Bioorganic & Medicinal Chemistry Letters 26 (2016) 5103–5109

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Temozolomide analogs with improved brain/plasma ratios – Exploring the possibility of enhancing the therapeutic index of temozolomide

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ARTICLE INFO

Article history:
Received 21 July 2016
Revised 17 August 2016
Accepted 19 August 2016
Available online 21 August 2016

Keywords:
Temozolomide
Glioblastoma
Chemotheraphy
O^6-Methyl-2′-deoxyguanosine (O^6-MedG)

ABSTRACT

Temozolomide is a chemotherapeutic agent that is used in the treatment of glioblastoma and other malignant gliomas. It acts through DNA alkylation, but treatment is limited by its systemic toxicity and neutralization of DNA alkylation by upregulation of the O^6-methylguanine-DNA methyltransferase gene. Both of these limiting factors can be addressed by achieving higher concentrations of TMZ in the brain. Our research has led to the discovery of new analogs of temozolomide with improved brain:plasma ratios when dosed in vivo in rats. These compounds are imidazotetrazine analogs, expected to act through the same mechanism as temozolomide. With reduced systemic exposure, these new agents have the potential to improve efficacy and therapeutic index in the treatment of glioblastoma.

The standard of care for glioblastoma multiforme (GBM) is debulking surgery followed by radiation and alkylating chemotherapy, frequently temozolomide (TMZ). Patients are treated with a standard regimen of 150 or 200 mg/m^2/day for five consecutive days, to be repeated every four weeks. As detailed in a recent review[1] this treatment is palliative rather than curative, with TMZ providing an overall survival increase of just 2.5 months over radiation alone. The median overall survival in GBM is only 14.6 months and a large percentage of patients have negligible benefit from TMZ treatment.[1]

Known toxicities from TMZ treatment include thrombocytopenia, lymphopenia and neutropenia.[1] These dose-limiting toxicities prevent dose escalation beyond a certain point, limiting a potentially curative treatment. The development of secondary or acquired resistance, whereby a patient fails to respond following initial response, may be related to this limitation. Both primary and secondary resistance to TMZ treatment is most often due to overexpression or upregulation of the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT), which effectively mitigates the cytotoxicity that would result from TMZ treatment.[2] Therefore, an ability to dose higher would mitigate the issue of MGMT upregulation.

TMZ is a prodrug that harbors an imidazotetrazine which is stable at the acidic pH of the stomach. In patients, it is readily absorbed with 100% oral bioavailability within 1–2 h of administration.[3,4] Once in circulation, the slightly alkaline environment of the blood and tissues causes spontaneous hydrolysis to form the active metabolite MTIC [3-methyl-(triazen-1-yl)imidazole-4-carboxamide]; MTIC rapidly breaks down to form the reactive methyldiazonium ion (diazomethane) that alkylates DNA. 5-Aminoimidazole-4-carboxamide (AIC) is formed as a side product. This is illustrated in Figure 1 where O^6-methyl-2′-deoxyguanosine (O^6-MedG) is highlighted as the end-product since it is the most cytotoxic. Interestingly, O^6 methylation of guanine represents only 5% of methylated adducts following TMZ administration.[4] N^7-methyl guanine represents 70% and N^1- and N^3-methyl adenine and N^3-methyl cytosine together form 25% of adducts. N^7- and N^3-methyl purines are rapidly repaired by base excision repair.[4]

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http://dx.doi.org/10.1016/j.bmcl.2016.08.064
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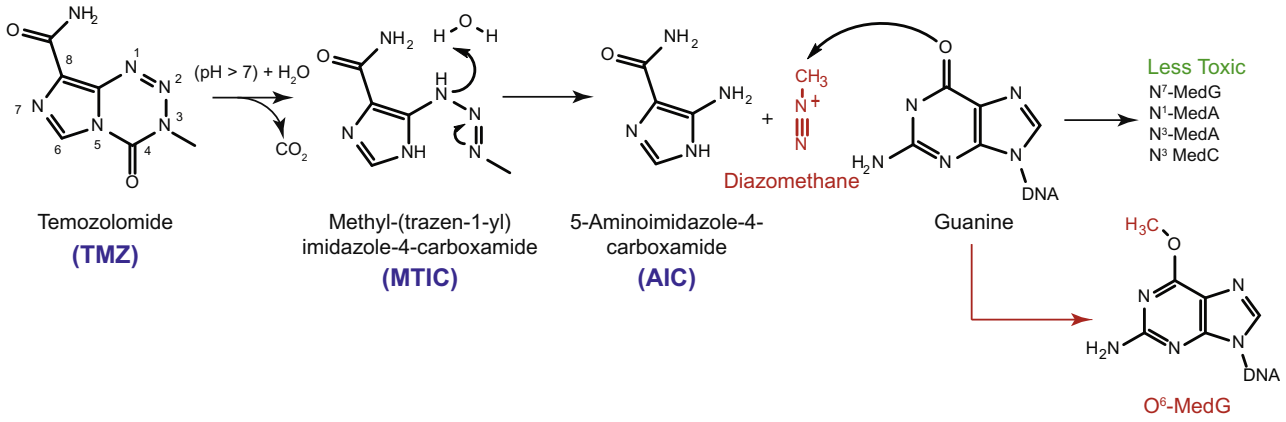


Figure 1. Mechanism of action of TMZ, illustrating the release of diazomethane, which alkylates DNA.

