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Article

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Activity of 2-Aryl-2-(3-indolyl)acetohydroxamates Against Drug-Resistant Cancer Cells

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Abstract: Many types of tumor, including glioma, melanoma, non-small cell lung, esophageal, head and neck cancer, among others, are intrinsically resistant to apoptosis induction and poorly responsive to current therapies with proapoptotic agents. In addition, tumors often develop multi-drug resistance based on the cellular efflux of chemotherapeutic agents. Thus, novel anticancer agents capable of overcoming these intrinsic or developed tumor resistance mechanisms are urgently needed. We describe a series of 2-aryl-2-(3-indolyl)acetohydroxamic acids, which are active against apoptosis- and multidrug-resistant cancer cells as well as glioblastoma neurosphere stem-like cell cultures derived from patients. Thus, the described compounds serve as a novel chemical scaffold for the development of potentially highly effective clinical cancer drugs.

Introduction

Apoptosis-resistant cancers represent a major challenge in the clinic as most of the currently available chemotherapeutic agents work through the induction of apoptosis and, therefore, provide limited therapeutic benefits for the patients affected by these malignancies.[1,2] Cancers with such intrinsic resistance to proapoptotic stimuli include the tumors of the lung, liver, stomach, esophagus, pancreas as well as melanomas and gliomas.[3] For example, patients afflicted by a type of gliomas, known as glioblastoma multiforme,[4,5] have a median survival expectancy of less than 14 months when treated with a standard protocol of surgical resection, radiotherapy and chemotherapy with temozolomide, carmustine or cisplatin.[6] Because glioma cells display resistance to apoptosis, they respond poorly to such conventional chemotherapy with proapoptotic agents.[5,7]

Resistance to apoptosis is also an intrinsic property of tumor metastases. Successful treatment of metastases remains an important clinical challenge as 90% of cancer patients die from metastatic cancer spread.[8] By acquiring resistance to anoikis, a cell death process resulting from the loss of contact with extracellular matrix or neighboring cells,[8] metastatic cells display poor sensitivity to apoptosis induction and are thus poorly responsive to conventional proapoptotic chemotherapeutic agents.[5,9,10] One solution to apoptosis resistance entails the complementation of cytotoxic therapeutic regimens with cytostatic agents and thus a search for novel cytostatic anticancer drugs that can overcome cancer cell resistance to apoptosis is an important pursuit.[12-15]

Often, tumors are initially susceptible to cancer agents and patients respond to chemotherapy but eventually experience a relapse in spite of the continuing treatment. In such instances of acquired resistance tumors generally become refractory to a broad spectrum of structurally and mechanistically diverse antitumor agents and this phenomenon is referred to as multidrug resistance (MDR).[16,17] MDR usually results from upregulation of certain protein pumps, such as P-glycoprotein (P-gp) in cancer

cells, causing a decreased intracellular drug concentration. MDR is a major factor that contributes to the failure of chemotherapy, for example with such widely used anticancer drugs as the vinca alkaloids[18] or the taxanes.[19]

Our recent studies of a reaction of indole derivatives with β-nitrostyrenes in polyphosphoric acid (PPA)[20] led to the discovery of an efficient synthesis of 2-aryl-2-(3-indolyl)acetohydroxamates. Although 2,2-diarylacetohydroxamates had been previously synthesized and studied as HDAC inhibitors,[21,22] compounds in which one of the two aromatic rings is an indole moiety had not been reported in the literature. Thus, 2-aryl-2-(3-indolyl)acetohydroxamate was revealed to be a new chemotype prompting our thorough investigation of biological properties of compounds incorporating this structural feature. Although HDAC inhibition was not observed with these compounds (data not shown), these studies led to the discovery of significant activity associated with a number of synthesized compounds against cancer cell lines displaying resistance to various types of proapoptotic stimuli as well as glioblastoma neurosphere stem-like cell cultures derived from patients. It was also found that the active analogues exhibited their antiproliferative activity through a cytostatic non-apoptotic mechanism of action and maintained their potency against multi-drug resistant cells, which are poorly responsive to important clinical cancer drugs taxol and vinblastine. Although the detailed mechanistic studies aimed at the elucidation of mode(s) of action of the 2-aryl-2-(3-indolyl)acetohydroxamates are currently pursued in our labs, the compelling evidence for the effectiveness of these compounds against the apoptosis- and multidrug resistant cancer cells prompts us to disclose our findings in the present paper.

Results and Discussion

Chemistry

2-Aryl-2-(3-indolyl)acetohydroxamates (**3**, Figure 1) were identified to be intermediates in our recently discovered transannulation of indoles to 2-quinolones carried out by reacting 2-substituted indoles with β-nitrostyrenes in PPA at 100 ºC.[20] It was found that if the reaction temperature kept at 70 ºC, compounds **3** could be isolated as the main reaction products (Figure 1A, Tables 1 and 2). The reaction scope was found to allow for the introduction of a variety of substituents R^1, R^2, R^3 and R^4 into the 2-aryl-2-(3-indolyl)acetohydroxamate scaffold **3**. In addition, the recognition of limited access to a number of specific substituted indoles that would be required for systematic structure-activity relationship (SAR) analyses prompted the development of an alternative route based on an *in situ* Fisher indole synthesis utilizing arylhydrazines **4** and ketones **5** (Figure 1B). In this multicomponent variation, compounds **4** and **5** are reacted at 100 ºC to allow for the indole formation and then the reaction temperature is lowered to 70 ºC prior to the introduction of β-nitrostyrenes **2**. Thus, the availability of two complementary approaches to compounds **3** permits the synthesis of analogues with the desired identity and positioning of substituents R^1, R^2, R^3 and R^4 on the 2-aryl-2-(3-indolyl)acetohydroxamate scaffold facilitating the development of these compounds as medicinal agents. Since the synthesized compounds have four diversification points, a four-dimensional tagging system is employed for labeling the products. Thus, the reaction of hydrazine **4aa** with ketone **5f** produces indole **1aaf**, which in the subsequent reaction with nitrostyrene **2n** affords hydroxamic acid **3aafn**.

