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O-Benzyl-*N*-(9-acridinyl)hydroxylamines

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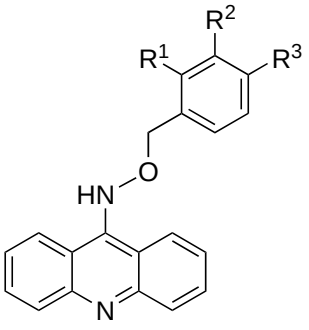
Abstract

A series of *O*-benzyl-*N*-(9-acridinyl)hydroxylamines was prepared, isolated, and evaluated for biological activity using both thermal denaturation and MTT assays. Changes in the thermal denaturation temperature of genomic calf-thymus DNA ranged from +6.6 °C to +20.2 °C. MTT assays on SNB-19 glioblastoma cells provided biological activity that ranged from 17.4 µM to 33.2 µM. Both evaluation methods of biological activity indicate that substitution of the benzyl group by either electron-withdrawing or electron-donating groups provides a measureable benefit in these assays. The two assays agreed on the magnitude of the interaction for each substitution pattern.



R^1 = H, CH_3, Cl
R^2 = H, CH_3O, CH_3, Cl, NO_2
R^3 = H, CH_3O, CH_3, Cl, Br, NO_2

Keywords: Aminoacridines, hydroxylamines, thermal denaturation, MTT assay, QSAR

Introduction

Aminoacridines have been known for some time as DNA intercalators. Lerman[1] proposed an intercalating model for this class of compounds that has been used to describe the initial interactions resulting in biological activity. In this model, the acridine subunit intercalates between adjacent nucleotide base pairs in duplex DNA and results in the lengthening, stiffening, and unwinding of the complex. The substituents on the acridine nucleus are located outside of the intercalation pocket, allowing for potential interactions with other compounds. The biological activity of this class of compound was further elucidated to involve a ternary complex with Topoisomerase II.[2]

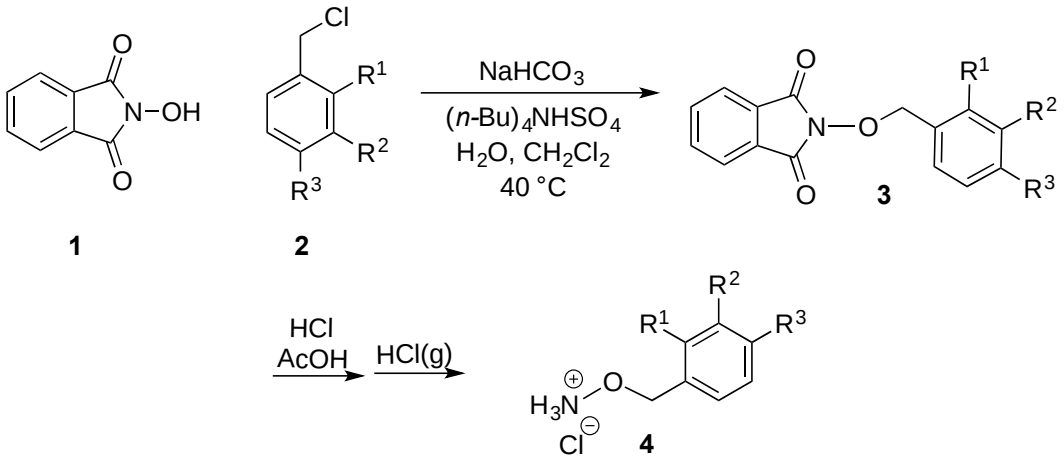
9-Aminoacridines, such as the 9-pyridinylaminoacridines,[3] 9-aminomethylacridines,[4] acridones,[5] 9-thioacridines,[6] and 9-anilinoacridines[8] have been studied for their ability to intercalate DNA. These, and other, studies have verified that the proposed model of binding is valid; a strong intercalation of the acridine with duplex DNA requires that the intercalator exist as a cation under physiological conditionsm[7] be planar and aromatic, and possess substitution that is directed away from the site of intercalation.

In our continued exploration of the effect of substitution on the acridine nucleus and the ability of the resulting compounds to interact with duplex and/or quadruplex DNA, we sought to prepare a series of O-benzyl-N-acridinylhydroxylamines to study the effect of the hydroxylamine on the biological activity of this class of compound.[9] Our interest in the hydroxylamine as a linker between a substituted aryl system and the acridine nucleus was predicated by the electronic characteristics of the hydroxylamine and the potential additional hydrogen-bonding sites in the group.

