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Anticancer potential of aminomethylidene-diazinanes I. Synthesis of arylaminomethylidene of diazinetriones and its cytotoxic effects tested in glioblastoma cells

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Abstract

Diazinane and aryl moieties with vinylamine linkers were synthesized to investigate the importance of their structural variations as potential anti-glioblastoma agents. Structural variations incorporated on to the diazinane moiety included oxa and thio derivatives, each with a variety of nitrogen-bound substituents. The size and shape of the aromatic moiety was varied, with the final variation introducing two carbonyl groups, yielding a substituted anthraquinone. Readily available diazinanes and aryl amines were used as an advantageous foundation. Several parameters were calculated whilst engineering these compounds, including: Clog P, molecular polarizability, polar surface area, minimal molecular projected area, and pK_a. In addition, a simple and efficient procedure was developed to synthesize these compounds. It was demonstrated that a vinylamine with 1,3-diazinane-2,4,6-trione and 1-anthraquinone moiety is the most promising drug candidate causing almost 70% of LN229 tumor cell death at 1 µg/ml. In addition, its molecular polarizability, polar surface area and minimal molecular projected area indicate a possible potential of this molecule for crossing BBB.

Keywords

Glioblastoma; Anticancer; Diazinane; Carbamoyl; Merbarone

1. Introduction

Gliomas are aggressive tumors of the CNS arising from the supportive tissue in the brain and account for nearly 70% of all brain malignancies. They are highly vascularized with a high proclivity of infiltrating surrounding normal brain parenchyma. It is estimated that 1 in 161 individuals born today will develop brain or nervous system cancer at some point in their lives, and nearly 24,000 men and women in the U.S. are diagnosed with brain tumor every

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year, resulting in around 16,000 deaths.¹ Glioblastoma multiforme (GBM) is the most aggressive form of glial tumors with most patients dying in less than one year after diagnosis.² For newly diagnosed patients that are treated with the current standard of care for GBM, Temozolomide, the median progression free survival is just 6.9 months, and median overall survival is 14.6 months.³ Although temozolomide treatment increases patient median survival by an average of 2.5 months,⁴ over 50% of GBM patients develop temozolomide resistance, and recurrent GBMs are practically incurable.⁵ In addition, Bevacizumab (trade name, Avastin), which is a humanized monoclonal antibody targeting VEGF, is often used in second-line therapy for the treatment of GBM patients who experience remission. Despite the 26% of patients who had partial responses to bevacizumab, most patients lived less than six months with no evidence of improvement in overall survival.⁶

Merbarone is a non-sedating barbituric acid derivative that crosses the blood brain barrier.⁷ It has been extensively evaluated for its clinical potential against GBM; however, phase II clinical trials proved it to be an ineffective treatment.⁷ This compound is a catalytic inhibitor of topoisomerase II, the primary target of most cancer agents, and acts by blocking the DNA cleavage response of topoisomerase II.^{8,9} Merbarone is the first of its kind to specifically inhibit this response, to not affect protein-DNA binding and to induce single strand breaks in DNA apparently without binding to the DNA.^{8,10} Though merbarone's specific structure is no longer being sought out for its anti-tumor activity, its unique abilities merit further investigation of its derivatives.

Brain tumors are particularly difficult to treat due to distinct anatomical and physiological traits of neural tissue and vasculature. The blood-brain tumor barrier (BTB), although more permeable than blood-brain barrier (BBB), still represents the major obstacle preventing chemotherapeutic agents from reaching therapeutically relevant concentrations. Although multiple strategies have been proposed over the years to enhance BBB and BTB permeability their efficacy for glioblastoma treatment is still very low.^{11–16} The exceedingly grim prognosis of GBM patients due to the lack of successful drug delivery indicates that there is urgent need for new chemotherapeutics against glioblastoma.¹⁷ One of our research interests is in the implementation of structural variations of arylaminomethylene-diazinane moieties as new pharmacophores in design and development of potent anti-glioblastoma agents.