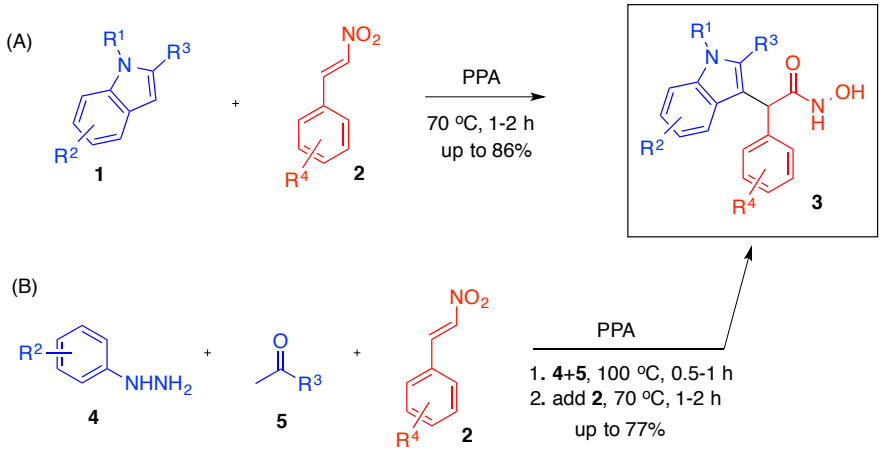


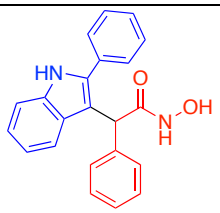
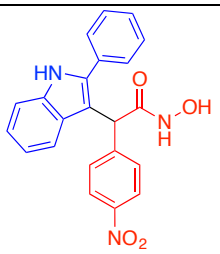
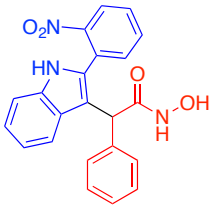
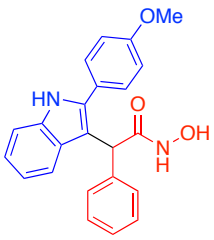
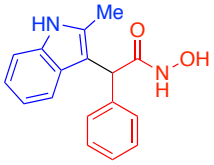
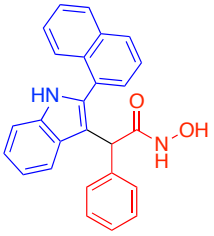
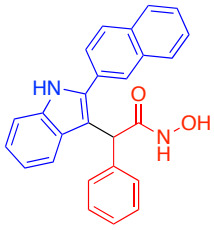
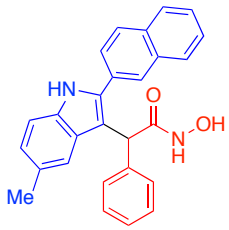
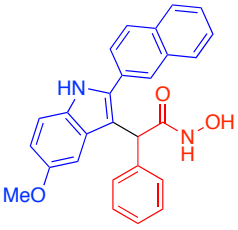
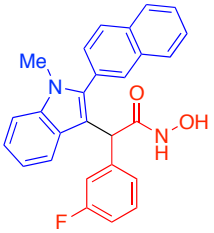
Figure 1. Two synthetic approaches toward 2-aryl-2-(3-indolyl)acetohydroxamates **3**

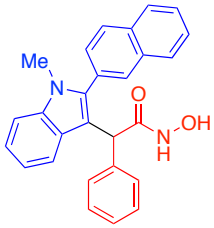
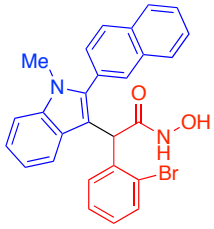
Pharmacology

(a) SAR analyses

The evaluation of an initially synthesized series of compounds **3** for a variety of activities led to the identification of double-digit micromolar antiproliferative potencies associated with the parent acetohydroxamate **3aaaa** (Table 1). This finding led to an exploration of the SAR analyses by synthesizing the first generation compounds **3** containing diverse substituents at different positions in the 2-aryl-2-(3-indolyl)acetohydroxamate skeleton and testing this series for *in vitro* growth inhibition using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay[23] against two cell lines, human HeLa cervical and MCF-7 breast adenocarcinomas (Table 1). It emerged from these experiments that the substitution on the benzene ring of the indole moiety ($R^2 \neq H$) was not tolerated (e.g., **3abfa** and **3acfa**), whereas the nitrogen could be derivatized ($R^1 \neq H$) with only a small activity drop (e.g., **3bafa** vs **3aafa**). The key SAR finding resulted from the variations of the C2-position of the indole moiety ($R^3 \neq$ Ph as in **3aaba**, **3aaca**, **3aada**, **3aaea** and **3aafa**) and identification of single-digit micromolar potencies associated with compounds containing the β-naphthyl substituent at this position (as in **3aafa**).

Table 1. Structures, synthetic yields (methods A or B) and antiproliferative activities of the first generation compounds **3**

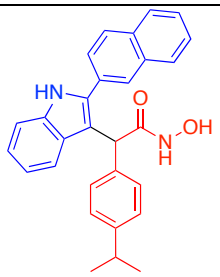
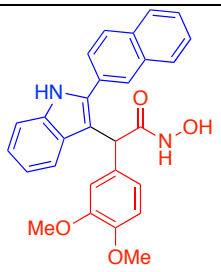
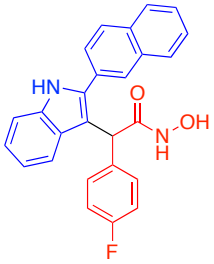
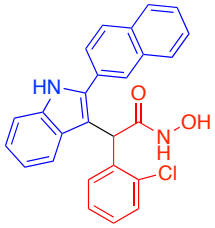
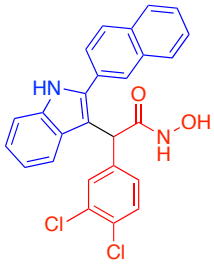
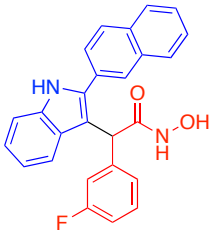
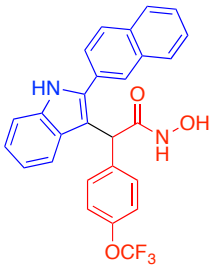
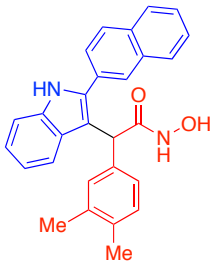
# logP	structure	% yield (method)	cell viability GI_{50},[a] μM HeLa	MCF7	# logP	structure	% yield (method)	cell viability GI_{50},[a] μM HeLa	MCF7
3aaaa 4.1		82 (A) 76 (B)	23.0 ± 2.6	31.0 ± 0.6	**3aaab** 4.1		73 (A) 68 (B)	>50	>50
3aaba 4.1		68 (A) 61 (B)	>50	>50	**3aaca** 4.1		43 (A) 35 (B)	>50	>50
3aada 2.7		46 (A) 27 (B)	>50	>50	**3aaea** 5.2		76 (A) 70 (B)	25.7 ± 1.6	32.0 ± 0.9
3aafa 4.1		85 (A) 73 (B)	3.6 ± 0.5	3.4 ± 0.3	**3abfa** 5.7		79 (A) 73 (B)	>50	>50
3acfa 5.2		28 (B)	>50	>50	**3bafc** 5.5		54 (A)	36.8 ± 0.4	25.7 ± 1.4