Results and Discussion

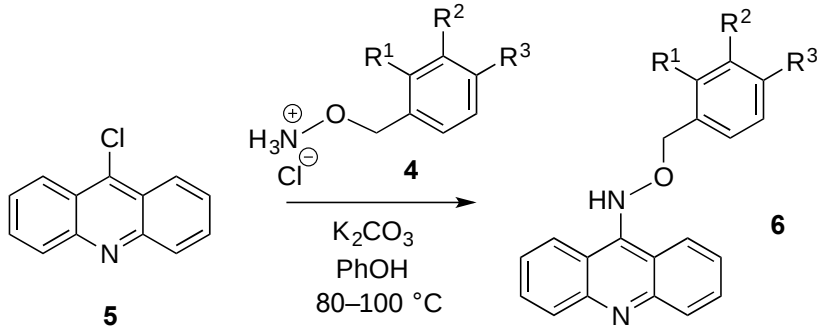
The title compounds were prepared from the commercially available benzyl halides using a slight modification of the method by Bonaccorsi and Giorgi (Scheme 1).[10] Alkylation of *N*-hydroxyphthalimide (**1**) with an appropriately substituted benzyl chloride **2** produced the *N*-benzyloxyphthalimides **3** in good yields as white or off-white powders. Acidic hydrolysis of **3** in acetic acid followed by precipitation of product by bubbling anhydrous hydrogen chloride gas through the reaction mixture gave the expected *N*-benzylhydroxylamines as the hydrochloride salts **4**. In many cases, the yield of the unmodified reaction gave only trace amounts of product. Improvement of the reaction yield in the decomposition of the phthalimide was accomplished by repeatedly recharging the reaction with additional hydrochloric acid. Yields for this step were variable and seemed to be loosely correlated to the electronic effect of the substituents on the benzyl group.

The desired title compounds, *O*-benzyl-*N*-(9-acridinyl)hydroxylamines were prepared by reacting the prepared hydroxylamines with 9-chloroacridine[11,12] (**5**) as shown in Scheme 2. Initial attempts in this reaction resulted in relatively poor yields of **5**, likely due to the limited availability of the free base of compounds **4**. Use of potassium carbonate as a weak base in the reaction mixture dramatically improved the yield of the reaction. The desired compounds, **6**, were isolated by extraction and purified by multiple chromatographic separations using radial chromatography.



1 **2** **3** **4**

Scheme 1. Synthetic scheme for production of hydroxylamine salts used in this study. R^1 = H, CH_3, Cl; R^2 = H, CH_3O, CH_3, Cl, NO_2; R^3 = H, CH_3O, CH_3, Cl, Br, NO_2.



5 **4** **6**

Scheme 2. Reaction of hydroxylamines **4** with 9-chloroacridine (**5**).

Spectroscopic analysis of the products in chloroform-*d* indicated severe line broadening for both proton and carbon resonances in the acridine ring system. This was particularly pronounced in the proton-NMR spectra.[13] Those same signals, however, sharpened in acetone-d_6 hinting that restricted rotation about the N–O or C9–N bond might exist in **6**. Moreover, it was observed that symmetrically related hydrogen atoms identified by 2-D NMR experiments on the acridine nucleus were identified as magnetically non-equivalent (Figure 1). For example, separate resonances for the protons on C1 and C8 of the acridine in **6j** occur at 8.06 ppm and 8.98 ppm, respectively. The acidic hydrogen was identified as the resonance at 9.33 ppm by shaking the acetone-d_6 solution with a drop of D_2O.

The existence of symmetrically related but non-magnetically equivalent hydrogens on the acridine nucleus posed a further issue about the structure of the isolated compound. It was theorized that the structure for the product **6g**, for example, could be represented either as the hydroxylamine or as its tautomer, the oxime (Scheme 3). Free rotation about the C9–N bond in the hydroxylamine should allow the symmetrically related protons to exhibit a single resonance, assuming the rotation is fast on the NMR timescale. However, if the compound were to exist as the tautomeric oxime, then rotation about the C9–N bond wpi;d be very restricted and the apparent symmetrically related acridine hydrogen atoms would not be magnetically equivalent.

