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Chalcones and Carbon Dots as Emerging Breakthroughs in Glioblastoma Multiforme Treatment

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ABSTRACT

Glioblastoma Multiforme (GBM) is a very aggressive brain tumor that is resistant to treatment and has a diverse population of genetically unstable, deeply infiltrating cells. Although significant efforts have been made to find new anti-GBM drugs, these efforts have largely been unsuccessful in human trials because of problems with effectiveness, selectivity, or physicochemical properties. The successful eradication of GBM stem cells (GSCs) is of high priority because GBM stem cells are in charge of patient relapse and tumor regrowth after therapy. Analysis of five published science journals has led to the conclusion that utilizing chalcones as the treatment for Glioblastoma and carbon dots as the carriers could change the approach to curing Glioblastoma. All of the authors examined have either conducted research with chalcones and carbon dots or have reported their effectiveness from other published papers. The purpose of this paper will be to bring awareness to the potential of chalcones and carbon dots, seeing as they have been scarcely mentioned in the literature regarding Glioblastoma research.

Introduction

Glioblastoma (GBM) is the most prevalent and deadly primary brain tumor in adults. After diagnosis, the typical survival is just 14 to 15 months despite the extensive care which, namely: surgery (when possible), chemotherapy in the form of temozolomide (TMZ), radiotherapy, or, in some cases, alternating electric field therapy (Thakur, 2022). Thousands of treatment trials and in-depth studies targeted at figuring out the genetic and epigenetic causes of the illness have not resulted in a significant improvement in patient outcomes over the years. Due to the highly invasive nature of GBM, the inability to completely remove the tumor, the absence of effective chemotherapy agents that can cross the blood-brain barrier at therapeutic levels, and the presence of GBM stem-like cells that are resistant to treatment, which cause tumor recurrence and patient relapse, have all been cited as reasons for treatment failure (Veliz, 2022).

The first part of an effective treatment is being able to reach the tumor location by crossing the Blood Brain Barrier (BBB). Lack of effective medications capable of crossing the BBB at therapeutic levels has been one of the biggest barriers to effective GBM therapy. The other half of the battle is to prevent tumor relapse. While GSCs—also known as GBM stem-like cells or tumor initiating cells—represent just a tiny population of cells inside the tumor, they have the ability to divide asymmetrically, giving birth to both new GSCs and the more differentiated cells that make up the majority of the tumor (Veliz, 2022). Researchers are wary of the recorded advancement of brain tumor drug development programs since previous attempts to treat brain tumors have failed, as seen by the low FDA approval rate of CNS medications compared to non-CNS therapies. A growing amount of research, however, have concentrated on finding viable targets that may be exploited in BBB permeable treatment to combat the intra-tumoral molecular heterogeneity of GBM. Notably, significant progress has been achieved in comprehending the processes that control the onset and development of GBM. The discoveries have subsequently aided in the creation of manageable anti-GBM medicines that can follow an unbroken development route, be guided to advanced stage clinical settings, and be quickly translated into more personalized, cell-type-specific, effective, and safe therapies for GBM. (Thakur, 2022).

Moreover, during the past ten years, several researchers have concentrated on small-molecule inhibitors in an effort to overcome the aforementioned difficulties and increase the range of therapy choices for GBM. As a result, the majority of challenges faced in the development of GBM drugs have been overcome through the use of effective drug design techniques to create small molecule inhibitors with promising preclinical and early characteristics. (Thakur, 2022). The research conducted in the papers examined will provide evidence as to why the research of chalcone variations and implementation of carbon dots is a step in the right direction for the effective treatment of GBM.

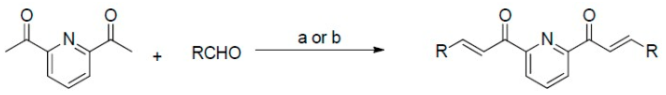
Review of Literature

This research will dissect the significance of chalcones and carbon dots in the right against GBM. Four of the sources that will be referenced have all conducted research with glioma cells using chalcones. However, not all of them use carbon dots. Rather, they use liposomes, which serve the same purpose as carbon dots—to bypass the BBB.