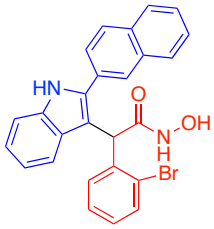
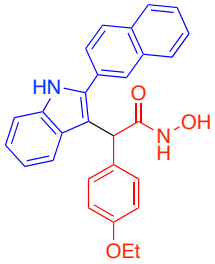
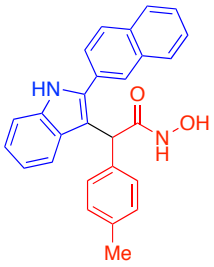
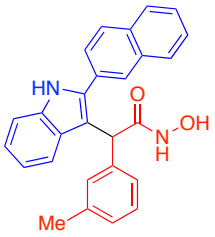
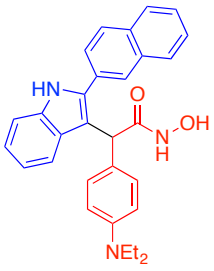
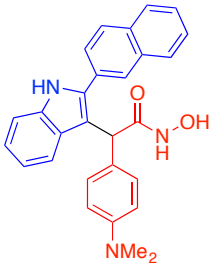
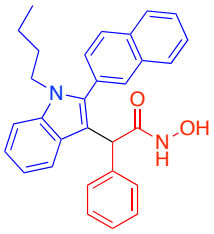
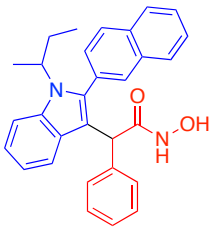
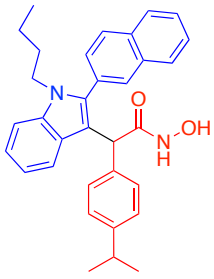
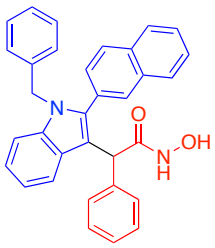
3bafa		75 (A)	19.7 ± 1.7	10.0 ± 0.3	**3bafd**		36 (A)	24.0 ± 0.2	11.2 ± 0.9
5.4					6.2				

[a] Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control ± SD from two independent experiments, each performed in 4 replicates, as determined by the MTT assay.

Based on the initial SAR in Table 1, the second generation compounds **3** were synthesized and they all contained an R^2 = β-naphthyl, while R^1 and R^4 remained variable. These experiments led to the identification of a number of compounds possessing single-digit micromolar (e.g., **3aafe**, **3aafk**, **3aafm**, **3aafn**, **3aafo** and **3aafp**) or even submicromolar (e.g., **3aafe** and **3aafp**) activites, all containing meta and/or para-positioned R^4. The addition of an R^1 = alkyl (e.g., **3cafa**, **3dafa**, **3cafe** and **3eafa**) did not appear to be detrimental with GI_{50} values still in the single-digit micromolar region. Because of the significant lipophilicities associated with our acetohydroxamates and thus the possibility that the activities were a function of their lipophilic character, logP values were calculated for each analogue using three different methods, all giving similar results (Tables 1 and 2). The significant activity was indeed present among both less lipophilic analogues (e.g., **3aafc** with logP = 4.1) and those with higher lipophilicity (e.g., **3cafe** with logP = 8.4), thus ruling out such a possibility.

Table 2. Structures, synthetic yields (methods A or B) and antiproliferative activities of second generation compounds **3**

#	structure	% yield	cell viability[a]		#	structure	% yield	cell viability[a]	
logP		(method)	GI_{50}, µM		logP		(method)	GI_{50}, µM	
			HeLa	MCF7				HeLa	MCF7
3aafe		73 (A)	0.68	1.4	**3aaff**		60 (A)	26.9	2.4
6.7		61 (B)	±0.04	± 0.1	4.9		56 (B)	± 0.3	± 0.1
3aafg		76 (A)	18.2	6.3	**3aafh**		84 (A)	31.0	4.9
5.4		64 (B)	± 0.9	± 1.1	5.9		72 (B)	± 0.2	± 0.1
3aafi		45 (A)	31.7	12.2	**3aafc**		75 (A)	32.9	20.4
6.5		43 (B)	± 1.7	± 1.0	4.1		64 (B)	± 1.1	± 0.1
3aafj		56 (A)	27.4	10.2	**3aafk**		70 (A)	2.7	2.5
6.3		52 (B)	± 0.2	± 0.1	6.1		59 (B)	± 0.1	± 0.1

3aafd		57 (A)	13.0	2.7	**3aafl**		81 (A)	17.3	8.5
6.2		55 (B)	± 0.4	± 0.0	5.6		72 (B)	± 0.2	± 0.3
3aafm		80 (A)	6.3	4.9	**3aafn**		76 (A)	6.9	7.4
5.7		72 (B)	± 1.3	± 0.2	5.7		69 (B)	± 0.4	± 0.6
3aafo		53 (A)	4.1	7.9	**3aafp**		45 (A)	0.60	1.1
6.0		50 (B)	± 0.2	± 0.7	5.3		43 (B)	±0.02	± 0.0
3cafa		83 (A)	5.6	7.8	**3dafa**		80 (A)	9.8	9.3
6.8			± 0.3	± 0.6	6.7			± 0.8	± 0.3
3cafe		68 (A)	6.6	7.7	**3eafa**		75 (A)	5.4	5.5
8.4			± 0.5	± 0.4	7.0			± 0.3	± 0.2

[a] Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control ± SD from two independent experiments, each performed in 4 replicates, as determined by the MTT assay.

Finally, to assess the importance of the hydroxamic acid moiety, **3aafa** was converted to nitrile **6** by treating the former with PCl_3 and further to amide **7** by partial hydration of **6** in 80% PPA (Figure 2). The evaluation of nitrile **6** and amide **7** for antiproliferative activity revealed a 6- and 3-fold lower potencies associated with these compounds as compared with hydroxamate **3aafa**, thus underscoring the importance of the hydroxamic acid moiety but not its criticality.

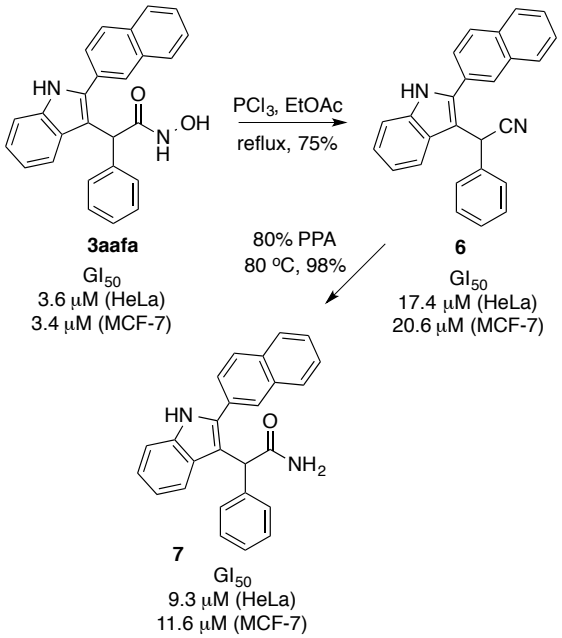


Figure 2. Synthesis of non-hydroxamate analogues of **3**

(b) Activity against cells exhibiting various types of resistance to proapoptotic stimuli

As part of the ongoing efforts in our lab aimed at identification of compounds active against cancer cell displaying resistance to proapoptotic agents,[24-27] the selected 2-aryl-2-(3-indolyl)-acetohydroxamates were evaluated for *in vitro* growth inhibition against a panel of additional cancer cell lines including those resistant to various proapoptotic stimuli, such as human T98G and U87 glioblastoma[28,29] and human A549 non-small-cell lung cancer (NSCLC),[30] as well as an apoptosis-sensitive tumor model, such as human Hs683 anaplastic oligodendroglioma,[28] used as reference. The

obtained GI_{50} values associated with potent hydroxamates are shown in Table 3. The data reveal that for the most part these compounds retain the single-digit antiproliferatve GI_{50} values in this challenging cancer cell panel. Further analysis of the results from Tables 2 and 3 combined shows that the hydroxamates do not discriminate between the cancer cell lines based on the apoptosis sensitivity criterion and display comparable potencies in both cell types, indicating that apoptosis induction is not the primary mechanism responsible for antiproliferative activity in this series of compounds.