The discovery of TMZ represented an improvement over agents such as dacarbazine. Both dacarbazine and TMZ work through the active metabolite MTIC, but dacarbazine requires metabolic dealkylation[5] while TMZ generates MTIC through spontaneous hydrolysis in basic media.[5] The small molecular weight of TMZ facilitates penetration into the central nervous system (CNS). In humans, the AUC_{CSF}/AUC_{plasma} has been reported to be 20–29%.[3,6] In rats, the AUC_{brain}/AUC_{plasma} (B/P ratio) has been measured at 35–39% with TMZ being widely distributed in tissues as well.[7] We hypothesized that if TMZ levels could be focused primarily to the brain, with limited systemic exposure, such a profile would attenuate toxicities, leading to a possible increase in the maximum tolerated dose (MTD). A higher dose of TMZ with reduced side-effects can mitigate against adaptive resistance due to MGMT upregulation.

Our experience with β- and γ-carbolines[8–10] had led to the knowledge that compounds that embody these scaffolds have inherently superior brain penetration. Additionally, such scaffolds have been used to explore anti-Alzheimer's agents[11] as well as anti-psychotic agents,[12,13] indications which necessitate good brain penetration. We proposed the use of such privileged structures as 'carrier agents' to target TMZ to the brain. Our goal was to synthesize and test compounds with increased brain concentration of the imidazotetrazine analogs, with reduced plasma exposure compared to TMZ.

Before embarking on the synthesis and screening of our proposed molecules, we used TMZ to establish protocols for in vivo and in vitro monitoring of compound levels and activity. Healthy male Sprague Dawley rats ($n = 3$) were dosed intravenously with 10 mg/kg of TMZ and drug levels in the brain and plasma were analyzed by LC–MS/MS at three time points (30, 90 and 180 min post dose). While TMZ was present in the brain and plasma, concentrations in the brain were much lower than the plasma (Fig. 2). The B/P ratio was 0.34 (Table 1, Supplementary Table 1), matching previously reported levels[7] and suggesting that TMZ does not efficiently pass through the blood brain barrier (BBB).

We next monitored the activity of TMZ in vitro in the U87 MG human glioblastoma cell line by quantifying the amount of O^6-MedG present after incubation with the compound. Cells were incubated with 10 or 100 μM TMZ for 3 h at 37 °C and then washed and lysed with a commercial buffer (ThermoScientific Cat # K0721). Genomic DNA was extracted using an equal volume of isopropyl alcohol. DNA was washed with isopropyl alcohol, dried and hydrolyzed. To hydrolyze the DNA, 100 μg of genomic DNA was incubated at 37 °C for 30 min in ddH_2O (2 mg/ml), 50 μl buffer (50 mM ammonium acetate, 0.2 nM $ZnCl_2$, pH 5.3), 10 μl nuclease P1 (0.4 unit/μl) and 8 μl alkaline phosphatase (1 unit/μl in 10 mM Tris pH 7.4). An internal standard, 15 ppb [H]O^6-MedG, was added to hydrolyzed DNA and the mixture was centrifuged for 15 min at 15,000*g* at 4 °C and analyzed by LC–MS/MS. Incubation of cells with TMZ showed a dose-dependent increase in O^6-MedG (Fig. 3) indicating that TMZ hydrolyzes in vitro and the released diazomethane methylates guanine residues in cell culture.

We also monitored for N^7-methyl-2′-deoxyguanosine (N^7-MedG). We found that treatment of U87 MG cells with TMZ resulted in methylation at N^7, and the extent of N^7-MedG formation was more abundant than O^6-MedG (data not shown) as reported in the literature.[4] However, O^6-MedG monitoring is more relevant in the context of TMZ treatment, since the formation of

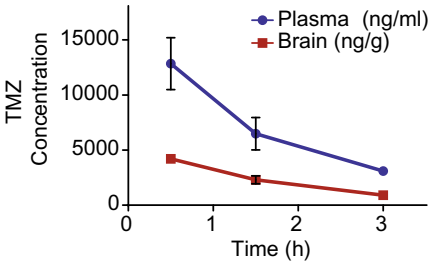


Figure 2. Average drug levels in the plasma and brains of rats dosed with 10 mg/kg TMZ. Rats ($n = 3$ per time point) were dosed IV with TMZ and brain and plasma samples were collected at 30, 90 and 180 min post dose. Levels of TMZ were analyzed by LC–MS/MS. Values shown in the graph are the average TMZ levels in plasma (blue) and brain (red). Error bars represent the standard deviation of the three replicates.