The sources used in the analysis of the paper come from thirty-nine different authors who all have either a master's or doctoral degree in pharmaceutical sciences, biotechnology, medicinal chemistry, organic chemistry, neurosurgery, or health sciences. Amandeep Thakur, Ram Sharma, Sachin Sharma, Kunal Nepali, and Jing Ping Liou are from Taipei Medical University. Chetna Faujdar is from Jaypee Institute of Information Technology. Basant Malik is from Dr. Reddy's Laboratories. Eduardo A. Veliz, Anastasiia Kaplina, Sajini D. Hettiarachchi, Athina L. Yoham, Carolina Matta, Sabrin Safar, Meghana Sankaran, Esther L. Abadi, Emel Kirbas Cilingir, Frederic A. Vallejo, Winston M. Walters, Steven Vanni, Roger M. Leblanc, Lorenzo Sansalone, Nadia G. Myrthil, Vasileios Stathias, Ingrid I. Torrens, Stephan C. Schürer, and Regina M. Graham are from the University of Miami. Daniel Mendanha, Joana Vieira de Castro, Bruno M. Costa, Helena Ferreira, and Nuno M. Neves are from the University of Minho. Joana Moreira, Honorina Cidade, and Madalena Pinto are from the University of Porto. Giovanna Calabrese, Giovanna de Luca, Giuseppe Nocito, Maria Giovanna Rizzo, and Sabrina Conoci are from the Università degli Studi di Messina. Sofia Paola Lombardo and Giulia Chisari are from the Istituto Oncologico del Mediterraneo. Emanuele Stefano Forte is from Iom Ricerca. Luigi Sciuto is from AO Universitaria Policlinico. All the sources are from a published scientific journal, and all have been published in the last three years.

Chalcones

Chalcones are aromatic ketones that have been shown to lessen the malignant properties of certain cancers, such as glioblastoma. As a natural source, chalcones are primarily responsible for the pigmentation of certain plants. However, with a synthetic approach, chalcones demonstrated to be a "privileged structure" for its extensive range of biological properties, including antibacterial, anti-inflammatory, antioxidant, and anticancer action. (Mendanha, 2021). This family of compounds was thoroughly investigated for use in the treatment of cancer due to its straightforward chemistry, simplicity in synthesis, and abundance of replaceable hydrogens that facilitate chemical changes. With regard to their anticancer properties, several studies have demonstrated that chalcone derivatives can exhibit antiproliferative action on cancer cells, and a number of molecular targets have previously been discovered and researched. Recently, symmetric bis-chalcones (Figure 1) were demonstrated to be potent inhibitors of the breast cancer resistance protein (BCRP/ABCG2); however, the anti-cancer effects of bis-chalcones have not been investigated in GBM. However, using a synthetic technique, chalcones showed several anti-glioma properties. Symmetric bis-chalcones have recently been shown to be powerful inhibitors of the breast cancer resistance protein (BCRP/ABCG2), but their anti-cancer effects in GBM have not yet been studied. (Veliz, 2022).



4a R = Ph Ph = Phenyl
4b R = 2-OMe-Ph
4c R = 3-OMe-Ph
4d R = 4-OMe-Ph
4e R = 2,3-DimethoxyPh
4f R = 2,4-DimethoxyPh
4g R = 3,4-DimethoxyPh
4h R = 3,5-DimethoxyPh
4i R = 3,4-(OCH_2O)
4j R = 3,4,5-TrimethoxyPh
4k R = 4-MOM-Ph
4l R = 4-OH-Ph
4m R = 3-OMe-4-MOM-Ph
4n R = 4-OH-3-OMe-Ph
c
4o R = 4-OBn-3-OMe-Ph
4p R = 4-DimethylaminoPh
4q R = 4-Dimethylaminocinnamyl
4r R = 2,3,4-TrimethoxyPh
4s R = 2,4,5-TrimethoxyPh
MOM = OCH_2OCH_3

Figure 1. Reaction scheme for the synthesis of bis-chalcones. Reagents and conditions: (a) 20%NaOH, MeOH, RT; (b) cat. Piperidine, MeOH, ref lux; (c) Trifluoroacetic acid/conc. HCl, Dichloromethane (Veliz, 2022).