2. Results and discussion

Previously, we used the key structures of merbarone, a conjugate of thiobarbituric acid and aniline, joined by an amide linker, to construct a series of novel compounds. We examined these *N*-aryl-1,3-diazine-5-carboxamides anti-glioblastoma effects in vitro.¹⁸ Our spectroscopic studies show that these compounds, in an acidic media, are exclusively in enol/enamine form (Fig. 1). From these results, we proposed that the actual pharmacophore moieties are the ones that are attached to the enol/enamine linker (Scheme 1). If this is correct, the substitution of the OH group of the enol/enamine (vinylamine) form with hydrogen will then yield a new enamine (vinylamine) linker with many possibilities to study new structural varieties of these biologically active compounds (Scheme 1).

The general approach for the preparation of pyrimidinetrione with methylene substituents in the 5-position is *via* Knoevenagel condensation of pyrimidinetrione, *N*-mono and disubstituted pyrimidinetriones with aryl aldehydes.¹⁹ If the same reaction pathway is feasible for condensation of *N*-arylformamide, then a similar synthetic procedure can be applied in preparation of the latter. It is known that arylformamides can be easily prepared by the direct reaction of arylamines and formic acid.²⁰ This method was used for direct preparation of *N*-aryl formamides studied here. However, two isomers anti (**Ba**, Fig. 2) and syn (**Bb**, Fig. 2) are prepared with *syn*-isomer dominance (60–80%), as estimated by their proton NMR spectra. To further explore the potential of Knoevenagel type condensation with *N*-arylformamides, several reaction following experiments were performed *via* NMR.

Several reaction environments such as water, ethanol, THF, TFA (trifluoroacetic acid), DMSO, DMF, and acetic acid were explored. In almost all of these reactions, formation of the product was observed. However, some conditions lead to low solubility (water), the inability to increase reaction temperature (TFA), and difficult product purification (DMSO). Acetic acid was selected as the least problematic and most reliable reaction environment (Fig. 3).

Our spectroscopic study indicated that this is a viable synthetic approach for these valuable compounds, with practical conversion accomplished in just 48 hours at reflux. To complete the condensation reaction, an additional 24 hours of reflux in acetic acid is required. This rivals the aromatic aldehyde condensation reaction with pyrimidinetrione **C** where the condensation is completed within few hours and isolated yields are almost quantitative. Nevertheless, condensation with arylformamides in acetic acid gave improved isolated yields of up to 80%.

Considering that arylformamides are made from aromatic amines and formic acid, a three-component, one-pot reaction in formic acid was explored first. Once again, NMR was utilized in reaction following experiments to seek out optimal reaction conditions. This process was carried out by taking aliquots at precise time intervals from the refluxing formic acid. The solvent was evaporated and a ¹H NMR of the solid residue was recorded in DMSO-*d*₆. During the course of the Knoevenagel condensation, an aryl formamide was initially formed as an intermediate and then was immediately consumed before producing the desired product. The amount of intermediate remained almost constant in the reaction mixture after 30 minutes and began to diminish after several hours. After 24 h, the intermediate and starting materials were completely absent from the reaction mixture (Fig. 4). There was no peaks indicative of a by-product seen throughout the course of the reaction, suggesting that isolated yields were quantitative. Hence, Method B became the method of choice for preparation of these compounds (Scheme 2).

Using these optimized conditions, we explored the potential of our proposed structural skeleton for glioblastoma activity. For this study, aromatic size was the only thing manipulated on the carbon skeleton; substituents on the aromatic rings were controlled. Four different structural motives were selected: structural **Motif A** with phenyl and biphenyl moieties, structural **Motif B** with a naphthyl moiety, structural **Motif C** with an anthracenyl moiety, and structural **Motif D** with an anthraquinyl moiety (Scheme 3). These structures

differ by size and aromatic conjugation. In the final motif, we added oxidation/reduction capability. For all structures, R₁ and (or) R₂ are either hydrogen or methyl groups, and X is either oxygen or sulfur.

If we consider (Scheme 1) as a plausible structural comparison to our previous studies, then none of the compounds with structural **Motif A** should show biological activity. Due to the low solubility of these compounds in both water and organic solvents, testing at 100 µg/ml was not possible with the highest tested concentration being 10 µg/ml. At this concentration as well as 1 µg/ml, no notable activity was observed. High cell viability (greater than 94%) was observed for all compounds (Table 1).