Table 3. Antiproliferative properties of potent hydroxamates against cancer cell lines displaying apoptosis resistance and representing cancers with dismal prognoses

compound	GI_{50} *in vitro* values (μM)[a]			
	glioma			lung carcinoma
	Hs683	U87	T98G	A549
3aafa	8.9 ± 0.4	9.5 ± 0.3	36.4 ± 1.9	2.8 ± 0.4
3aafe	6.1 ± 1.0	5.0 ± 0.5	8.8 ± 0.5	3.3 ± 0.6
3aafk	4.7 ± 1.0	6.7 ± 1.5	7.5 ± 0.8	2.9 ± 0.6
3cafa	10.8 ± 0.5	6.7 ± 0.3	12.3 ± 0.8	5.7 ± 0.5
3eafa	11.2 ± 0.9	9.1 ± 0.3	10.6 ± 0.4	5.8 ± 0.8
3aafp	5.1 ± 0.5	21.3 ± 1.6	1.9 ± 0.2	1.5 ± 0.3

[a] Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control ± SD from two independent experiments, each performed in 4 replicates, as determined by the MTT assay.

Our previous experience of working with cells resistant to various proapoptotic stimuli shows that generally a certain population of cells becomes rapidly eliminated with proapoptotic agents used at low concentrations leading to low GI_{50} values. However, these high potencies can be somewhat misleading as there often remains a significant portion of cells that resists the effects of the proapoptotic agents

even at concentrations 100- or 1000-fold of their GI_{50}s.[31] It was thus instructive to compare the hydroxamates with common proapoptotic agents for their ability to affect such resistant populations. Indeed, as can be seen in Figure 3, hydroxamates **3aafa** and **3aafp** have potent growth inhibitory properties against most of the cells in U87 and A549 cultures and, with increasing concentration, rapidly reach antiproliferative levels of a non-discriminate cytotoxic agent phenyl arsine oxide (PAO). In contrast, common proapoptotic agents taxol and podophyllotoxin have no effect on proliferation of ca. 50% of cells in these cultures at concentrations up to 100 μM.

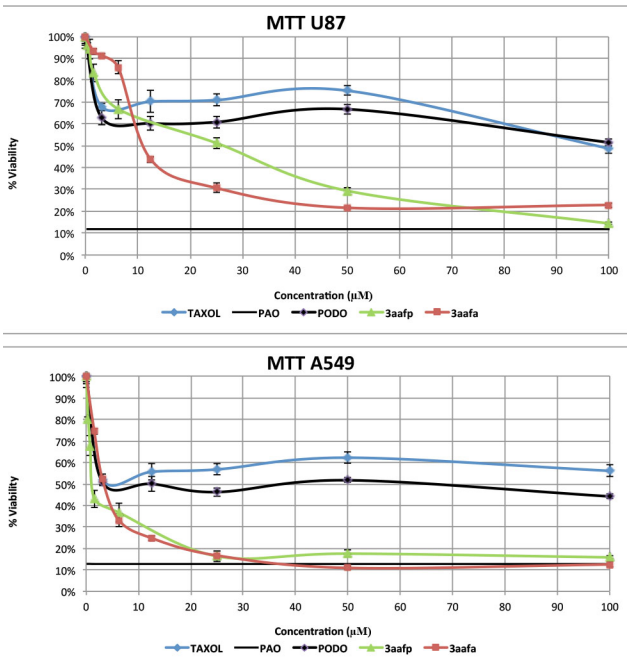


Figure 3. Activity of **3aafp** and **3aafa** against cell populations resistant to proapoptotic agents.

(c) Quantitative videomicroscopy

To obtain insight into the effectiveness of 2-aryl-2-(3-indolyl)acetohydroxamates against apoptosis-resistant cancers, computer-assisted phase-contrast microscopy[12,13,15] (quantitative videomicroscopy) was employed to observe the phenotypic morphological changes in cancer cells as they are treated with these compounds. Figure 4 shows that acetohydroxamate **3aafa** inhibits cancer cell proliferation

without inducing cell death when assayed at concentrations slightly exceeding the GI_{50} values (25 μM) in SKMEL-28 melanoma and U373 glioblastoma cells, both exhibiting resistance to various proapoptotic stimuli.[28,32] Based on the phase contrast pictures obtained by means of quantitative videomicroscopy, a global growth ratio (GGR) was calculated, which corresponds to the ratio of the mean number of cells present in a given image captured in the experiment (in this case after 24, 48 and 72 h) to the number of cells present in the first image (at 0 h). The ratio obtained in the **3aafa**-treated experiment was then divided by the ratio obtained in the control. The GGR value of ca. 0.3 in both of these two cell lines indicates that 30% of cells grew in the **3aafa**-treated experiment as compared to the control over a 72 h observation period. Thus, the GGR calculations are consistent with the MTT colorimetric data and indicate that it is the cytostatic properties associated with the hydroxamates that are responsible for their antiproliferative effects against apoptosis-resistant cancer cells at least at relevant concentrations (slightly above the GI_{50} values).