The most crucial aspect of chalcones is their ability to prevent a tumor relapse. Recent investigations employing both natural and synthesized chalcones against GBM have demonstrated the chemicals' potential therapeutic efficacy. These compounds have been shown to trigger a variety of cell death pathways, including caspase-mediated apoptosis, autophagy, methuosis, and unfolded protein response-mediated cell death (UPR). Numerous chalcones with potential growth-inhibitory action in human cancer cell lines have previously been described. A375-C5 (melanoma), MCF-7 (breast adenocarcinoma), and NCI-H460 (non-small cell lung cancer) cells all showed antiproliferative activity in response to chalcones tested. The most promising substance's capacity to be encapsulated in liposomes while retaining its biological function was also explored (Mendanha, 2021). Another way of doing this is by stifling the growth of GSCs through weaponizing UPR. The UPR is an adaptive system set up to lessen ER stress caused by the tumor microenvironment, oncogene activation, rapid cell proliferation, and anti-cancer therapy. The resistance to radiation and temozolomide in GBM is specifically influenced by the UPR. On the other hand, the UPR causes cell death if protein homeostasis cannot be recovered. In one of the studies referenced, a new bis-chalcone (4j) was identified that can "weaponize" the UPR to encourage GBM stem cell death (Figure 2). 4j is a potential lead chemical for medication development, and targeting the UPR provides a unique approach for treating this fatal illness (Sansalone, 2019). Chalcones such as 4j trigger a response similar to ER stress, which induces the UPR, therefore stimulating cell death at the location of GSCs. Furthermore, results later showed the cytotoxicity of the chalcones were enough to discourage neurosphere reformation (Figure 3).

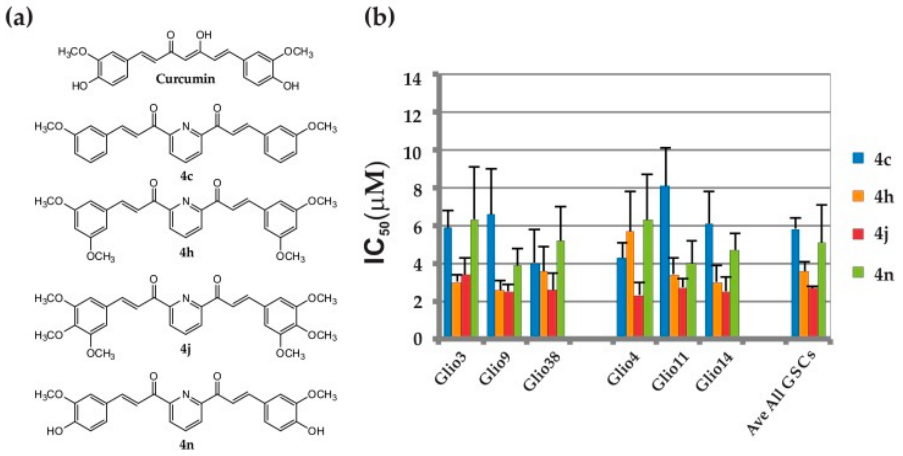


Figure 2. Bis-chalcones 4c, 4h, 4j, 4n induces robust cell death in 6 GSC lines. (**a**) structures of 4c, 4h, 4j, and 4n; (**b**) IC50 of each bis-chalcone for Glio3, Glio4, Glio9, Glio11, Glio14 and Glio38 (Sansalone, 2019).

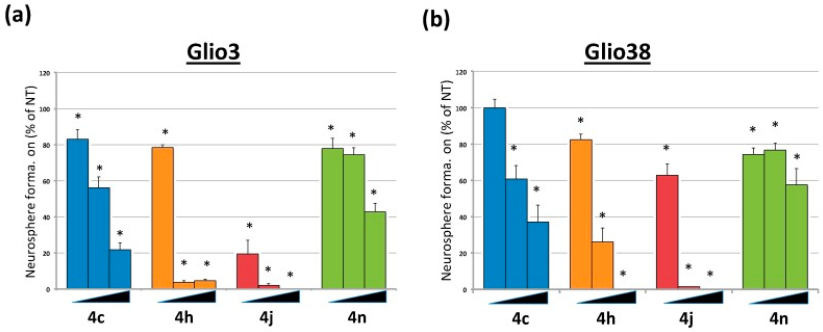


Figure 3. Bis-chalcones 4c, 4h, 4j, 4n significantly reduce neurosphere formation. GSCs Glio3 and Glio38 were plated as single cells in 96-well plates and treated with increasing concentration of 4c, 4h, 4j, 4n (100 nM, 250 nM or 500 nM) and the number of neurospheres counted on day 14. Data is presented as percent neurospheres relative to non-treated controls. (**a**) Glio3; (**b**) Glio38. * $p < 0.05$ (Sansalone, 2019).