The ability of a drug to reach its therapeutic target site is essential in drug design. By quantifying physicochemical parameters such as lipophilicity (ClogP), acidity (pK_a), polar surface area (PSA), and polarizability, a proper prediction can be made on the bioefficiency of these compounds. It is now accepted that a lipophilicity with a ClogP value between 4 and 7 might be one of the requirements for reasonable biological activity of small organic compounds.²¹ For polar surface area (PSA), research suggests a PSA greater than 140 Å² tends to be too polar for cell membrane penetration, while the PSA must be less than 90 Å² in order to cross the blood-brain barrier.^{22,23} According to our calculations, compounds with a phenyl moiety **VA1–VA5** have a PSA below 90 Å², indicating that their cell permeability should be relatively high. Their calculated ClogP is relatively low, suggesting very low cell absorption (uptake) capability. Considering optimal drug absorption is typically seen in compounds that have a ClogP between 2 and 5,^{24,25} we accurately predicted low cell absorption and poor anti-tumor activity (94–97% cell viability). The pK_a in comparison with pH of the biological media is a standard approach to determine solubility of the drug in water media, and percentage of the molecule ionization.²⁶ In our case, **VA1**, **VA2**, **VA4**, and **VA5** are partially ionized at testing pH, indicating relatively low cell absorption. However, **VA3** is in neutral (unionized) form, implying that high cell absorption is possible (Table 1).

Polarizability, a molecular characteristic that quantifies the ability of a molecular electronic system to be distorted by an external (biomolecule) field, is widely used for modeling molecular properties and biological activity.²⁷ In order to be administered, absorbed, and disposed of, a drug must be soluble in water media to some degree. In many instances, finding the appropriate solvent for drug administration requires a considerable amount of time and trial and error approach. Recently, Fujisawa and coworkers have used computed molecular polarizability in correlation with drug solubility to ease this process.²⁸ Their research shows that polarizability can be used as a relative measure of solubility in drugs with similar structural features, as is the case in our compounds. If we compare the relative polarizability of compounds **VA1** to **VA5** (Table 1), with gradual increase of polarizability and identical trending in lipophilicity, one can argue that the best activity should be observed in **VA5**. To the contrary, none of these compounds show noticeable glioblastoma activity, with 10 µg/mL being the highest tested concentration.

Our initial studies with compounds **VA1–VA5** suggest that their inherently low lipophilicity, polarizability, and polar surface area are the major reasons for their inactivity. It is important to find an optimal correlation between these molecular parameters to obtain the greatest

biological outcome possible.²⁹ From minimal data, it is reasonable to assume that the values for all the previously discussed parameters should be greatly increased to improve our compounds activity against glioblastoma. The first modification was aimed at increasing the lipophilicity and polarizability of our compounds. For this, the original phenyl moiety was replaced with a biphenyl group (Table 2). Computed lipophilicity and polarizability of the compounds increased substantially while PSA remained identical. The importance of molecular polarizability and lipophilicity was evident with the increased biological activity seen in this compound class. The simplest of all compounds in this class **VA6** showed minimal glioblastoma activity (85.5% cell viability) at 10 µg/ml with no activity observed at 1 µg/ml (Table 2). Apart from **VA9**, a sulfur analog of **VA6**, none of these compounds show good activity at 10 µg/ml without any noticeable activity at 1 mg/ml. Compound **VA9** (13.8% cell viability, 10 µg/ml) has the highest calculated lipophilicity (2.56, Table 2) of all analogs in the group. This definitively indicates that structural modifications of the phenyl moiety of our original compound **VA1** is required to optimize the activity of the biologically interesting compounds.

The next structural modifications were to increase the overall aromaticity of the initial compound by replacing the existing phenyl moiety of **VA1** with the polycyclic aromatic hydrocarbons, naphthalene (**Motif B**) and anthracene (**Motif C**). Variations of the C(2,3) fused compounds are presented in Table 3. These compounds possess fewer degrees of freedom than chain substituents which is associated with an increase in ligand-receptor binding energy and overall drug potency.³⁰ Consequently, the increased molecular rigidity and the capacity for π - π stacking are factors that decreased overall solubility and lipophilicity.^{30,31} In fact, our most active analog from our first modification (**VA9**, Table 2) with the new naphthyl moiety cannot be tested due to solubility issues (**VA14**, Table 3). With the addition of an *N*-methyl group, **VA15** we saw only minimal activity (90% cell viability) at 10 µg/ml.