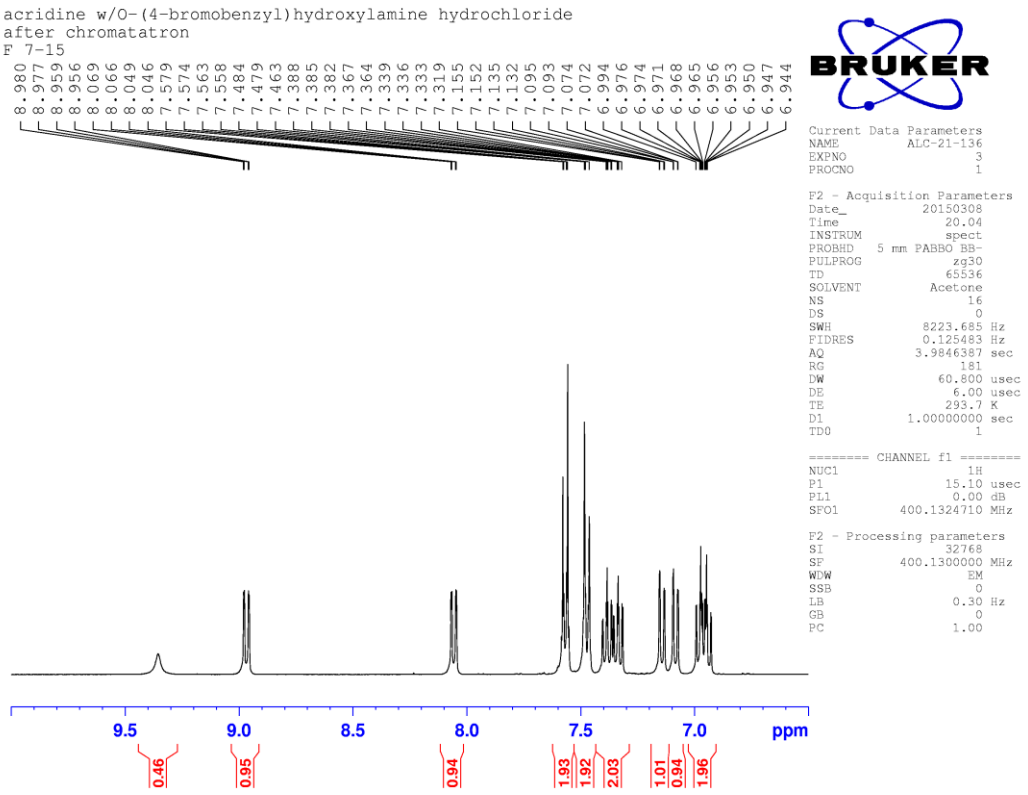
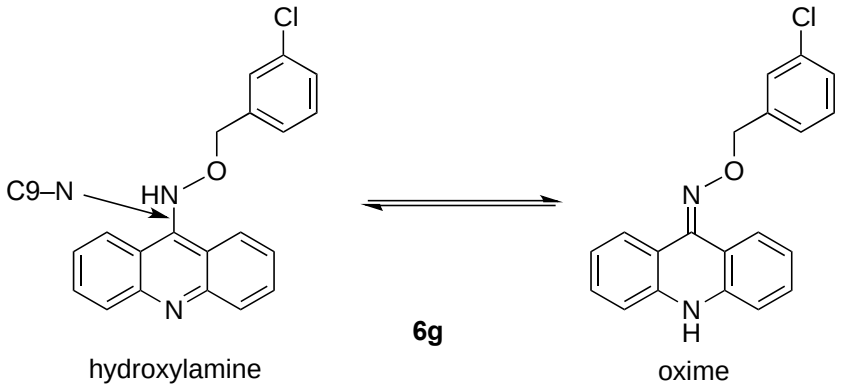


Figure 1. ^{1}H-NMR spectrum of **6j** in acetone-d_6 at 400 MHz.



Scheme 3. Restricted rotation in **6g** results in non-equivalent hydrogen atoms on the acridine ring system. The hydroxylamine would be expected to freely rotate compared to the tautomeric oxime.

While nitrogen inversion[14] would still allow for an exchange that would mimic rotation, sp^2-hybridized inversion is significantly higher in energy that it would likely not occur to a large extent.

Measurement of the interaction of the title compounds **6** with double-stranded genomic calf thymus DNA was conducted by evaluation of the change in the thermal denaturation temperature of the DNA.[15] These measurements were taken with mixtures containing 1.5 x 10^{-6} M DNA and 1.5 x 10^{-6} M **6a-6l** in a pH 6 phosphate buffer (0.01 M). A small amount of DMSO was added to maintain solubility (~5% DMSO). Use of the buffer

solution at pH 6 was necessary given that the 9-hydroxylaminoacridines had an estimated pK_a 6.3 and were therefore significantly less soluble at pH 7.[7]