A possible new chalcone derivative (1) with cytotoxicity, antiproliferative properties, and anti-invasion properties toward GBM cells was mentioned in another paper (Figure 4). Additionally, chalcone 1 showed less cytotoxicity toward the brain endothelial cell line at therapeutic dosages. It was successful to retain Chalcone 1's therapeutic action while encapsulating it in liposomes. As a result, the creation of liposomes containing chalcone derivatives has the potential to offer a fresh option for treating GBM. Given the observed selectivity, at the same doses of the other two derivatives, chalcone 1 showed more cytotoxicity in GBM cells than in brain endothelium cells.

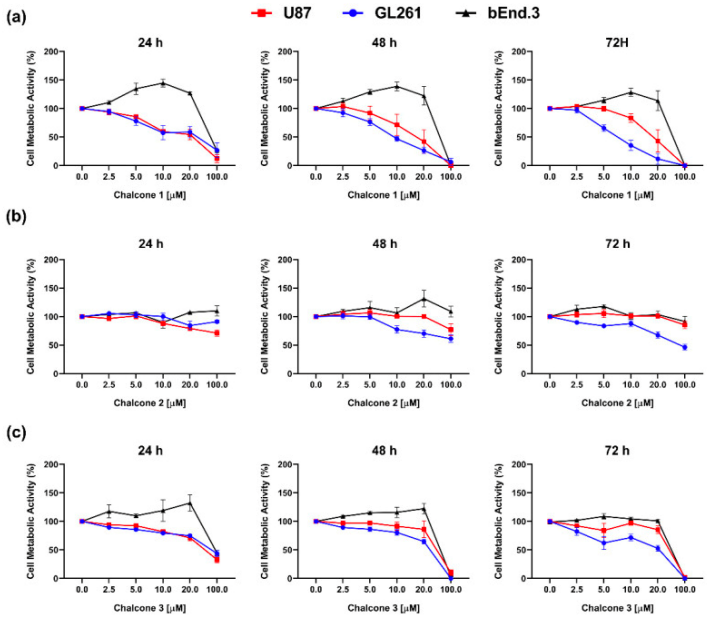


Figure 4. Cytotoxic effect of chalcone derivatives—Cell metabolic activity after treatment (24, 48, and 72 h) with chalcones **1** (**a**), **2** (**b**), and **3** (**c**) on GBM cell lines (U87 and GL261) and a brain endothelial cell line (bEnd.3) by MTS assay. Results are normalized to control (1% DMSO) and presented as mean ± SEM of three independent experiments (Mendanha, 2021).

Carbon Dots

Due to their unique physicochemical characteristics, carbon dots (C-dots) have attracted considerable attention in biomedical applications since they were unintentionally discovered during the electrophoretic purification of arc-discharged single-walled carbon nanotubes. C-dots have remarkable biocompatibility, size-dependent photoluminescence, good photostability, and size tunability in the nanometer range. These nanocrystalline graphitic carbon allotropes have a core with a strong sp2 character, virtually isotropic nanoparticles with diameters under 10 nm, and a high surface oxygen concentration. They are significantly different from nanodiamond particles, which have a diamond (sp3) core and a sp2 carbon exterior and are generated in difficult-to-obtain environments. Instead, the primary distinction between C-dots and graphene quantum dots is related to their shape, with the former being spherical particles and the latter being best characterized as zero-dimensional graphene disks. Top-down and bottom-up approaches are two general categories for the synthetic methods used to make C-dots (Figure 5). The former involves the physical or chemical fragmentation of initial carbonaceous materials (for example, electrochemical synthesis, chemical oxidation, arc discharge, and laser ablation); the latter begins with molecular precursors and includes, among other things, ultrasound and hydrothermal treatments, microwave-assisted synthesis, and pyrolization). Functional groups like carboxyl, hydroxyl, or amino groups are frequently present on the surface of C-dots, and the composition of these groups is greatly influenced by the nature of the precursors or the conditions of the reaction, which frequently results in significant differences in the properties of the nanoparticles.The inherent polar residues on the C-dots' surface provide them exceptional water solubility, which is particularly useful for creating nanobiological or nanomedical applications. Although the need for additives to give the NPs an affinity for the aqueous environment is no longer necessary, it is still preferable to change the functional moieties at the surface of the original carbon nanomaterials, for example, to improve their biocompatibility or give the C-dots new chemical functions. In both covalent and non-covalent surface treatments, the presence of accessible and reactive functional groups is heavily utilized, giving C-dots new