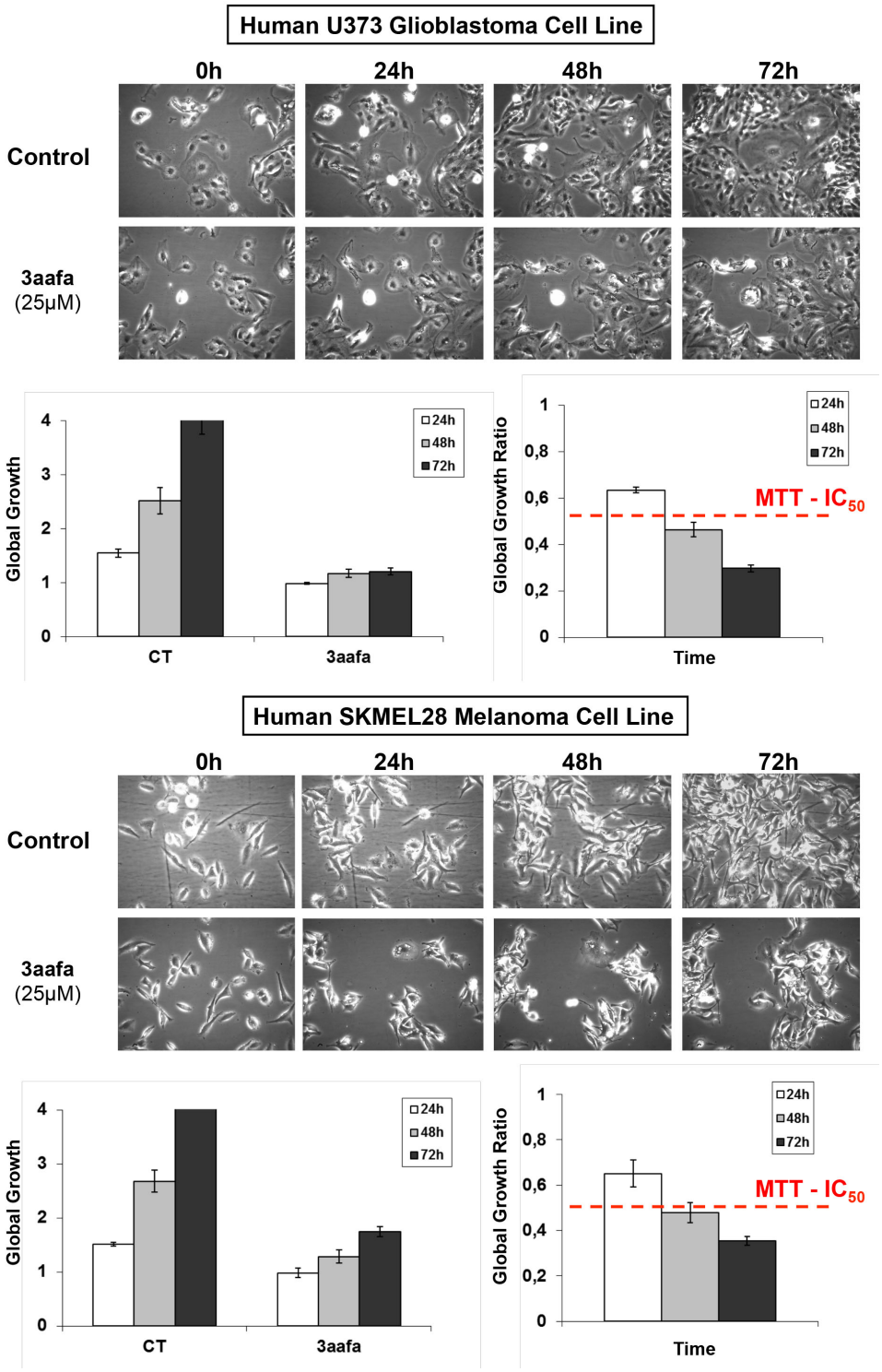


Figure 4. Cellular imaging of **3aafa** against SKMEL-28 melanoma and U373 glioblastoma cells illustrating the cytostatic antiproliferative mechanism.

(d) Redifferentiation of U87 cells to an astrocytic phenotype

To elucidate the fate of the cells whose growth has been arrested with the hydroxamates, the phenotypic morphological changes of U87 glioma cells were observed for a period of several weeks after the treatment with hydroxamate **3aafa** at the GI_{50} concentration. Interestingly, while untreated cells proliferated rapidly and quickly formed spheroids (Figure 5B), the treated cells ceased to replicate and appeared to undergo redifferentiation to a non-malignant state resembling a reactive astrocyte (data not shown) phenotype (Figure 5C). Although such redifferentiation processes are known, there are only a few small molecule agents reported to induce these epigenetic transformations.[33,34] The literature reports indicate that these redifferentiated cells possess significantly reduced tumorigenicity *in vivo*[33] and, thus, new chemical entities capable of triggering such phenotypic changes hold a promising but completely unexplored potential as anticancer agents.

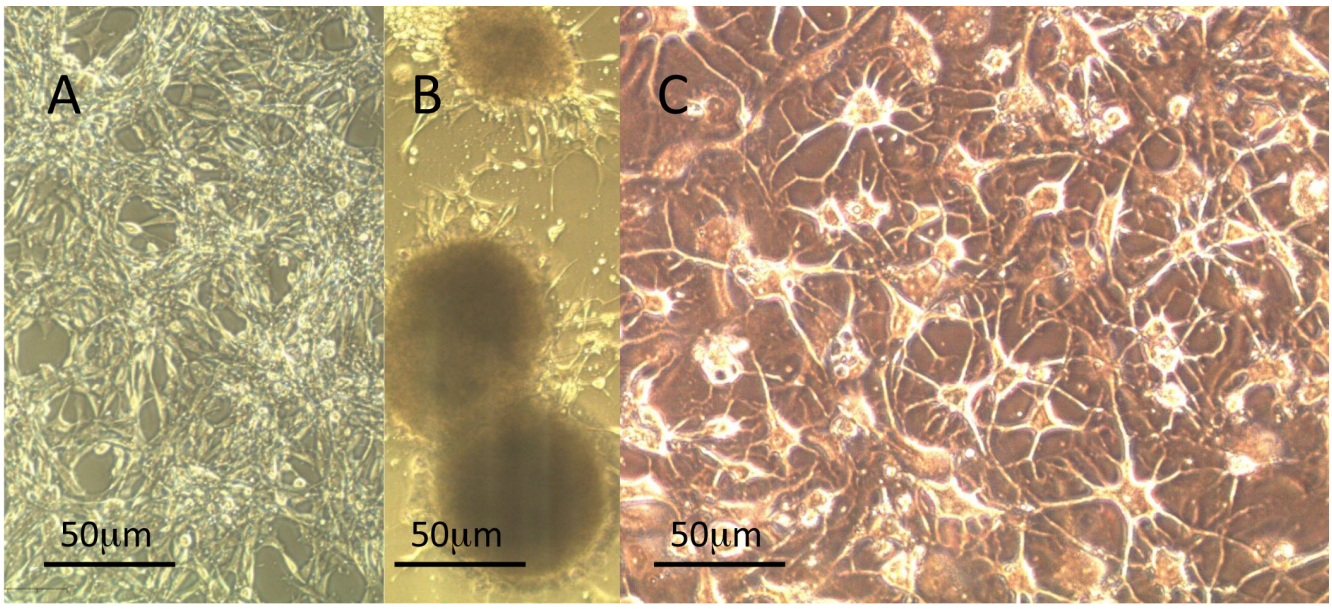


Figure 5. Redifferentiation of growth-inhibited malignant U87 cells to an astrocytic phenotype. (A) Three day old glioblastoma cancer cells. (B) Untreated, these grow into mini-tumors during the following three days. (C) After a 33-day treatment with 7 μM **3aafa**.

(e) Activity against MDR cells, glioblastoma neurosphere stem-like cell cultures derived from patients and normal fibroblasts.

Compared with the intrinsic drug resistance, as described above for such as cancers as glioblastoma and melanoma, a large variety of tumors can also develop resistance to anticancer drugs resulting in MDR as explained in the introduction. To assess whether the hydroxamates can overcome this resistance mechanism, selected hydroxamates were tested against MDR cells (Table 4). The MDR uterine sarcoma cell line MES-SA/Dx5 was utilized for this experiment. This cell line was established from the parent uterine sarcoma MES-SA, grown in the presence of increasing concentrations of doxorubicin and is known to be resistant to a number of P-gp substrates.[35] Both taxol and vinblastine displayed more than a thousand fold drop in potency when tested for antiproliferative activity against the MDR cell line as compared with the parent line (Table 4). In contrast, there was only a small variation in the sensitivities of the two cell lines towards the hydroxamates indicating their potential to overcome clinical multi-drug resistance.