Table 1
Mean PK parameters from the 3-point screening rat PK studies. Rats ($n = 3$ per time point) were dosed with 10 mg/kg of compound and brain and plasma samples were Collected at 30, 90 and 180 min post dose and analyzed by LC–MS/MS for drug levels. The AUC was calculated by summing the area under the three point curve created by connecting the 30, 90 and 180 time points with straight lines as shown in Figure 2. The B/P ratio was calculated by dividing the AUC_{brain} by the AUC_{plasma}

Molecule	PK parameters			
	AUC_{brain} (ng h/g)	AUC_{plasma} (ng h/ml)	B/P ratio	$AUC_{brainTMZ}/AUC_{brainMol}$
TMZ	5660.37	16,853.70	0.34	1.0
1	638.33	959.21	0.67	8.9
2	750.18	998.19	0.75	7.5
3	4005.62	4892.32	0.82	1.4
4	525.05	386.30	1.36	10.8
5	2467.21	650.33	3.79	2.3
6	11183.14	1073.59	10.42	0.5

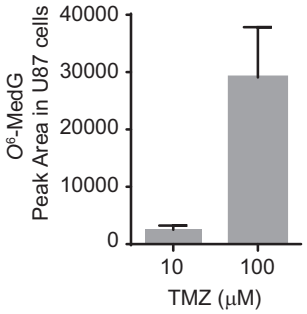


Figure 3. Treatment of U87 MG glioblastoma cells with TMZ results in dose-dependent methylation of to form O^6-MedG. U87 MG cells were cultured and treated with 10 or 100 μM TMZ for 3 h. Columns represent the mean ($n = 3$) O^6-MedG levels and error bars display the standard deviation.

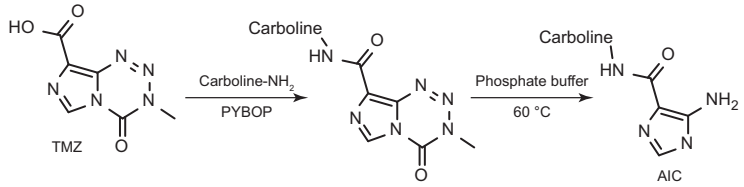
N^7-MedG, while more abundant, is rapidly repaired by base excision repair.[4]

The primary premise of our hypothesis required our new imidazotetrazine analogs to have improved brain penetration relative to TMZ and to efficiently methylate DNA. We, therefore, decided to use these two assays to screen new compounds for improved B/P ratios relative to TMZ and for efficacy in the U87 MG cellular assay. This would allow us to select a compound for further testing in vivo.

The structure of TMZ (Fig. 1) offers limited options for making analogs. Derivatizing at the 6-position is synthetically complex; moreover, depending on the substitution, it could perturb the electronics of the ring and may affect the reactivity of the molecule. We did not want to modify the 3-methyl group as this is the site of action of TMZ. The carboxamide group at the 8-position provided a possible handle for attachment of carboline moieties, with or without an intervening linker. The synthesis of 3-methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxylic acid was previously reported.[14,15] The carboxamide is distant from the 3-methyl group, which eventually forms diazomethane. We postulated that the 8-position carboxamide analogs would afford compounds that are not chemically compromised from generating diazomethane, and hence would be capable of acting through the same mechanism as TMZ. As a quick check, the 8-position *N*,*N*-dimethyl carboxamide analog of TMZ was made and tested in U87 MG cells for its capacity to hydrolyze and produce O^6-MedG. This compound performed similarly to TMZ in this assay (data not shown).

A general procedure for the synthesis of carboline-imidazotetrazine analogs and their corresponding AICs is described in Scheme 1. The key step was an amide coupling of a carboline with an appropriately placed amine 'handle' with the 8-acid analog of TMZ using PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate). The final product was isolated by extraction and drying, followed by trituration with acetonitrile. This imidazotetrazine amide analog was further used to synthesize the corresponding AIC by warming a solution in phosphate buffer at 60 °C for 4 h followed by purification by reverse phase HPLC. The synthesis of the β- and γ-carbolines (Compounds **1–6**) are described in Supplemental Information.

In the γ-carboline series, we explored attachment of the imidazotetrazine at the 2- and the 5-positions through an amino ethyl linker, as shown in Figure 4. Compound **1** has the imidazotetrazine attached to the γ-carboline at the 5-position, while compounds **2** and **3** have the attachment at the 2-position. As with TMZ, these



Scheme 1. General process for synthesis of carboline-imidazotetrazines and the corresponding AIC.