(bio)sensing capabilities, enhanced photoluminescence, expanded in vitro and in vivo bioimaging, as well as drug-delivery and theranostic capabilities (Calabrese, 2021).

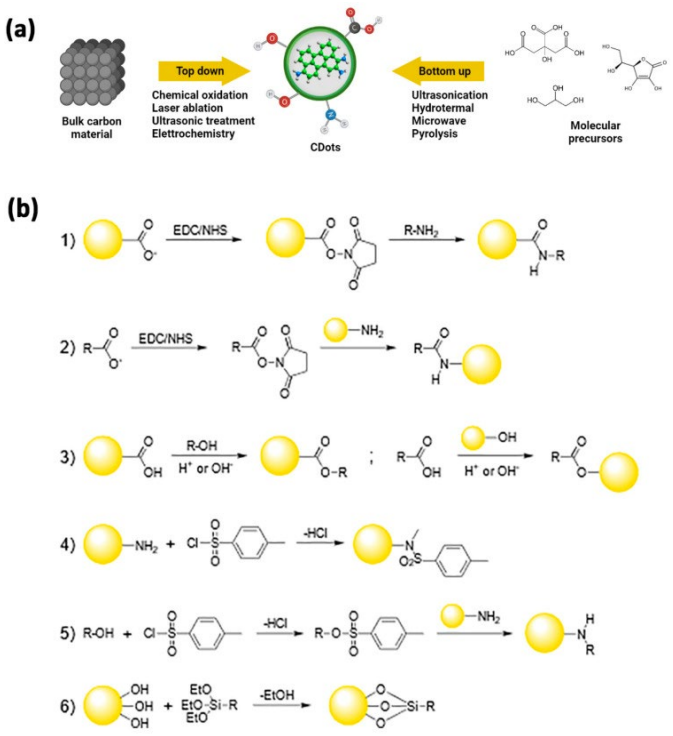


Figure 5. (**a**) General approaches for the synthesis of C-dots. In a top-down approach, C-dots are synthesized by transforming bulk carbon material into ultra-small powders via oxidation, laser ablation, ultrasounds, or electrochemical methods. In a bottom-up approach, C-dots are synthesized via physical or chemical treatments of molecular precursors that may get ionized, dissociated, evaporated, or sublimated and then condense/react to form C-dots, or via hydrothermal, sonochemical, pyrolytic treatments. (**b**) Reactions schemes for covalent strategies to C-dots functionalization: amide coupling (**1**,**2**), esterification (**3**), sulfonamide formation (**4**); tosylate-leaving group in nucleophilic substitution (**5**), silylation (**6**) (Calabrese, 2021).

For glioblastoma, the other problem researchers must overcome is the Blood-brain barrier, or BBB. BBB, blood-brain tumor barrier (BBTB), and a somewhat insignificant increased permeability and retention (EPR) impact are the three primary barriers to treating brain tumors. As the brain tumor gets bigger, the EPR effect becomes visible. In reality, most solid tumors have abnormally high amounts of vascular growth factors, such as vascular-endothelial growth factor, and vascular permeability-enhancing factors, such as bradykinin, prostaglandin, and nitric oxide, as well as a deficiency in lymphatic drainage. The EPR effect is based on these qualities, which also cause new blood vessels to have a bigger lumen, broader fenestrations, and a rise in fluid pressure. These traits also provide the basis for invasive and quick tumor development. As a result, nanocarriers with the right particle size can penetrate brain tumors through the endothelial gaps on the microvessels, allowing for the extravasation and retention of macromolecules in tumors (Calabrese, 2021).