Given the ability of the hydroxamates to overcome drug resistance a few select compounds were further evaluated against glioma cells grown in neurosphere conditions, which are known to promote the growth of stem-like cells from human glioma tissue. Indeed, the neurospheres show the ability of self-renewal by regrowing in culture from individual cells, can differentiate into multiple neural lineages and recapitulate human gliomas on both histological and genetic levels more faithfully than serum cultured glioma cell lines when injected into the brains of mice.[36-39] Because, neurosphere cells are generally resistant to radiation and chemotherapy,[40-43] the micromolar to submicromolar activity of of the hydroxamates against the glioma neurosphere cell cultures is noteworthy (Table 4). The glioma culture 031810 used is derived from a patient with glioblastoma who progressed on temozolomide due to high O^6-methylguanine-DNA-methyltransferase (MGMT) expression and thus shows high

resistance to this agent (Table 4). It is worthy of note, that the unmethylated MGMT promoter leading to such temozolomide resistance is found in about half of all GBM patients, who respond poorly to temozolomide chemotherapy.[44] To date, no alternative treatment exists for this group of patients.[44]

Table 4. Antiproliferative effect of selected compounds against MDR cells and patient-derived GBM neurosphere cells

compound	GI_{50} *in vitro* values (μM)		
	MES-SA[a]	MES-SA/Dx5[a]	GBM 031810[b]
Taxol	0.007 ± 0.001	9.8 ± 0.3	
Vinblastine	0.006 ± 0.001	5.0 ± 1.4	
Temozolomide			> 1000
3aafa	2.0 ± 0.2	4.0 ± 1.1	0.8 ± 0.6
3aafp	0.8 ± 0.1	1.6 ± 0.6	5.6 ± 0.8
3aafe	1.7 ± 0.4	4.9 ± 1.9	3.4 ± 0.7
3aafk	1.8 ± 0.4	2.2 ± 0.8	
3cafa	5.9 ± 1.7	2.7 ± 0.3	
3eafa	7.1 ± 0.1	8.5 ± 0.9	

[a] Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control ± SD from two independent experiments, each performed in 4 replicates, as determined by the MTT assay. [b] Average GI_{50} ± SD from three GI_{50} determinations.

Finally, selected hydroxamates were tested against the normal human dermal (NHDF) and lung (NHLF) fibroblast cell lines in comparison with the cancerous glioma and NSCLC cells (Figure 6). The compounds displayed a modest but noteworthy selectivity in inhibiting the growth of cancer cells with **3aafa** and **3aafp** being particularly ineffective at inhibiting the proliferation of the normal NHDF

cell line (Figure 6). These results show that the selectivity of the hydroxamates toward cancer cells is structure-dependent and can be optimized to select the best candidates for the forthcoming *in vivo* tests in animal models.

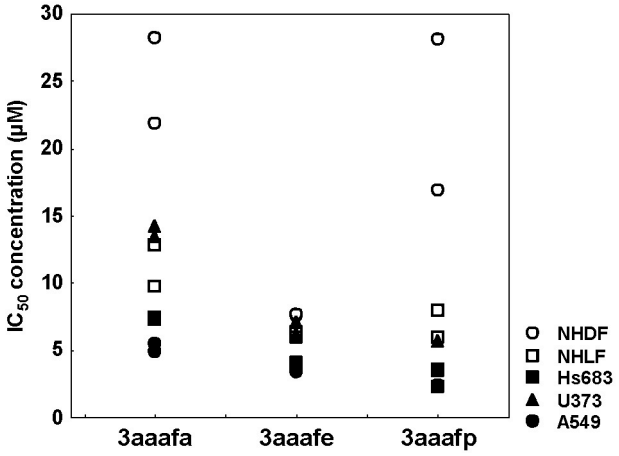


Figure 6. Activity of selected analogues toward non-cancerous and cancerous cell lines. The results were obtained using two independent experiments (both shown in Figure) in sextuplicates. Non-cancerous fibroblast cell lines are presented with open symbols, while cancer cell lines are presented with filled symbols.

Conclusion

Drug resistance is one of the main causes for the failure of cancer chemotherapy, affecting patients with a broad variety of tumors. Resistance to chemotherapy can be intrinsic, in which cancers such as glioma, melanoma or NSCLC, among others, fail to respond to the first chemotherapy given. Resistance can also be acquired, in which tumors innately respond to chemotherapy but eventually become refractory to a broad spectrum of structurally and mechanistically diverse antitumor agents. The results presented herein demonstrate the potential 2-aryl-2-(3-indolyl)acetohydroxamates for the treatment drug-resistant cancer, regardless of whether the latter harbors intrinsic and acquired resistance mechanisms. The structural scaffold associated with these compounds represents a new

chemotype, whose further investigation is warranted by the described findings and should be facilitated by the straightforward synthetic methodologies developed to accommodate systematic SAR studies as well as preparation of specific designed analogues. The ongoing work includes further optimization of compound potency, elucidation of mechanisms responsible for cytostatic and redifferentiation effects as well as experiments involving animal models of drug-resistant human cancer.

Experimental Section

General Experimental.

Reagents, solvents and catalysts were purchased from commercial sources (Acros Organics and Sigma-Aldrich) and used without purification. All reactions were performed in oven-dried flasks open to the atmosphere and monitored by thin layer chromatography on TLC precoated (250 μm) silica gel 60 F254 glass-backed plates (EMD Chemicals Inc.). Visualization was accomplished with UV light. Filtration was performed using silica gel (32-63 μm, 60 Å pore size). ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-400 and Bruker DRX-500 spectrometers. Chemical shifts (δ) are reported in ppm relative to the TMS internal standard. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Indoles: 2-phenyl-1*H*-indole (**1aaa**), 2-(2-nitrophenyl)-1*H*-indole (**3aab**), 2-(4-methoxyphenyl)-1*H*-indole (**3aac**), 2-methyl-1*H*-indole (**3aad**), 2-(naphthalen-1-yl)-1*H*-indole (**1aae**), 2-(naphthalen-2-yl)-1*H*-indole (**1aaf**), 1-methyl-2-(naphthalen-2-yl)-1*H*-indole (**1baf**), and 5-methoxy-2-(naphthalen-2-yl)-1*H*-indole (**1acf**) were purchased from commercial sources and used as received. Procedures for preparation of 5-methyl-2-(naphthalen-2-yl)-1*H*-indole (**1abf**), 1-butyl-2-(naphthalen-2-yl)-1*H*-indole (**1caf**), 1-(*sec*-butyl)-2-(naphthalen-2-yl)-1*H*-indole (**1daf**), 1-benzyl-2-(naphthalen-2-yl)-1*H*-indole (**1eaf**) are provided below. Ketones: acetophenone (**5a**), *o*-nitroacetophenone (**5b**), *p*-methoxyacetophenone (**5c**), acetone (**5d**), 1-acetylnaphalene (**5e**), and 2-

acetylnaphalene (**5f**) were obtained from commercial sources and used as received. Arylhydrazines: pehylhydrazine (**4aa**), *p*-tolylhydrazine (**4ab**), and *p*-anisylhydrazine (**4ac**) were obtained from commercial sources and used as received. Nitroalkenes: (2-nitrovinyl)benzene (**2a**), 1-nitro-4-(2-nitrovinyl)benzene (**2b**), 1-fluoro-3-(2-nitrovinyl)benzene (**2c**), 1-bromo-2-(2-nitrovinyl)benzene (**2d**), 1,2-dimethoxy-4-(2-nitrovinyl)benzene (**2f**), 1-chloro-2-(2-nitrovinyl)benzene (**2h**), 1,2-dichloro-4-(2-nitrovinyl)benzene (**2i**), 1-(2-nitrovinyl)-4-(trifluoromethoxy)benzene (**2j**), 1-methyl-4-(2-nitrovinyl)benzene (**2m**), *N*,*N*-dimethyl-4-(2-nitrovinyl)aniline (**2p**) were acquired from commercial sources and used as received. 1-Isopropyl-4-(2-nitrovinyl)benzene (**2e**), 1-fluoro-4-(2-nitrovinyl)benzene (**2g**), 1,2-dimethyl-4-(2-nitrovinyl)benzene (**2k**), 1-ethoxy-4-(2-nitrovinyl)benzene (**2l**), 1-methyl-3-(2-nitrovinyl)benzene (**2n**) were synthesized using a reported procedure,[45] as well as *N*,*N*-diethyl-4-(2-nitrovinyl)aniline (**2o**).[46] Elemental analyses were performed using a CHN-1 analyzer. HRMS analyses were performed on ESI Bruker Maxis. The synthesized compounds were at least 95% pure according to elemental analyses and/or HPLC chromatograms.