The thermal denaturation curves for many of the title compounds indicated a strong initial interaction with genomic DNA characterized by a significant decrease in the absorbance of the solution as the temperature increased to 50 °C. Then, as the temperature continued to increase beyond 50 °C, a bimodal denaturation curve was observed. The first inflection occurred at the melting point for the uncomplexed genomic calf thymus DNA and the second occurred at the proposed denaturation temperature of the DNA-**6** intercalative complex. It is interesting to note that the thermal denaturation temperature changes range from 6.6 °C (weakly intercalating) to 20.2 °C (strongly intercalating). In addition, the majority of the compounds exhibited thermal denaturation temperature changes greater than 15 °C. This is suggestive of a mono-intercalating compound possessing a propensity to fully intercalate genomic DNA, rather than an associative binding to the grooves or phosphate backbone of genomic DNA. The denaturation temperatures for each of the compounds in the study are indicated in Table 1.

Table 1. Title compounds prepared in this study and their measured interactions with calf thymus DNA and SNB-19 glioblastoma cell line

Compound	R^1	R^2	R^3	Yield, %[a]	ΔTm, °C[c]	MTT, μM
6a	H	CH_3O	H	64	9.1	nm
6b	H	H	CH_3O	0[b]	nm[d]	nm
6c	CH_3	H	H	49	15.5	17.7 ± 0.2
6d	H	CH_3	H	71	19.0	20.7 ± 0.5
6e	H	H	CH_3	40	19.0	22.2 ± 1.7
6f	H	H	H	16	6.6	33.2 ± 0.6
6g	Cl	H	H	15	18.2	17.4 ± 0.2
6h	H	Cl	H	46	18.5	18.0 ± 0.2
6i	H	H	Cl	30	20.2	17.0 ± 0.4
6j	H	H	Br	51	18.1	18.5 ± 4.3
6k	H	NO_2	H	41	15.1	31.8 ± 0.1
6l	H	H	NO_2	38	nm	30.3 ± 1.4

[a]Isolated and purified yield. Compounds were greater than 99% pure prior to biological analysis. [b]Trace amounts were observable in the crude mixture by ^{1}H-NMR spectroscopy. Only trace amounts of this compound were isolated. No attempt was made to improve the yield of this reaction. [c]Thermal denaturation change of calf thymus DNA-compound complex versus genomic calf thymus DNA in pH 6 phosphate buffer. Values presented indicate the average of at least two measurements. [d]not measured.

An MTT assay using the SNB-19 glioblastoma cell line was performed in triplicate for each of the title compounds. The reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2*H* tetrazolium bromide), a yellow tetrazole, to its insoluble formazan was observed and resulted in a color change that was easily monitored by a change in absorbance at 560 nm.[16] The reduction of the dye is dependent on NAD(P)H-dependent oxidoreductase enzymes that are found in large amounts within the cytosolic compartment of the cell. These enzymes are indicative of the number of viable cells within a sample as they are tied to cell proliferation. The

reduction of the MTT dye, then, would be expected to increase with cellular metabolic activity due to elevated NAD(P)H flux. The results of the MTT assay versus the SNB-19 glioblastoma cell line on the title compounds (**6**) are collected in Table 1.

Construction of a quantitative structure activity relationship (QSAR) using the calf-thymus DNA ΔT_m values and the IC_{50} values from the MTT assay was attempted using various parameters. When each assay was correlated to the Hammett sigma values[17] of the benzyl substituents, very similar information was obtained. The addition of either an electron-withdrawing or electron-donating group caused an increase in biological activity from that of the unsubstituted compound. This indicates that any substitution on the benzyl ring resulted in an increase in activity. In fact, the specific nature of the substituents had little impact on the overall activity of the compound.

Yet, when the thermal denaturation values, ΔT_m, were plotted against the MTT assay results, IC_{50}, the correlation was observed as shown in Figure 3. A slight preference for *ortho* substitution, with respect to the MTT assay, seems to be present, though significantly more data are needed to confirm this effect. In short, however, this evaluation indicates a correlation between the MTT assay results and the change in thermal denaturation temperature for calf-thymus DNA.