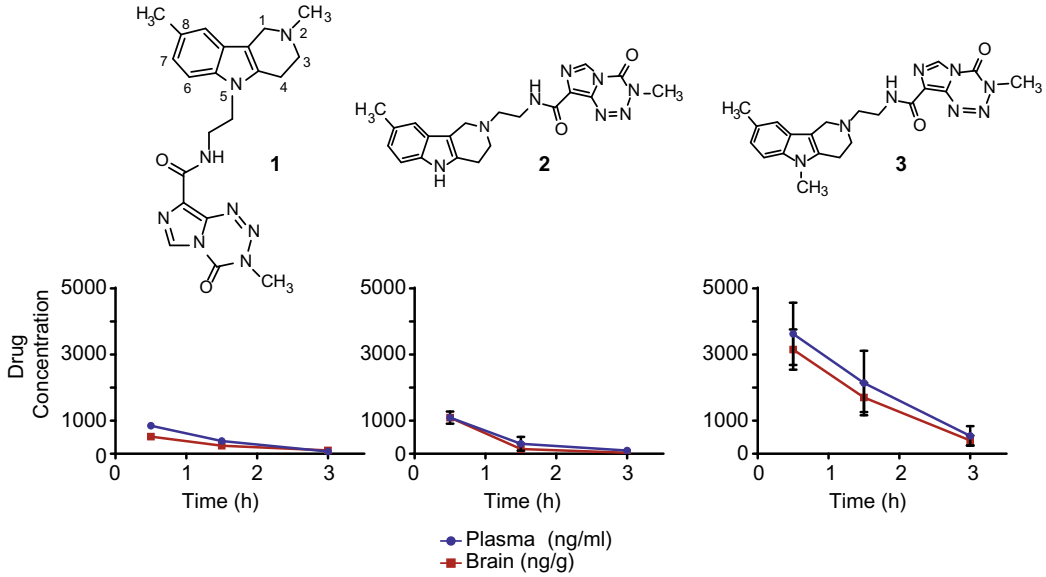


Figure 4. SAR progression of γ-carbolines.

compounds were administered IV to rats and compound levels were monitored at the previously specified time points (30, 90 and 180 min). Compound **1** showed a B/P ratio of 0.67 (Table 1), which was an improvement over TMZ (B/P ratio of 0.34). A comparison of drug concentration in the brain revealed that at an identical dose, TMZ had 9-fold higher amounts of the drug in the brain than **1** (Table 1, Supplementary Table 1). Compound **2** had a B/P ratio of 0.75 but absolute concentration in the brain was low (7.5-fold below TMZ). Our SAR progression led to **3** (the 5-methyl analog of **2**) that maintained a high B/P ratio of 0.8 while simultaneously improving the absolute levels of compound in the brain (TMZ levels were only 1.4-fold higher than levels of **3**). This result correlates with the calculated physicochemical properties of **2** and **3**; methylating the free N–H of **2** decreases the topological polar surface area (TPSA) from 110.98 to 100.12 and increases *c*Log*P* from 0.57 to 0.9.[16] We expected this change to enhance the brain penetration of **3**. With compound **3**, we accomplished our original goal of utilizing a carboline as a carrier molecule to favor brain partitioning of an imidazotetrazine analog.

We next turned our attention to β-carbolines, as exemplified by compounds **4**, **5** and **6** (Fig. 5A). Going from compound **4** to **5** and **6**, we made some major modifications to the physicochemical properties by: (a) using a shorter linker between the imidazotetrazine and the carboline (which reduced the molecular volume and flexibility), (b) converting a basic amine to a neutral amide and (c) introducing two lipophilic chlorines (which resulted in an increased *c*Log*P*). Such changes have been documented to increase brain penetration.[17] Compounds **5** and **6** have B/P ratios of 3.79 and 10.42, respectively, compared to a B/P ratio of 0.34 for TMZ (Table 1). Brain levels of these two compounds were comparable to that of TMZ (Table 1 and Supplementary Table 1). Importantly, compounds **5** and **6** had comparable or better brain levels with concomitantly reduced plasma levels as compared to TMZ (Fig. 5B). These leads could be useful as tool compounds to explore in vivo efficacy.

We compared the calculated and observed properties of the γ- and β-carbolines with that of TMZ (Table 2). Optimal brain penetration occurs in *c*Log*P* range of 1.5–2.7. While TMZ has a negative *c*Log*P* due to the number of polar heteroatoms, the small molecular weight and compact molecular volume allow for some brain penetration, resulting in a B/P ratio of 0.34. Attachment of the imidazotetrazine to a carboline increased *c*Log*P*; this could explain the improvement in B/P ratios. Major improvements to

Table 2
Physico-chemical properties like *c*Log*P* and p*K*a can explain improved brain penetration of compounds **5** and **6**

Compound	Structure	*c*Log*P*[a]	Carboline N p*K*a[a]	B/P ratio
TMZ		−0.99		0.34
1	γ-Carboline	1.16	9.0	0.67
2	γ-Carboline	0.57	8.8	0.75
3	γ-Carboline	0.9	8.8	0.82
4	β-Carboline	1.21	8.8	1.36
5	β-Carboline	1.04	−4.2	3.17
6	β-Carboline	1.77	−4.3	10.42

[a] ACD Labs PhysChem profiler was used to calculate *c*Log*P* and p*K*a.