Compound 1abf: A mixture of 4-methylphenylhydrazine (**4ab**) (1.22 g, 10 mmol) and 2-acetylnaphthalene (**5f**) (1.70 g, 10 mmol) was vigorously stirred at 100-110 °C in 80% PPA (3-5 g) for 40 min. When the reaction was complete based on TLC analysis the mixture was cooled down to rt, poured into water (50 mL), and neutralized with aqueous ammonia. The formed precipitate was filtered, dried in vacuum, and used without additional purification. Yield 2.44 g (9.5 mmol, 95%); m.p. = 212-213 °C (toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.43 (br. s, 1H), 8.08 (s, 1H), 7.93-7.86 (m, 4H), 7.56-7.48 (m, 2H), 7.46 (s, 1H), 7.30-7.34 (m, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 6.9 (s. 1H), 2.49 (s. 3H); ^{13}C NMR (100 MHz , $CDCl_3$) *δ*: 133.7, 129.8, 128.9, 128.7, 128.4, 128.1, 127.9, 126.8,

126.7, 126.4, 126.3, 125.4, 124.9, 124.4, 123.9, 120.6, 118.2, 111.0, 21.6; HRMS calc`d for $C_{19}H_{16}N$ $(M+H)^+$: 258.1277, found 258.1276.

Compound 1caf: To a stirred solution of KOH (2.24 g, 40 mmol) in DMSO (20 mL was added 2-(2-naphthyl)indole (**1aaf**) (2.43 g, 10 mmol), and the mixture was stirred for 45 min. Then, *n*-butyl bromide (2.7 g, 20 mmol) was added and the stirring was continued for additional 45 min. The mixture was diluted with water (20 mL) and extracted with benzene (3 x 50 mL). Combined organic layers were washed with water (3 x 100 mL), dried with $CaCl_2$ and concentrated in vacuum to obtain the titled compound as yellowish oil. Yield 2.60 g (8.7 mmol, 87%); ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.99-7.92 (m, 4H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.65 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.58-7.55 (m, 2H), 7.45 (d, *J* = 8.2 Hz, 1H), 7. 28 (ddd, *J* = 7.4, 7.6, 1.1 Hz, 1H), 7.19 (ddd, *J* = 7.4, 7.4, 0.9 Hz, 1H), 6.65 (s, 1H), 4.25 (t, *J* = 7.5 Hz, 2H), 1.74 (m, 2H), 1.21 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz , $CDCl_3$) *δ*: 141.5, 137.7, 133.4, 132.9, 130.9, 128.5, 128.4, 128.3, 128.2, 127.9, 127.4, 126.6, 126.5, 121.7, 120.7, 119.9, 110.2, 102.7, 44.1, 32.3, 20.1, 13.8; HRMS calc`d for $C_{22}H_{22}N$ $(M+H)^+$: 300.1747, found 300.1749.

Compound 1daf: To a stirred solution of KOH (2.24 g, 40 mmol) in DMSO (20 mL was added 2-(2-naphthyl)indole (**1aaf**) (2.43 g, 10 mmol), and the mixture was stirred for 45 min. Then, *sec*-butyl bromide (2.7 g, 20 mmol) was added and the stirring was continued for additional 60 min. The mixture was idluted with water (20 mL) and extracted with benzene (3 x 50 mL). Combined organic layers were washed with water (3 x 100 mL), dried with $CaCl_2$ and concentrated in vacuum to obtain the titled compound as colorless solid. Yield 2.52 g (8.4 mmol, 84%); m.p. = 103-104 °C (petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.99-7.91 (m, 4H), 7.69, (d, *J* = 7.9 Hz, 1H), 7.63 (dd, *J* =

8.4, 1.5 Hz, 1H), 7.59-7.55 (m, 3H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.27 (ddd, *J* = 7.6, 7.5, 0.7 Hz, 1H) 7.18 (t, *J* = 7.3 Hz, 1H), 6.66 (s, 1H), 4.15-4.13 (m, 2H), 2.15-2.04 (m, 1H), 0.69-067 (m, 6H); ^{13}C NMR (100 MHz , $CDCl_3$) *δ*: 141.8, 138.0, 133.4, 132.9, 131.22, 128.7, 128.4, 128.3, 128.2, 127.9, 127.6, 126.6, 126.5, 121.6, 120.7, 119.9, 110.7, 103.0, 51.5, 29.1, 22.9, 20.2; HRMS calc`d for $C_{22}H_{22}N$ $(M+H)^+$: 300.1747, found 300.1750.

Compound 1eaf: To a stirred solution of KOH (2.24 g, 40 mmol) in DMSO (20 mL was added 2-(2-naphthyl)indole (**1aaf**) (2.43 g, 10 mmol), and the mixture was stirred for 45 min. Then, benzyl bromide (3.4 g, 20 mmol) was added and the stirring was continued for additional 45 min. The mixture was diluted with water (20 mL) and extracted with benzene (3 x 50 mL). Combined organic layers were washed with water (3 x 100 mL), dried with $CaCl_2$ and concentrated in vacuum to obtain the titled compound as colorless solid. Yield 3.07 g (9.2 mmol, 92%); m.p. = 144-146 °C (toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.89-7.84 (m, 3H), 7.76-7.70 (m, 2H), 7.57 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.51-7.49 (m, 2H), 7.32-7.24 (m, 4H), 7.20-7.17 (m, 2H), 7.08 (d, *J* = 6.8 Hz, 2H), 6.77 (s, 1H), 5.43 (s, 2H); ^{13}C NMR (100 MHz , $CDCl_3$) *δ*: 142.0, 138.4 (2C), 133.3, 133.0, 130.2, 128.9 (2C), 128.5, 128.4, 128.3 (2C), 127.8, 127.4, 127.2, 126.6, 126.5, 126.2 (2C), 122.2, 120.7, 120.4, 110.7, 102.9, 48.1; HRMS calc`d for $C_{25}H_{20}N$ $(M+H)^+$: 334.1590, found 334.1595.

Preparation of 2-aryl-2-(3-indolyl)acetohydroxamates 3. General Method A. A mixture of a selected indole **1** (1 mmol) and a selected nitrostyrene **2** (1.2 mmol) in 80% PPA (3-4 g) was stirred at 65-70 ºC for 1 h. The disappearance of the starting indole was monitored by TLC. After the indole has reacted completely, the mixture was cooled to rt, poured in water (50 mL) and treated with saturated NH_4OH to pH 8. The formed precipitate was filtered and recrystallized from the indicated solvent.