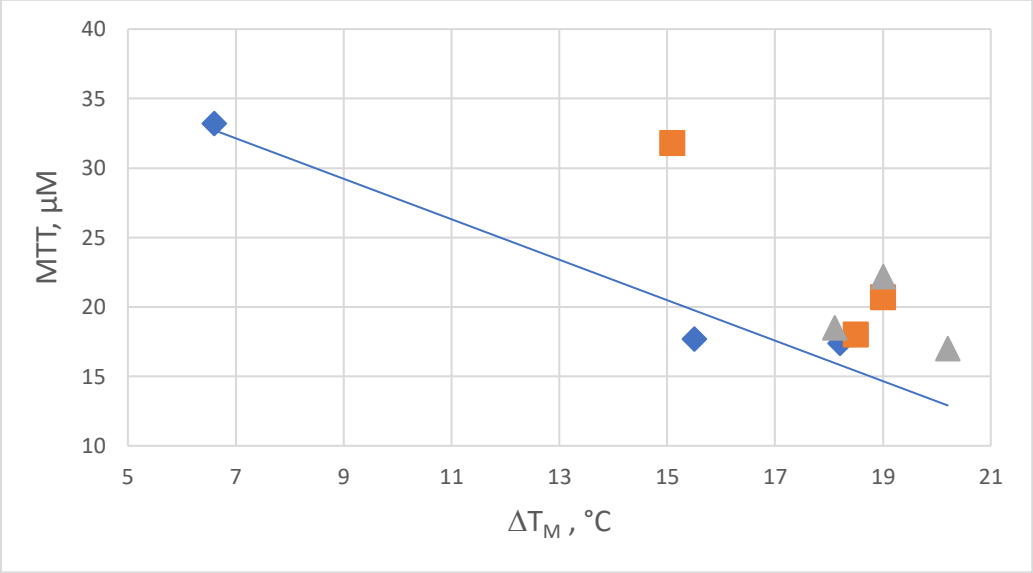


Figure 3. Correlation of ΔT_m values and MTT IC_{50} results. Data are represented as *ortho*- (blue diamonds), *meta*- (orange squares), or *para*- (gray triangles) substituted compounds. The trendline is indicated for the *ortho* substituted compounds.

The correlation reveals that substitution at the benzyl group of **6** favors biological interaction of the compound. The location of the substituent on the benzyl ring did not appear to influence the outcome of that biological interaction (as evidenced by the similar results seen for **6c-e** and **6g-i**, see Table 1). This is likely due to the fact that the benzyl ring is not conjugated to the acridine ring system (the intercalating portion of the molecule.) However, the fact that a difference was noted in the assays based on the presence of a substituent may hint that a conjugated version of the title compounds could possess significantly more pronounced effects. Such analyses on a fully conjugated system are currently being performed.

Conclusions

A series of *O*-benzyl-*N*-(9'-acridinyl)hydroxylamines has been prepared and evaluated for their physical and biological properties as potential antitumor agents. Preparation of these compounds from the hydrochloride salts of the substituted hydroxylamines requires the addition of an excess of weak base in the phenol reaction solvent. Purification of the compounds using multiple separations on radial chromatography provided useful quantities of the pure compound **6** used for the evaluation of physical, spectroscopic, and biological properties. Thermal denaturation data for the series indicated a significantly strong interaction that will be further explored. The implication of the strong intercalative interaction with genomic DNA was confirmed by MTT assays.

The title compounds are an interesting series of 9-substituted aminoacridines worthy of further study. In addition to their biological activity, these compounds possess restricted conformations in the solution phase. We are currently exploring the preparation, isolation, and evaluation of crystalline compounds for evaluation by diffraction. Obtaining a crystal structure of the compound would settle the question of the existence of the title compounds as hydroxylamines or oximes. Evaluation of these conformers could also provide additional insight into the observed effect of substitution on the benzyl group.

Experimental Section

General. Benzyl chloride, substituted benzyl chlorides, *N*-hydroxyphthalimide, 9-chloroacridine (**5**) and other reagents were obtained commercially from Sigma Aldrich, and were used after a determination of purity via ^{1}H NMR spectroscopy. Compounds **3** and **4** were prepared using the method of Bonaccorsi and Giorgi.[10] All solvents used were dried prior to use and their purity verified via spectroscopic methods. Hydrogen chloride gas was generated as needed through the addition of concentrated sulfuric acid to sodium chloride. Radial chromatography was performed using a Harrison Associates Chromatotron® on 2 mm-thick silica gel plates containing fluorescent indicator that were pre-cleaned with MeOH and stored at elevated temperatures prior to use. NMR spectra were obtained on a Bruker Avance II (400 MHz for ^{1}H) multinuclear FT-NMR. The resonances for each atom in each compound were identified by 1D and 2D NMR techniques. Infrared spectra were collected using a Thermo Scientific iD$_5$ ATR ZnSe cell. All UV-visible data were measured using an Agilent UV-visible diode-array spectrophotometer with a Peltier-temperature controller. MTT assay data were collected using published procedures.[16]