Benzene fusing at C(3,4) of the original compound **VA1**, yielded a 2-naphthyl moiety (Table 4). Estimated physical properties do not differ from the 1-naphthyl compounds, however their estimated minimal projection area (MPA) was substantially different. It has been suggested that drugs with a relatively large projection area are less well transported than other substrates.³² Considering the MPA of our 2-naphthyl compounds **VA16–VA20**, these compounds should be more transportable and therefore more active in comparison with similar compounds (1-naphthyl moiety). **VA16** and **VA17** (Table 4) both show moderate glioblastoma activity (56–82.4%) at the 10 µg/mL concentration that was not present in corresponding compounds **VA11** and **VA12** (Table 3), respectively.

These findings indicate that with increased aromaticity and further enhancement of molecular electronic properties, improved glioblastoma activity is possible. We saw a noticeable increase in both the lipophilicity and minimal project area even though the size of these molecules was increased with only carbon and hydrogen. Once again, these electronic molecular changes resulted in lower solubility of our first compound **VA21** in this series and we were unable to evaluate its activity against glioblastoma (Table 5). For all other compounds in this series, some activity was observed, though **VA21** might structurally be the best candidate. The addition of a methyl group to the diazinetrione moiety slightly

increased solubility, making **VA22** possible to test. This compound produced the best results of the series showing cell viability of 60.3% at 1 µg/mL. The sulfur analog of this compound **VA25** has comparable activity with cell viability of 75% (Table 5).

Based on the data presented in Table 5, it can be postulated that the anthracene scaffold is the framework to our pharmacophore of these structural variations. Solubility of the unsubstituted anthracene model compound **VA21** is too low to be tested at even 10 µg/mL. By introducing two carbonyl groups in the 9 and 10 positions, the number of hydrogen bond acceptor possibilities increases by 4, which should result in a more water soluble compound. Indeed, the compound had increased solubility, and we were able to test the resulting compound **VA26** at both 10 µg/mL and 1 µg/mL with glioblastoma cell viability 17.2 and 82.9%, respectively (Table 6). The two carbonyl groups also increase the size of the molecule (MPA) and decreased lipophilicity. All studied structural analogs of **VA26** showed activity at 10 µg/mL with lowest cell viability measured for the sulfur analog **VA29** (12.3%, Table 6). Negligible glioblastoma activity was observed for all compounds in this group at 1 µg/ml concentration.

While exploring the structural properties of **VA26** derivatives, we found that by structuring this molecule to be slightly smaller in size, we can substantially influence the electronic and physical properties in our favor. This physical modification resulted in a new group of compounds with strong intramolecular hydrogen bonding between its two carbonyl groups (Scheme 4). Presence of the intramolecular bonding is demonstrated by the higher chemical shift of the vinylamine hydrogen of **VA33** in comparison with **VA26**. The chemical shifts are 14.12 and 11.95 ppm, respectively. Due to the molecule being frozen in one conformation, hydrogen bonding is limited and should increase the lipophilicity of this compound. Our calculated ClogP values agree with this prediction (Table 7) as they are all higher. The greatest glioblastoma activity observed was **VA33** with cell viability at 10 µg/mL and 1 µg/mL of 22.1 and 31.9%. Unfortunately, the thio analog **VA36** is not sufficiently soluble to evaluate its activity. All other compounds of this group showed some degree of cell viability reduction.