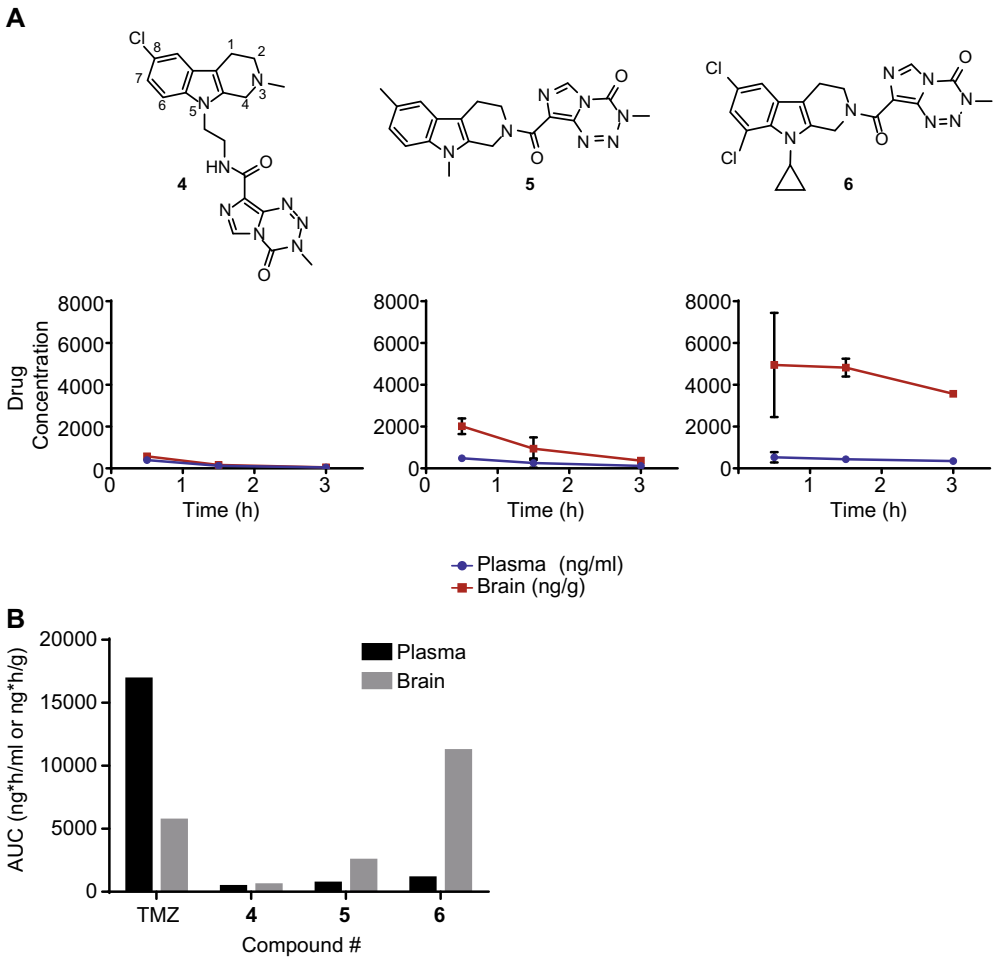


Figure 5. (A) SAR progression of β-carbolines. (B) Graphical representation of the improvement in brain:plasma ratio.

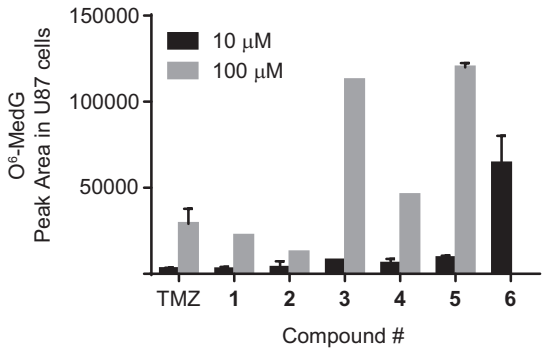


Figure 6. O^6-Methylation of guanine by TMZ and its carboline analogs in U87 MG glioblastoma cells. U87 MG cells were incubated for 3 h with TMZ or the carboline analogs (10 μM shown in black or 100 μM shown in grey) and formation of O^6-methylguanine was monitored by LC–MS/MS. The average peak area is shown in the graph. Error bars (where shown) represent the standard deviation of at least 2 replicates. Where error bars are not shown, only singlicate experiments were done. Due to the inherent complexity of this assay, duplicates were done for compounds that were contenders for further in vivo evaluation, e.g., compound **5**.

