitors acting on the epidermal growth factor receptor: Their relevance for cancer therapy. Pharmacol. Res. 2020, 161, 105164.
16. Chistiakov, D.A.; Chekhonin, I.V.; Chekhonin, V.P. The EGFR variant III mutant as a target for immunotherapy of glioblastoma multiforme. Eur. J. Pharmacol. 2017, 810, 70–82.
17. Lo, H.-W. EGFR-targeted therapy in malignant glioma: Novel aspects and mechanisms of drug resistance. Curr. Mol. Pharmacol. 2010, 3, 37–52.
18. Keller, S.; Schmidt, M.H.H. EGFR and EGFRvIII promote angiogenesis and cell invasion in glioblastoma: Combination therapies for an effective treatment. Int. J. Mol. Sci. 2017, 18, 1295.
19. Ye, F.; Gao, Q.; Cai, M.-J. Therapeutic targeting of EGFR in malignant gliomas. Expert Opin. Ther. Targets 2010, 14, 303–316.
20. Khwaza, V.; Mlala, S.; Oyedeji, O.O.; Aderibigbe, B.A. Pentacyclic triterpenoids with nitrogen-containing heterocyclic moiety, privileged hybrids in anticancer drug discovery. Molecules 2021, 26, 2401.
21. Salvador, J.A.R.; Leal, A.S.; Alho, D.P.S.; Gonçalves, B.M.F.; Valdeira, A.S.; Mendes, V.I.S.; Jing, Y. Highlights of pentacyclic triterpenoids in the cancer settings. In Studies in Natural Products Chemistry, 1st ed.; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, The Netherlands, 2014; Volume 41, pp. 33–73.
22. Hodon, J.; Borkova, L.; Pokorny, J.; Kazakova, A.; Urban, M. Design and synthesis of pentacyclic triterpene conjugates and their use in medicinal research. Eur. J. Med. Chem. 2019, 182, 111653.

23. Radwan, M.O.; Ismail, M.A.H.; El-Mekkawy, S.; Ismail, N.S.M.; Hanna, A.G. Synthesis and biological activity of new 18β glycyrrhetinic acid derivatives. Arab. J. Chem. 2016, 9, 390–399.
24. Kavitha, C.V.; Agarwal, C.; Agarwal, R.; Deep, G. Asiatic acid inhibits pro-angiogenic effects of VEGF and human gliomas in endothelial cell culture models. PLoS ONE 2011, 6, e22745.
25. Garanti, T.; Stasik, A.; Burrow, A.J.; Alhnan, M.A.; Wan, K.W. Anti-glioma activity and the mechanism of cellular uptake of asiatic acid-loaded solid lipid nanoparticles. Int. J. Pharm. 2016, 500, 305–315.
26. Wang, J.; Li, Y.; Wang, X.; Jiang, C. Ursolic acid inhibits proliferation and induces apoptosis in human glioblastoma cell lines U251 by suppressing TGF-β1/miR-21/PDCD4 pathway. Basic Clin. Pharmacol. Toxicol. 2012, 111, 106–112.
27. Markov, A.V.; Zenkova, M.A.; Logashenko, E.B. Modulation of tumour-related signaling pathways by natural pentacyclic triterpenoids and their semisynthetic derivatives. Curr. Med. Chem. 2017, 24, 1277–1320.
28. Paduch, R.; Kandefer-Szerszeń, M. Antitumor and antiviral activity of pentacyclic triterpenes. Mini-Rev. Org. Chem. 2014, 11, 262–268.
29. Sathya, S.; Sudhagar, S.; Sarathkumar, B.; Lakshmi, B.S. EGFR inhibition by pentacyclic triterpenes exhibit cell cycle and growth arrest in breast cancer cells. Life Sci. 2014, 95, 53–62.
30. Wang, S.-R.; Fang, W.-S. Pentacyclic triterpenoids and their saponins with apoptosis-inducing activity. Curr. Top. Med. Chem. 2009, 9, 1581–1596.