3. Conclusion

Combining 1,3-diazinane-2,4,6-trione and either a benzene, biphenyl, naphthalene, anthracene, or anthraquinone through a vinylamine linker resulted in the formation of several new potential anticancer compounds. From our studies, an unsubstituted 1,3-diazinane-2,4,6-trione and its thio isomers on one side of the vinylamine linker and a larger aromatic moiety on the other, are the requirements for high glioblastoma activity. To further evaluate these compounds, we used a combination of calculated physicochemical properties such as ClogP, polarizability, and minimal molecular projection area. These three parameters served as our guide while designing these new and interesting compounds. The estimated optimal parameters for these three descriptors are a ClogP between 1.5 and 3.5, 35–50 Å² for polarizability, and a minimal projection area of 40–60 Å². The most active compounds share the anthracene variation, and more precisely, the anthraquinone moiety. The most promising candidate is 5-[[9,10-dioxo-9,10-dihydroanthracen-1-yl]amino]methylidene-1,3-diazinane-2,4,6-trione (**VA33**) having glioblastoma activity with 31.9% cell viability at 1

µg/ml. Given these promising results, we are extensively investigating additional structural modifications to these compounds in order to optimize their efficiency against cancer cell viability and to determine their ability to cross BBB and BTB.

4. Experimental section

4.1. Chemistry

4.1.1. General remarks—All starting materials were reagent grade purchased from Sigma–Aldrich or Ark Pharm. ¹H NMR spectra were recorded on Varian Mercury Plus 400 MHz instrument in CDCl₃, DMSO-*d*₆, CDCl₃-TFA, DMSO-*d*₆-TFA, and in TFA with internal D₂O tube as NMR solvents using the solvent chemical shifts as an internal standard. Electrospray Mass Spectroscopy (EMS) was performed on MDS Sciex 3200 Qtrap LC/MS/MS system in positive or negative mode with methanol or acetonitrile as solvents. 1-Methylbarbituric acid was prepared from methylurea and malonic acid in acetic anhydride under microwave conditions.³³ 1-Phenyl-barbituric acid and 1-(4-nitrophenyl)barbituric acid were prepared from phenylurea (or 4-nitrophenylurea) and diethyl malonate in sodium ethoxide/ethanol reaction media.³⁴ All molecular physical properties were calculated using Marvin Sketch software. The calculation method is based on atomic and fragment method contribution.³⁵

4.2. General procedure for preparation of arylformamides

4.2.1. Preparation of N-(anthracen-2-yl)formamide (VA21FA)—Formic acid (5 ml, 6.1 g, 0.13 mol) and anthracen-2-amine (192 mg, 1 mmol) was refluxed overnight. Excess formic acid was evaporated under reduced pressure to form a solid residue. The solid residue was mixed with *t*-butyl methyl ether (5 ml) and sonicated at room temperature. Any insoluble product was separated by filtration, washed with *t*-butyl methyl ether (3×1 ml) and dried at 110 °C for 30 min to give pure product in 90% isolated yield. Product is a mixture of *syn*- and *anti*-amide isomers in ratio 1:0.3.³⁶ ¹H NMR (400 MHz) δ (*syn*-isomer) 10.46 (1H, s, NH), 8.51 (1H, s, CHO) (*anti*-isomer) 10.42 (1H, d, *J* = 7.2 Hz, NH), 9.01 (1H, d, *J* = 7.2 Hz, CHO) and 7.4–8.6 (9H of anthracene hydrogens for both isomers) ppm.

4.3. General procedure for preparation of arylformamides

Method A: From corresponding arylformamides.

4.3.1. Preparation of 5-[(1,1'-biphenyl)4yl]amino)methylidene]-1,3-diazinane-2,4,6-trione (VA6)—Acetic acid (15 ml) and a mixture of 1,3-diazinane-2,4,6-trione (128 mg, 1 mmol) and *N*-{[1,1'-biphenyl]-4-yl}formamide (VA6FA, 197 mg, 1 mmol) was refluxed for three days (~70 h) resulting in a clear, dark brown reaction mixture. The solvent was evaporated under reduced pressure resulting in a brown solid residue. Acetone (25 ml) was added to the solid and the resulting mixture was sonicated and room temperature for several hours. The solid product from the acetone suspension was separated via vacuum filtration. The resulting light brown solid was washed with acetone (4 × 5 ml) and dried at 110 °C for two hours to give 97% pure product in 81% (250 mg) yield.