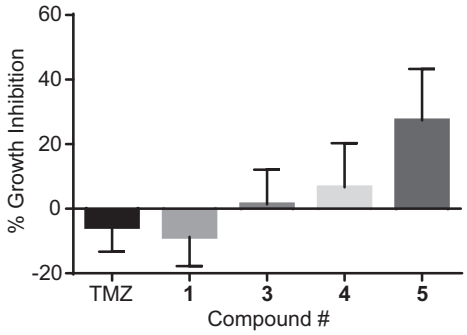


Figure 7. Inhibition of growth of U87 MG glioblastoma cells by TMZ and compounds **1–5**. U87 MG cells were treated with 100 μM compound for 72 h. Growth of drug-treated cells was compared with growth of vehicle-treated cells using the CellTiter Cell Proliferation Kit (Promega). Growth inhibition by compounds was calculated by dividing the average growth of drug treated cells ($n = 3$) by the growth of vehicle treated cells ($n = 3$, normalized to 100%). Error bars represent the standard deviation of the three replicates.

B/P ratios were accomplished by reducing the molecular volume as in compounds **5** and **6**. In these molecules, we attached the imidazotetrazine directly to the carboline through an amide bond at the carboline-nitrogen. In doing so, the basicity of this nitrogen is abrogated as well.

We next tested our lead compounds for their ability to form O^6-MedG in vitro in U87 MG cells as described above for TMZ. All compounds were tested at both 10 and 100 μM except for **6**, which could not be dosed at 100 μM due to solubility limitations. A positive result in this assay would reveal the ability of compounds to hydrolyze and release diazomethane and alkylate DNA. Compounds **1** and **2** had similar levels of O^6-MedG as TMZ, while lead compounds from our in vivo screen that had improved B/P ratios over TMZ (**3**, **5** and **6**) had increased levels of O^6-MedG as compared to TMZ (Fig. 6). We attributed this improvement to the enhanced cell permeability of these lead compounds as compared to TMZ, under the assumption that the same physicochemical properties that result in improved brain penetration would also improve cell permeability. We chose to do further evaluations with compound **5**.

TMZ kills cells by hydrolyzing and releasing the active diazomethane that methylates purine bases in DNA to such an extent that cells are unable to repair and apoptosis is induced. TMZ is known to have little effect on the proliferation of U87 MG cells at 100 μM.[18] Given that our lead compounds showed enhanced O^6-MedG formation in U87 MG cells as compared to TMZ, we thought that they could be more cytotoxic than TMZ and inhibit cell proliferation. To test this hypothesis, we measured the proliferation of U87 MG cells in the presence of 100 μM TMZ or our compounds for 72 h (MTS CellTiter 96 Cell Proliferation Assay, Promega). We confirmed that 100 μM TMZ did not inhibit the proliferation of U87 MG cells (Fig. 7). Compounds **1**, **3** and **4** did not significantly affect the proliferation of U87 MG cells but compound **5** inhibited the growth by 27.3% (Fig. 7).

We proceeded forward with our lead compound **5** to test its efficacy in an in vivo orthotopic mouse xenograft model of glioblastoma with U87 MG cells. One million luciferase expressing U87 MG cells were surgically implanted into the brains of female Harlan Nude mice and tumor volume was monitored by MRI over time. Dosing began on Day 26 post implantation when the mean tumor volume was 10.1 mm^3 for each group (Fig. 8). TMZ was formulated in 50% PEG400 and administered IV at 50 μmol/kg as