The BBB is made up of a monolayer of highly specialized endothelial cells, which is nearly completely encircled by the end-feet of astrocytes and partially covered by pericytes and basement membrane. By limiting the passive reception of big, hydrophilic molecules and eliminating poisons, it creates a highly restrictive barrier that keeps the CNS in a stable state. It further severely restricts medication entry into the brain by acting as a physical (tight junctions), metabolic (enzymes), and immune barrier. Carbon-dots are potential nanocarriers that are simple to

functionalize with cancer-fighting medicines and ligands that target tumors. Drug delivery systems (DDS) have been developed using carbon dots (C-dots) to increase the bioavailability and tolerance of medications, as well as their solubility, half-life, and accumulation at the tumorous site. With the idea that the amino group would act as a "handle" to help with covalent attachment to carbon-dots, one researcher created a series of 4′-amino chalcones and assessed their cytotoxicity toward GSCs. After that, they produced 31 chalcones, including 5 new chalcones (22 4′-amino and 9 4′ derivatives), and discovered that 13 of them had an IC50 in all GSC lines < 10 M (Veliz, 2022).

To carry out the experiment, they created three sets of 31 chalcones and tested them against glioblastoma stem-like cells (GSC, Glio3, Glio9, and Glio38). Five of the 31 chalcones—3d, 3g, 3h, 3j, and 3m—were new chalcones, two of which showed action against GSCs. They discovered a total of 14, with IC50 values in all three GSC lines of around 10 M or less, that caused robust GSC mortality. In addition, several of these chalcones were not very harmful to human MSCs without cancer. These 14 chemicals' physicochemical characteristics point to their potential to cross the BBB (Table 1). The findings imply that some of the chalcones may be used as lead compounds for additional structural optimizations to develop more effective and less toxic derivatives for treating GBM on their own or as a covalently attached nanoparticle formulation (Veliz, 2022).

Table 1. Physicochemical properties relating to BBB crossing. Values were obtained using online software Molinspiration (www.molinspiration.com accessed on 28 July 2020). Molecular weight (MW), topical polar surface area (TPSA), lipophilicity (log*P*), hydrogen-bond (H-bond).

Chalcone	MW	TPSA	LogP	H-Bond Donors	H-Bond Acceptors	Rotatable Bonds
			Optimal	Values		
	<450 g/mol	<60-70 À	<5	<3	<7	<8
3d	325.36	64.64	2.89	1	5	6
3e	313.35	70.80	2.52	2	5	6
3f	283.33	61.56	2.93	2	4	5
3g	325.36	64.64	3.07	1	5	6
30	268.27	88.92	2.82	2	5	4
3p	224.26	55.99	1.54	2	3	3
3q	224.26	55.99	1.47	2	3	3
3u	241.26	43.09	3.03	2	2	3
3v	257.72	43.09	3.54	2	2	3
3w	257.72	43.09	3.56	2	2	3
3x	302.17	43.09	3.67	2	2	3
4b	284.31	55.77	3.37	1	4	5
4c	314.34	65.00	2.96	1	5	6
4d	356.37	71.08	2.99	0	6	8
5a	313.35	70.80	2.50	2	5	6

Conclusions

In summary, this paper highlighted the importance of chalcones and carbon dots in the realm of glioblastoma research. Due to glioblastoma's unique properties, it is one of the deadliest brain tumors. Since common treatments such as surgery, chemotherapy, and radiotherapy are usually ineffective, it has become necessary to seek out new ways to treat glioblastoma. However, two problems have hindered progress: GBM stem cells, which give rapid regenerative properties to the tumor, thus allowing for a relapse, and the brain-blood barrier. Chalcones and carbon dots are the solution to both of these issues. As reviewed in the referenced publications, chalcones have been proven to mitigate GBM relapse by prompting the UPR response, which kills GBM stem cells while minimal threat to other brain cells. In the other publications, carbon dots were also proven to be a safe and effective way to cross the BBB. The combination of these two methods is, without a doubt, a favorable development in the field of glioblastoma research.

Acknowledgements

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