^1H NMR (400 MHz) δ 11.90 (1H, d, J = 14 Hz, vinyl moiety NH), 10.99 (1H, s, diazine moiety NH), 10.85 (1H, s, diazine moiety NH), 8.56 (1H, d, J = 14 Hz, vinyl moiety CH), 7.72 (2H, d, J = 8.4 Hz, H_2), 7.67 (2H, d, J = 7.2 Hz, H_{ortho}), 7.59 (2H, d, J = 8.4 Hz, H_3), 7.45 (2H, t, J = 7.2 Hz, H_{meta}), and 7.35 (1H, t, J = 7.2 Hz, H_{para}) ppm. EMS^+ (CH_3OH), m/z 308 ($\text{M}+1$), 368 ($\text{M}+\text{Na}+\text{K}$).

Method B: Three component reaction from corresponding arylamine, formic acid, and corresponding diazinane.

4.3.2. Preparation of 5-[[[(9,10-dioxo-9,10-dihydroanthracen-1yl)

amino]methylidene]1,3-diazinane-2,4,6-trione (VA33)—Formic acid (6 ml, 7.32 g, 0.16 mol), 1-aminoanthraquinone (223 mg; 1 mmol), and 1,3-diazinane-2,4,6-trione (128 mg, 1 mmol) was combined and refluxed overnight (approximately 30 h). In the course of the reaction, an orange precipitate start to form. Excess of formic acid was evaporated under reduced pressure. The remaining dark orange solid was mixed with acetone (20 ml) and sonicated at room temperature for several hours. The fine, powdery material that formed was separated by filtration, washed extensively with acetone (6×3 ml) and dried at 110°C for 3 h. The resulting product was greater than 97% pure. Isolated yield 94% (340 mg). ^1H NMR δ 1.10 (1H, d, J = 13.6 Hz, vinyl moiety NH), 11.09 (1H, s, diazinane moiety NH), 10.99 (1H, s, diazinane moiety NH), 8.73 (1H, d, J = 13.6 Hz, vinyl moiety CH), 8.24 (2H, t, J = 8.4 Hz) 8.17 (1H, dd, J_1 = 7.6 Hz, J_2 = 1.6 Hz), 8.05 (1H, d, J = 7.2 Hz), and 7.89–7.97 (3H, m) ppm. EMS^+ (CH_3OH), m/z 398 ($\text{M}+\text{NH}_4+\text{NH}_4$), 412 ($\text{M}+\text{NH}_4+\text{Na}$), 422 ($\text{M}+\text{Na}+\text{K}$). Elemental analysis for $\text{C}_{19}\text{H}_{11}\text{N}_3\text{O}_5$: Calc: C, 63.16; H, 3.07; N, 11.63. Found: C, 63.06; H, 3.17; N, 11.41

4.4. Cell Culture

We used human glioblastoma cell line LN-229 (ATCC CRL-2611) which according to our previous studies is very suitable for testing drug toxicity. The cell line was maintained as a semi-confluent monolayer culture in DMEM supplemented with 50 U/ml penicillin, 50 ng/ml streptomycin, and 10% fetal bovine serum (FBS) at 37°C in a 5% CO_2 atmosphere. Prior to treatment, cells were plated on 24-well tissue culture plates (Falcon) at 2×10^4 cells *per* well. The cells were treated with different compounds (see Table 1), which were re-suspended in DMSO to prepare 10 mg/ml stock solutions, which were subsequently suspended in cell culture media to reach final concentrations of 1 and 10 $\mu\text{g}/\text{ml}$, respectively, and the cells were exposed to the compounds for 72 h. Cell viability was assessed by Guava/Express plus and Guava/ViaCount per the manufacturer's recommendations (Millipore). The data represents average cell survival values from two experiments in which at least 1000 cells *per* repetition were analyzed.^{37,38}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2017.08.020>.

Fig. 1.
Amide and enol/enamine forms of previously studied *N*-aryl-1,3-diazine-5- carboxamide.

Fig. 2.
Two isomers of *N*-arylfarmides.

Fig. 3. ^1H NMR reaction following in acetic acid between formamide **B** and pyrimidinetrione **C** (2 eqvs).

Fig. 4.
 ^1H NMR reaction following: preparation of enamides **D**.

Scheme 1.