31. Laszczyk, M.N. Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy. Planta Med. 2009, 75, 1549–1560.
32. Rufino-Palomares, E.E.; Pérez-Jiménez, A.; Reyes-Zurita, F.J.; García-Salguero, L.; Mokhtari, K.; Herrera-Merchán, A.; Medina, P.P.; Peragón, J.; Lupiáñez, J.A. Anti-cancer and anti-angiogenic properties of various natural pentacyclic tri-terpenoids and some of their chemical derivatives. Curr. Org. Chem. 2015, 19, 919–947.
33. Khwaza, V.; Oyedeji, O.O.; Aderibigbe, B.A. Ursolic acid-based derivatives as potential anti-cancer agents: An update. Int. J. Mol. Sci. 2020, 21, 5920.
34. Zou, J.; Lin, J.; Li, C.; Zhao, R.; Fan, L.; Yu, J.; Shao, J. Ursolic acid in cancer treatment and metastatic chemoprevention: From synthesized derivatives to nanoformulations in preclinical studies. Curr. Cancer Drug Targets. 2019, 19, 245–256.
35. Hordyjewska, A.; Ostapiuk, A.; Horecka, A.; Kurzepa, J. Betulin and betulinic acid: Triterpenoids derivatives with a powerful biological potential. Phytochem. Rev. 2019, 18, 929–951.
36. Chen, H.; Gao, Y.; Wang, A.; Zhou, X.; Zheng, Y.; Zhou, J. Evolution in medicinal chemistry of ursolic acid derivatives as anticancer agents. Eur. J. Med. Chem. 2015, 92, 648–655.

37. Ciftci, H.I.; Emirdag Ozturk, S.; Ali, T.F.S.; Radwan, M.O.; Tateishi, H.; Koga, R.; Ocak, Z.; Can, M.; Otsuka, M.; Fujita, M. The first pentacyclic triterpenoid gypsogenin derivative exhibiting anti-ABL1 kinase and anti-chronic myelogenous leukemia activities. Biol. Pharm. Bull. 2018, 41, 570–574.
38. Ciftci, H.I.; Radwan, M.O.; Ozturk, S.E.; Ulusoy, N.G.; Sozer, E.; Ellakwa, D.E.; Ocak, Z.; Can, M.; Ali, T.F.S.; Abd-Alla, H.I.; et al. Design, synthesis and biological evaluation of pentacyclic triterpene derivatives: Optimization of anti-ABL kinase activity. Molecules 2019, 24, 3535.
39. Emirdağ-Öztürk, S.; Karayıldırım, T.; Çapcı-Karagöz, A.; Alankuş-Çalışkan, Ö.; Özmen, A.; Poyrazoğlu-Çoban, E. Synthesis, antimicrobial and cytotoxic activities, and structure-activity relationships of gypsogenin derivatives against human cancer cells. Eur. J. Med. Chem. 2014, 82, 565–573.
40. Park, J.H.; Liu, Y.; Lemmon, M.A.; Radhakrishnan, R. Erlotinib binds both inactive and active conformations of the EGFR tyrosine kinase domain. Biochem. J. 2012, 448, 417–423.
41. Wang, Y.; Xing, J.; Xu, Y.; Zhou, N.; Peng, J.; Xiong, Z.; Liu, X.; Luo, X.; Luo, C.; Chen, K.; et al. In silico ADME/T modelling for rational drug design. Q. Rev. Biophys. 2015, 48, 488–515.
42. Ciftci, H.I.; Bayrak, N.; Yıldız, M.; Yıldırım, H.; Sever, B.; Tateishi, H.; Otsuka, M.; Fujita, M.; Tuyun, A.F. Design, synthesis and investigation of the mechanism of action underlying anti-leukemic effects of the quinolinequinones as LY83583 analogs. Bioorg. Chem. 2021, 114, 105160.
43. Giamanco, K.A.; Matthews, R.T. The role of BEHAB/Brevican in the tumor microenvironment: Mediating glioma cell invasion and motility. Adv. Exp. Med. Biol. 2020, 1272, 117–132. [Google Scholar]