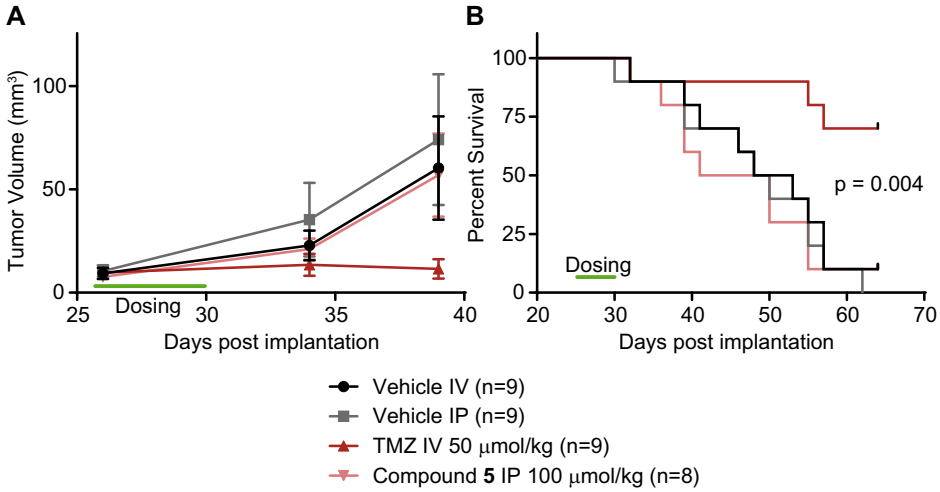


Figure 8. TMZ but not compound **5** inhibited tumor growth and increased survival in an orthotopic U87 mouse xenograft model. Days of dosing are indicated by the green line. (A) Average tumor volume of animals that survived to the day 39 is graphed. Error bars represent the standard deviation of the volumes of the number of animals indicated. Compounds were dosed QD×5 starting on day 26 when the average tumor volume was 10.1 mm^3. **B**. Groups were monitored for survival out to day 63 and the survival curves are plotted. Survival curves were compared with log-rank Mantel–Cox test ($p = 0.004$). The survival curve for TMZ treated group was compared to its vehicle control group ($p = 0.0059$) and the curves for compound **5** treated group and its vehicle control were not statistically different.

a positive control. The corresponding vehicle group was also dosed IV. Compound **5** was formulated in 20% *N*,*N*-dimethylacetamide/35% PEG400/30% propylene glycol/15% 0.9% sterile saline and administered IP at 100 μmol/kg (IP administration was used to avoid tail necrosis induced by IV dosing). The corresponding vehicle group was also dosed IP. Compounds were dosed once daily for five days (QD×5) and animals were monitored for tumor volume, body weight and mortality (mice found in moribund condition were euthanized).

As expected,[19–21] TMZ slowed tumor growth and significantly improved survival (Fig. 8A and B). Compound **5**, however, did not slow tumor growth (Fig. 8A) and the effects on survival were indistinguishable from those of the vehicle control groups (Fig. 8B). It was not possible to increase the dose of compound **5** due to tolerability issues including mortality, lack of grooming and low body carriage. With this negative result in hand, we looked for an explanation that would guide us in future experiments.

We demonstrated that compound **5** was present in rat brain. However, was it able to methylate DNA in the brain? To address this, TMZ and **5** were administered IV to rats at 10, 20 and 40 mg/kg (51.5, 103 and 206 μmol/kg for TMZ and 26.5, 53 and 106 μmol/kg for **5**) and brain tissue was collected and analyzed for O^6-MedG and drug concentration at 3 h post dose. Analysis of O^6-MedG levels revealed that while TMZ efficiently methylated DNA in vivo, dosing compound **5** did not result in measurable amounts of O^6-MedG at any dose tested (Fig. 9A). The in vivo concentration of each parent compound, however, was comparable at equimolar doses (103 μmol/kg TMZ and 106 μmol/kg **5**) (Fig. 9B).

We sought an explanation for the lack of in vivo activity of compound **5** (as measured by the formation of O^6-MedG) since our in vitro data suggested that this compound was capable of entering cells, hydrolyzing and releasing the diazomethane to alkylate DNA and cause cytotoxicity. One possible explanation is that, while compound **5** indeed reaches the brain when administered to rats, it is unavailable to enter cells because it is bound to lipid-rich brain tissue. TMZ, on the other hand, is present in the brain but is more available in the free fraction to enter cells and alkylate DNA. To test the proclivity of our compounds to bind to brain tissue, we incubated compounds at 10 μM with 200 μl homogenized mouse brain tissue in HBSS buffer for 1 h at 37 °C (1% final DMSO concentration). Samples were centrifuged and the percentage of compound in the insoluble tissue fraction and in the soluble supernatant were determined by LC–MS/MS. 50.3% (peak area 3322500) of TMZ was found in the soluble fraction while 49.7% (peak area 3279500) was found in the insoluble tissue fraction (Fig. 10, Table 3, Supplementary Table 2). Compound **5** tested under the same conditions was found to bind much more strongly to the tissue fraction with 97.8% of compound found in the insoluble tissue fraction (peak area 2111500) and only 2.2% (peak area 47345) found in the soluble fraction (Fig. 10, Table 3, Supplementary Table 2). The other β-carboline compounds were similarly highly tissue-bound (Fig. 10, Table 3, Supplementary Table 2). While the importance of the unbound brain concentration has been emphasized and discussed in the literature,[22] we had assumed the inherent instability of imidazotetrazines would result in viable concentrations of the reactive diazomethane at the site of action. Given this, we also measured the amount of the AIC in the tissue-bound and soluble fractions. The AIC of TMZ is similarly distributed in the tissue and soluble fractions as TMZ itself but the AIC molecules of our β-carboline compounds including compound **5** were highly tissue bound (Fig. 10, Table 3, Supplementary Table 2). This suggests that the hydrolysis of the imidazotetrazine is occurring in the tissue and any released diazomethane was likely quenched locally in the lipids and did not reach the intended site of action. We therefore concluded that a high unbound brain concentration is an important feature in the design of new compounds.