Structures of TMZ and merbarone together with the new proposed derivatives. Diazinane and aryl moieties that are linked through an enamine bridge.

Scheme 2.

Methods for preparation of arylaminomethylidene of diazinetriones.

Scheme 3.

Four structural motifs based on merbarone as a lead compound.

Scheme 4.
Vinylamine **VA33** with intramolecular hydrogen.

LN229 glioblastoma cell viability of diazinettriones with phenylaminomethylidenes moiety (VA1–VA5).

Table 1

Compound	X	R ¹	R ²	pK _a 1	pK _a 2	pK _a 3	ClogP	PSA	P	V1	V2
VA1	O	H	H	6.80	10.18	–	0.02	87.30	22.3	96.1	96.2
VA2	O	CH ₃	H	7.06	–	–	0.25	78.51	24.1	95.0	94.9
VA3	O	CH ₃	CH ₃	–	–	13.34	0.47	69.72	25.9	96.1	95.0
VA4	S	H	H	7.41	10.68	–	0.91	70.23	25.6	95.3	96.5
VA5	S	CH ₃	H	7.67	–	–	1.14	61.44	27.4	94.3	94.3

ClogP = calculated partition coefficient; PSA = polar surface area (2 D, Å³); P = polarizability (Å³); V1 = cell viability at 10 µg/ml; V2 = cell viability at 1 µg/ml.

LN229 glioblastoma cell viability of *diazinetrienes* with biphenylaminomethylidene moiety (**VA6–VA10**).

Table 2

Comp.	X	R ¹	R ²	pK _a 1	pK _a 2	pK _a 3	ClogP	PSA	P	V1	V2
VA6	O	H	H	6.80	10.18		1.67	87.30	33.1	85.5	92.6
VA7	O	CH ₃	H	7.06			1.90	78.51	35.0	93.5	96.9
VA8	O	CH ₃	CH ₃			13.34	2.12	69.72	36.8	94.6	94.8
VA9	S	H	H	7.41	10.63		2.56	70.23	36.5	13.8	95.3
VA10	S	CH ₃	H	7.67			2.28	61.44	38.4	89.6	97.0

ClogP = calculated partition coefficient; PSA = polar surface area (2 D, Å³); P = polarizability (Å³); V1 = cell viability at 10 µg/ml; V2 = cell viability at 1 µg/ml.

Table 3

LN229 glioblastoma cell viability of *diazinetriones* with naphthalen-1-ylaminomethylidene moiety (VA11–VA15).

Comp.	X	R ¹	R ²	pK _{a1}	pK _{a2}	pK _{a3}	ClogP	PSA	P	MPA	V1	V2
VA11	O	H	H	6.80	10.18		1.01	87.30	29.7	35.77	95.1	93.2
VA12	O	CH ₃	H	7.08			1.24	78.51	31.5	38.30	94.5	89.0
VA13	O	CH ₃	CH ₃			12.75	1.46	52.65	33.4	41.71	95.4	94.5
VA14	S	H	H	7.41	10.63		1.90	70.23	33.1	32.20	NT	NT
VA15	S	CH ₃	H	7.67			2.13	61.44	34.9	36.63	78.3	90.0

ClogP = calculated partition coefficient; PSA = polar surface area (2 D, Å²); P = polarizability (Å³); MPA = minimal projected area (Å²); V1 = cell viability at 10 µg/ml; V2 = cell viability at 1 µg/ml.

Table 4

LN229 glioblastoma cell viability of *diazinetrienes* with naphthalen-2-ylaminomethylidene moiety (VA16–VA20).

Comp.	X	R ¹	R ²	pK _a 1	pK _a 2	pK _a 3	ClogP	PSA	P	MPA	V1	V2
VA16	O	H	H	6.80	10.18		1.01	87.30	29.7	31.07	82.4	92.4
VA17	O	CH ₃	H	7.06			1.24	78.51	31.5	31.77	56.0	90.0
VA18	O	CH ₃	CH ₃			13.24	1.46	69.72	33.4	38.14	86.6	90.5
VA19	S	H	H	7.41	10.63		1.90	70.23	33.1	33.35	94.4	96.5
VA20	S	CH ₃	H	7.67			2.13	61.44	34.9	32.27	84.5	94.6

ClogP = calculated partition coefficient; PSA = polar surface area (2 D, Å²); P = polarizability (Å³); MPA = minimal projected area (Å²); V1 = cell viability at 10 µg/ml; V2 = cell viability at 1 µg/ml.