***O*-benzyl-*N*-(9'-acridinyl)hydroxylamines 6a-l.** The appropriate salt, **4a-l**, (7.02×10^{-4} mol) was treated with 9-chloroacridine (**5**) (4.68×10^{-4} mol). The reaction was carried out in molten phenol using 3.0 g of phenol per g of 9-chloroacridine (**5**). The reaction was heated between 80-100 °C for a period of 6-8 h, then cooled to rt and dissolved in CH_2Cl_2. The resulting orange or red organic solution was washed repeatedly with 0.25 M NaOH until greater than a 1:1 molar ratio of hydroxide to phenol was used. The organic phase was then washed with H_2O (once) and brine (once). The organic layer was dried over anhydrous Na_2SO_4, gravity filtered, and concentrated to a final volume of approximately 1 mL. This sample was then transferred to the top of a 5-cm column of silica gel constructed from a 10-mL syringe barrel and eluted with EtOAc. The orange eluate was collected, concentrated to a final volume of 0.5-1.0 mL, and subjected to radial chromatography (2 mm plate, silica gel, CH_2Cl_2:Et_2O 100:0 to 90:10 gradient elution). Compounds **6a-l** were obtained in pure form by evaporation of the solvent from the bands that eluted.

***O*-(3-Methoxybenzyl)-*N*-(9'-acridinyl)hydroxylamine (6a).** Yellow solid. Yield 64%, ^{1}H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.32 (s, 1H); 9.03 (d, J 8.4 Hz, 1H); 8.10 (d, J 8.0 Hz, 1H); 7.34 (m, 3H); 7.13 (m, 4H); 6.97 (m, 2H); 6.88 (m, 1H); 5.30 (s, 2H); 3.78 (s, 3H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 159.9; 143.5; 140.5; 140.4; 140.3; 138.1; 138.0; 131.9; 130.9; 129.8; 129.3; 124.6; 120.7; 120.0; 119.1; 118.1; 118.1; 115.4; 115.3; 115.0; 115.0; 114.9; 113.4; 113.0; 76.5; 54.5. IR (ATR-ZnSe) in cm^{-1}: 747; 964; 1157; 1265; 1474; 1598; 1614. HRMS: M+1, 331.1445 ($C_{21}H_{19}N_2O_2$; calc'd 331.1447). ΔT_m = 9.1°C.

***O*-(2-Methylbenzyl)-*N*-(9'-acridinyl)hydroxylamine (6c).** Yellow-red solid. Yield 49%, ^{1}H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.31 (s, 1H); 8.95 (m, 1H); 8.09 (d, J 8.4 Hz, 1H); 7.47 (d, J 8.0 Hz, 1H); 7.34 (m, 2H); 7.23 (m, 3H); 7.12 (m, 2H); 6.98 (m, 2H); 5.33 (s, 2H); 2.45 (s, 3H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 143.3; 140.4; 140.3; 138.1; 138.0; 136.8; 136.4; 131.7; 130.9; 130.0; 129.7; 129.2; 127.9; 125.7; 124.6; 120.6; 119.1; 118.2; 118.1; 115.4; 115.3; 115.0; 114.9; 114.9; 75.2. IR (ATR-ZnSe) in cm^{-1}: 1473. HRMS: M+1, 315.1496 ($C_{21}H_{19}N_2O$; calc'd 315.1497). ΔT_m = 15.5°C. MTT IC_{50} = 17.7±0.2μM.

***O*-(3-methylbenzyl)-*N*-(9'-acridinyl)hydroxylamine (6d).** Yellow solid. Yield 71%, ^{1}H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.31 (s, 1H); 8.99 (d, J 8.4 Hz, 1H); 8.09 (d, J 8.0 Hz, 1H); 7.33 (m, 5H); 7.10 (m, 3H); 6.98 (m, 2H); 5.27 (s, 2H); 2.34 (s, 3H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 143.3; 140.4; 140.4; 138.6; 138.1; 138.0; 137.7; 131.9; 130.9; 129.7; 128.7; 128.3; 128.2; 125.2; 124.6; 120.7; 119.1; 118.2; 118.1; 115.4; 115.3, 114.98, 115.0; 114.9; 76.8. IR (ATR-ZnSe) in cm^{-1}: 745; 770; 964, 1156; 1472; 1486; 1598; 1614. HRMS: M+1, 315.1477 ($C_{21}H_{19}N_2O$; calc'd 315.1497). ΔT_m = 19.0°C. MTT IC_{50} = 20.7±0.5μM.