In conclusion, the compounds presented here are imidazotetrazine analogs with B/P ratios that are up to 30-fold improved over TMZ, with concomitantly reduced plasma levels when dosed in rodents. Lead compounds were tested for their ability to alkylate DNA and form O^6-MedG, and found to be more efficacious than TMZ in U87 MG cells. While these results were encouraging, they

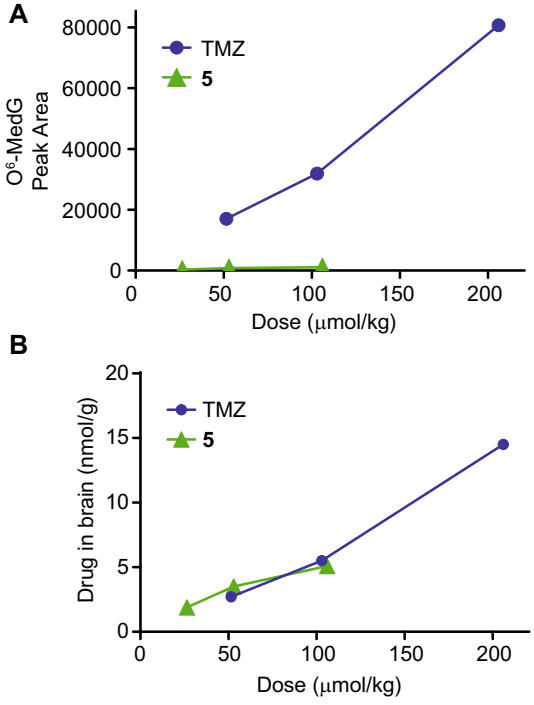


Figure 9. TMZ efficiently methylated guanine in brain tissue in vivo while compound **5** did not, despite similar exposure levels. Rats ($n = 1$ per compound per dose) were dosed with three different doses of TMZ or compound **5**. Brain tissue was collected 3 h post dose and analyzed for drug concentration and O^6-MedG. (A) Peak area from the LC–MS/MS analysis of the O^6-MedG quantification of rats dosed with TMZ (blue line) or compound **5** (green line). **(B)** Drug concentration present in brain of rats administered the indicated doses of TMZ (blue line) or compound **5** (green line).

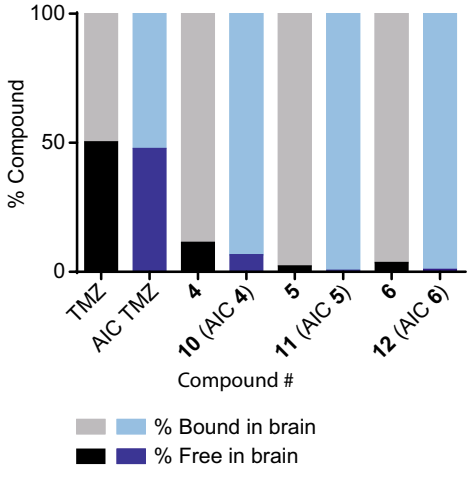


Figure 10. Carboline analogs of TMZ bind strongly to insoluble brain tissue. TMZ, compounds **4**, **5**, **6** and their corresponding AICs were incubated at 10 μM for 1 h with homogenized mouse brain tissue. Drugs levels were measured in the soluble supernatant fraction and the insoluble brain tissue fraction after centrifugation. The percentage of total **TMZ**, **4**, **5** or **6** present in the insoluble, or bound, fraction is shown in grey and the percentage of total **TMZ**, **4**, **5** or **6** present in the soluble, or free, fraction is shown in black. The percentage of total AIC compounds detected in the bound fraction is shown in turquoise and the percentage of total AIC compounds found in the soluble, or free fraction, is shown in dark blue.

Table 3
The percentages of TMZ or carboline analogs and their AICs that was present in the soluble supernatant or bound to mouse brain tissue. The percentages illustrated in Figure 10 are listed

Compound #	% Compound	
	Supernatant (%)	Tissue bound (%)
TMZ	50	50
AIC TMZ	48	52
4	11	89
10 (AIC **4**)	7	93
5	2	98
11 (AIC **5**)	1	99
6	3	97
12 (AIC **6**)	1	99

did not bear out when tested in vivo in an orthotopic U87 mouse xenograft model and in a DNA methylation assay in rat. We found a possible explanation for this lack of efficacy when we evaluated the proclivity of our compounds for binding brain tissue. In an ex vivo brain fractionation experiment, our lead compound **5** was almost completely tissue bound and unlikely to be available and reach the appropriate cells in vivo. Based on these results, replacing the carboline with other, less lipophilic brain penetrating groups should afford compounds with improved efficacy. Alternatively, the use of cleavable linkers which would release TMZ after entering the brain would be a novel way of trafficking TMZ while obviating the lipophilic nature of the carrier.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. It was funded solely by Medivation.

Acknowledgement

The authors thank Dr. Anil Agarwal for careful reading and editing of the manuscript and the Analytical and Purification group at Integral Bioscience for purification of compounds and for acquiring NMR data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.08.064.

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