LN229 glioblastoma cell viability of *diazinetrienes* with anthracen-1-ylaminomethylidene moiety (VA21–VA25).

Table 5

Compound	X	R ¹	R ²	pK _a 1	pK _a 2	pK _a 3	ClogP	PSA	P	MPA	V1	V2
VA21	O	H	H	6.80	10.18		2.00	87.30	37.2	39.91	NT	NT
VA22	O	CH ₃	H	7.06			2.23	78.51	39.1	44.08	9.4	60.3
VA23	O	CH ₃	CH ₃			13.23	2.45	69.72	40.9	44.08	60.8	88.1
VA24	S	H	H	7.41	10.63		2.89	70.23	40.6	43.66	11.0	83.3
VA25	S	CH ₃	H	7.67			3.12	61.44	42.5	45.43	3.7	75.0

ClogP = calculated partition coefficient; PSA = polar surface area (2 D, Å²); P = polarizability (Å³); MPA = minimal projected area (Å²); V1 = cell viability at 10 µg/ml; V2 = cell viability at 1 µg/ml.

Table 6

LN229 glioblastoma cell viability of *diazinetrienes* with 9,10-dioxo-9,10-dihydroanthracen-2-yl aminomethylidene moiety (**VA26–VA32**).

Comp.	X	R ¹	R ²	pK _a 1	pK _a 2	pK _a 3	ClogP	PSA	P	MPA	V1	V2
VA26	O	H	H	6.80	10.18		0.97	121.44	35.2	41.10	17.2	82.9
VA27	O	CH ₃	H	7.06		15.66	1.19	112.65	37.1	44.94	28.9	89.7
VA28	O	CH ₃	CH ₃			11.93	1.42	103.86	38.9	44.28	68.3	92.4
VA29	S	H	H	7.41	10.63		1.86	104.37	38.9	45.90	12.3	87.4
VA30	S	CH ₃	H	7.67			2.08	95.58	40.4	45.69	17.2	80.8
VA31	O	C ₆ H ₅	H	4.35		10.17	2.85	112.65	44.8	55.76	76.5	87.6
VA32	O	C ₆ H ₅ NO ₂	H	4.07		10.17	2.79	158.47	46.7	58.49	61.8	88.2

ClogP = calculated partition coefficient; PSA = polar surface area (2 D, Å²); P = polarizability (Å³); MPA = minimal projected area (Å²); V1 = cell viability at 10 µg/ml; V2 = cell viability at 1 µg/ml.

LN229 glioblastoma cell viability of diazinetrones with 9,10-dioxo-9,10-dihydroanthracen-1-yl moiety (VA33–VA39).

Compound.	X	R ¹	R ²	pK _a 1	pK _a 2	pK _a 3	ClogP	PSA	P	MPa	V1	V2
VA33	O	H	H	6.80	10.18		1.62	121.44	35.2	45.94	22.1	31.9
VA34	O	CH ₃	H	7.08		14.62	1.84	112.65	37.1	48.95	35.3	84.8
VA35	O	CH ₃	CH ₃			10.79	2.07	103.86	38.9	51.39	86.3	94.7
VA36	S	H	H	7.41		10.63	2.51	104.37	38.9	48.97	48.6	65.0
VA37	S	CH ₃	H	7.67		15.08	2.73	95.58	40.4	52.71	NT	NT
VA38	O	C ₆ H ₅	H	4.37		10.17	3.50	112.65	44.8	56.22	29.2	66.3
VA39	O	C ₆ H ₅ NO ₂	H	4.10		10.17	3.44	155.79	46.7	56.80	10.4	75.8

CllogP = calculated partition coefficient; PSA = polar surface area (2 D, Å³); P = polarizability (Å³); PA = minimal projected area (Å²); V1 = cell viability at 10 µg/ml; V2 = cell viability at 1 µg/ml.