***O*-(4-methylbenzyl)-*N*-(9'-acridinyl)hydroxylamine (6e).** Yellow solid. Yield 40%, ^{1}H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.31 (s, 1H); 8.98 (m, 1H); 8.11 (m, 1H); 7.37 (m, 4H); 7.14 (m, 4H); 6.96 (m, 2H); 5.27 (s, 2H); 2.33 (s, 3H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 143.2; 140.4; 138.1; 138.0; 137.1; 135.6; 131.8; 130.9; 129.7; 128.9; 128.2; 124.6; 120.7; 119.1; 119.1; 118.2; 115.4; 115.3; 115.0; 114.9; 76.6. IR (ATR-ZnSe) in cm^{-1}: 746, 965, 1157, 1472. HRMS: M+1, 315.1490 ($C_{21}H_{19}N_2O$; calc'd 315.1497). ΔT_m = 19.0°C. MTT IC_{50} = 22.2±1.7 μM.

***O*-benzyl-*N*-(9'-acridinyl)hydroxylamine (6f).** Yellow solid. Yield 16%, ^{1}H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.33 (s, 1H); 9.00 (m, 1H); 8.09 (m, 1H); 7.54 (m, 2H); 7.35 (m, 5H); 7.14 (m, 1H); 7.08 (m, 2H); 6.95 (m, 2H); 5.31 (s, 2H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 143.4; 140.5; 138.8; 138.1; 131.8; 130.9; 129.7; 128.3; 128.0; 127.6; 124.6; 120.7; 119.1; 118.1; 115.4; 115.30; 115.0; 114.9; 76.7. IR (ATR-ZnSe) in cm^{-1}: 1473. HRMS: M+1, 301.1341 ($C_{20}H_{17}N_2O$; calc'd 301.1341). UV λ_{max} 259 nm. ΔT_m = 6.6°C. MTT IC_{50} = 33.2±0.6 μM.

***O*-(2-chlorobenzyl)-*N*-(9'-acridinyl)hydroxylamine (6g).** Yellow solid. Yield 15%. ^{1}H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.37 (s, 1H); 9.02 (m, 2H); 8.05 (m, 2H); 7.61 (m, 2H); 7.36 (m, 8H); 7.15 (m, 2H); 7.09 (m, 1H); 6.97 (m, 3H); 5.05 (s, 2H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 143.9; 140.4; 140.4; 138.1; 138.0; 136.4; 132.9; 131.9; 131.0; 130.1; 129.8; 129.2; 129.2; 127.0; 124.6; 120.7; 119.2; 117.9; 115.5; 115.4; 115.0; 114.9; 114.8; 73.7. IR (ATR-ZnSe) in cm^{-1}: 746 1474. HRMS: M+1, 335.0952 ($C_{20}H_{16}N_2OCl$; calc'd 335.0951). ΔT_m = 18.2°C. MTT IC_{50} = 17.4±0.2 μM.

***O*-(3-chlorobenzyl)-*N*-(9'-acridinyl)hydroxylamine (6h).** Yellow solid. Yield 46%, ^{1}H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.37 (s, 1H); 9.00 (m, 1H); 8.08 (m, 1H); 7.40 (m, 6H); 7.10 (m, 2H); 6.98 (m, 2H); 5.32 (s, 2H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 143.8; 141.5; 140.4; 140.4; 138.1; 138.0; 133.6; 131.8; 131.0; 130.0; 129.8; 127.8; 127.5; 126.3; 124.6; 120.7; 119.2; 117.9; 117.9; 115.5; 115.4; 115.04; 115.0; 114.8; 114.8; 75.6. IR (ATR-ZnSe) in cm^{-1}: 747, 1474. HRMS: M+1, 335.0948 ($C_{20}H_{16}N_2OCl$; calc'd 335.0951). ΔT_m = 18.5°C. MTT IC_{50} = 18.0±0.2 μM

***O*-(4-chlorobenzyl)-*N*-(9'-acridinyl)hydroxylamine (6i).** Yellow-orange solid. Yield 30%, ^{1}H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.34 (s, 1H); 8.98 (m, 1H); 8.06 (m, 1H); 7.54 (m, 2H); 7.40 (m, 4H); 7.14 (m, 1H); 7.08 (m, 1H); 6.97 (m, 2H); 5.30 (s, 2H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 143.7; 140.4; 140.4; 138.1; 138.0; 137.8; 132.8; 131.8; 131.0; 129.8; 129.8; 128.3; 124.6; 120.7; 119.2; 118.0; 118.0; 115.4; 115.4; 115.0; 114.9;

114.8; 114.8; 75.6. IR (ATR-ZnSe) in cm^{-1}: 747; 964; 1473; 1489; 1598. HRMS: M+1, 335.0951 ($C_{20}H_{16}N_2OCl$; calc'd 335.0951). ΔT_m = 20.2°C. MTT IC_{50} = 17.0±0.4 µM.

***O*-(4-bromobenzyl)-*N*-(9'-acridinyl)hydroxylamine (6j).** Yellow solid. Yield 51%, ^1H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.34 (s, 1H); 8.97 (d, J 8.4 Hz, 1H); 8.06 (d, J 8.0 Hz, 1H); 7.57 (m, 2H); 7.48 (m, 2H); 7.37 (m, 2H); 7.14 (m, 1H); 7.08 (m, 1H); 6.96 (m, 2H); 5.28 (s, 2H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 143.7; 140.4; 140.4; 138.3; 138.1; 138.0; 131.8; 131.3; 131.0; 130.1; 129.8; 124.6; 121.0; 120.7; 119.2; 118.0; 117.9; 115.4; 115.4; 115.0; 114.9; 114.8; 114.8; 75.7 ppm. IR (ATR ZnSe) in cm^{-1}: 747; 964; 1008; 1473; 1486. HRMS: M+1, 379.0421 ($C_{20}H_{16}N_2OBr$; calc'd 379.0446). ΔT_m = 18.1°C. MTT IC_{50} = 18.5±4.3 µM.

***O*-(3-nitrobenzyl)-*N*-(9'-acridinyl)hydroxylamine (6k).** Yellow-orange solid. Yield 41%, ^1H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.40 (s, 1H); 9.01 (s, 1H); 8.39 (s, 1H); 8.18 (m, 1H); 8.05 (m, 1H); 7.95 (m, 1H); 7.69 (m, 1H); 7.39 (m, 2H); 7.16 (m, 1H); 7.10 (m, 1H); 6.98 (m, 2H); 5.46 (s, 2H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 148.4; 144.2; 141.5; 140.5; 138.1; 134.1; 131.8; 131.1; 129.9; 129.6; 124.6; 122.5; 122.3; 120.8; 119.3; 117.8; 117.8; 115.5; 115.5; 115.1; 115.0; 114.7; 114.7; 75.1. IR (ATR ZnSe) in cm^{-1}: 963; 1346; 1473; 1524; 1615. HRMS: M+1, 346.1194 ($C_{20}H_{16}N_3O_3$; calc'd 346.1192). ΔT_m = 15.1°C. MTT IC_{50} = 31.8±0.1 µM.

***O*-(4-nitrobenzyl)-*N*-(9'-acridinyl)hydroxylamine (6l).** Yellow solid. Yield 38%, ^1H NMR (400 MHz, acetone-d_6) δ, in ppm: 9.43 (s, 1H); 9.01 (m, 1H); 8.24 (m, 2H); 8.03 (m, 1H); 7.74 (m, 2H); 7.41 (m, 1H); 7.34 (m, 1H); 7.17 (m, 1H); 7.10 (m, 1H); 6.98 (m, 2H); 5.44 (s, 2H). ^{13}C NMR (100 MHz, acetone-d_6) δ, in ppm: 147.4; 146.9; 144.2; 140.5; 140.4; 138.1; 138.0; 131.8; 131.1; 129.9; 128.4; 123.4; 120.8; 119.3; 117.7; 117.7; 115.6; 115.5; 115.1; 115.0; 114.7; 114.6; 75.1. IR (ATR ZnSe) in cm^{-1}: 748; 1342; 1474; 1518. HRMS: M+1, 346.1172 ($C_{20}H_{16}N_3O_3$; calc'd 346.1192). MTT IC_{50} = 30.3±1.4 µM.

MTT Assay General Procedure

Growth inhibition was determined by the MTT colorimetric assay. Glioblastoma cells (SNB-19) were plated in 96-well plates at a density of 10 000 cells/mL and allowed to attach overnight (16-18h). Acridine solutions were prepared containing less than 2% DMSO and applied in medium for 24 h, removed, replaced with fresh medium, and the plates were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 for 4 days. MTT (50 µg) was added and the cells were incubated for another 4 h. Medium/MTT solutions were removed carefully by aspiration. The MTT formazan crystals were then dissolved in 100 µL of DMSO, and the absorbance of the resulting solution was determined on a plate reader at 560nm. IC_{50} values (concentration at which cell survival equals 50% of control) were determined from semilog plots of percent of control versus concentration.

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Supplementart Data

Supplementary data associated with this article can be found in the online version.

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