Preparation of 2-aryl-2-(3-indolyl)acetohydroxamates 3. General Method B. A mixture of a selected arylhydrazine **4** (1 mmol) and a selected methylaryl ketone **5** (1 mmol) in 80% PPA (2-3 g) was stirred at 100-110 ºC for 40 min. The disappearance of the starting arylhydrazine was monitored by TLC. After the arylhydrazine has reacted completely, the temperature was decreased to 65-70 ºC and a selected nitrostyrene **2** (1.2 mmol) was added. The mixture was stirred at this temperature for 1 h and the disappearance of the intermediate indole **1** was monitored by TLC. After the indole has reacted completely, the mixture was cooled to room temperature, poured in water (50 mL) and treated with saturated NH_4OH to pH 8. The formed precipitate was filtered and recrystallized from the indicated solvent.

Compound 3aaaa. Synthesized according to the general method A from 2-phenylindole (**3aaa**) and (2-nitrovinyl)benzene (**2a**) in 82% yield; Alternatively prepared according to the general method B starting from phenylhydrazine (**4aa**), acetophenone (**5a**) and (2-nitrovinyl)benzene (**2a**): 76%; m.p. = 220-221°C (toluene/petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ, ppm: 11.30 (br. s, 1H), 10.75 (br. s, 1H), 8.81 (br. s, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.54-7.48 (m, 4H), 7.41 (dd, *J* = 7.2, 7.1 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.27-7.16 (m, 5H), 7.06 (dd, *J* = 7.6, 7.4 Hz, 1H), 6.88 (t, *J* = 7.4 Hz, 1H), 5.10 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ, ppm: 168.6, 140.7, 136.2, 132.5, 128.6 (2C), 128.5 (2C), 128.0 (2C), 127.9 (2C), 127.7, 127.6, 126.1, 122.3, 121.1, 118.5, 110.9, 109.2, 46.0; EA: Calcd for $C_{22}H_{18}N_2O_2$: C 77.17, H 5.30, N 8.18. Found: C 77.33, H 5.22, N 8.11; HRMS calc`d for $C_{22}H_{18}N_2O_2Na$ $(M+Na)^+$: 365.1260, found 365.1272.

Compound 3aaab. According to the method A, starting from 2-phenyl-1*H*-indole (**3aaa**) and 1-nitro-4-(2-nitrovinyl)benzene (**2b**): 73%; According to the method B, starting from phenylhydrazine (**4aa**), acetophenone (**5a**) and 1-nitro-4-(2-nitrovinyl)benzene (**2b**): 68%; m.p. = 156-157 °C (toluene); ^{1}H

NMR (400 MHz, DMSO) δ, ppm: 11.44 (br. s, 1H), 10.89 (br. s, 1H), 8.96 (br. s, 1H), 8.14 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.49-7.46 (m, 4H), 7.42-7.36 (m, 5H), 7.09 (t, *J* = 7.2 Hz, 1H), 6.92 (t, *J* = 7.5 Hz,1H), 5.20 (s, 1H); ^{13}C NMR (100 MHz , DMSO) *δ*: 167.8, 148.6, 146.0, 136.7, 136.2, 132.2, 129.3 (2C), 128.7 (4C), 127.9, 127.4, 123.3 (2C), 121.6, 121.4, 118.9, 111.2, 108.0, 46.1; HRMS calc`d for $C_{22}H_{17}N_3O_4Na$ (M+Na)$^+$: 410.1111, found 410.1111.

Compound 3aaba. According to the method A, starting from 2-(2-nitrophenyl)-1*H*-indole (**3aab**) and (2-nitrovinyl)benzene (**2a**): 68%; According to the method B, starting from phenylhydrazine (**4aa**), 2-nitroacetophenone (**5b**) and (2-nitrovinyl)benzene (**2a**): 61%; m.p. = 118-119 °C (toluene); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.27 (br. s, 1H), 10.71 (br. s, 1H), 8.87 (br. s, 1H), 8.12 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.80-7.61 (m, 4H), 7.54 (d, *J* = 7.4 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.18-7.06 (m, 5H), 6.91 (t, *J* = 7.4 Hz, 1H), 4.77 (s, 1H); ^{13}C NMR (100 MHz , DMSO) *δ*: 166.4, 147.6, 139.9, 136.2, 134.2, 133.7, 133.1, 129.8, 127.8 (2C), 127.7 (3C), 127.0 (2C), 126.1, 124.5, 121.4, 118.5, 111.2, 110.9, 45.9; HRMS calc`d for $C_{22}H_{17}N_3O_4Na$ (M+Na)$^+$: 410.1111, found 410.1109.

Compound 3aaca. According to the method A, starting from 2-(4-methoxyphenyl)-1*H*-indole (**3aac**) and (2-nitrovinyl)benzene (**2a**): 43%; According to the method B, starting from phenylhydrazine (**4aa**), 4-methoxyacetophenone (**5c**) and (2-nitrovinyl)benzene (**2a**): 35%; m.p. = 133-134 °C (toluene); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.22 (br. s, 1H), 10.73 (br. s, 1H), 8.81 (br. s, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.27-7.17 (m, 5H), 7.06-7.01 (m, 3H), 7.86 (t, *J* = 7.5 Hz, 1H), 5.04 (s, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO) *δ*: 169.1, 159.4, 141.3, 136.7, 136.5, 130.4 (2C), 128.5 (2C), 128.4 (2C), 128.3, 126.6, 125.4, 122.6, 121.3, 118.9, 114.6 (2C), 111.2, 109.0, 55.7, 46.6; HRMS calc`d for $C_{23}H_{20}N_2O_3Na$ (M+Na)$^+$: 395.1373, found 395.1366.

Compound 3aada. According to the method A, starting from 2-methyl-1*H*-indole (**3aad**) and (2-nitrovinyl)benzene (**2a**): 46%; According to the method B, starting from phenylhydrazine (**4aa**), acetone (**5d**) and (2-nitrovinyl)benzene (**2a**): 27%; m.p. = 110-112 °C (toluene); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 10.86 (br. s, 1H), 10.79 (br. s, 1H), 8.86 (br. s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.37 (s, 1H), 7.26-7.20 (m, 5H), 6.94 (ddd, *J* = 7.4, 7.4, 0.6 Hz, 1H), 6.83 (t, *J* = 7.2 Hz, 1H), 4.93 (s, 1H), 2.30 (s, 3H); ^{13}C NMR (100 MHz , DMSO) *δ*: 168.7, 140.5, 135.1, 133.2, 128.1 (2C), 127.9 (2C), 127.7, 126.1, 119.9, 119.8, 118.0, 110.2, 108.5, 45.3, 11.9; HRMS calc`d for $C_{22}H_{17}N_3O_4Na$ $(M+Na)^+$: 303.1104, found 303.1103.

Compound 3aaea. According to the method A, starting from 2-(1-naphthyl)-1*H*-indole (**3aae**) and (2-nitrovinyl)benzene (**2a**): 76%; According to the method B, starting from phenylhydrazine (**4aa**), 1-acetylnaphthalene (**5e**) and (2-nitrovinyl)benzene (**2a**): 70%; m.p. = 110-112 °C (toluene); ^{1}H NMR (400 MHz, DMSO, 338K) δ, ppm: 11.28 (br. s, 1H), 10.41 (br. s, 1H), 8.63 (br. s, 1H), 8.03-7.99 (m, 2H), 7.79-7.50 (m, 5H), 7.40-7.33 (m, 2H), 7.26-7.07 (m, 6H), 6.93 (t, *J* = 7.4 Hz, 1H), 4.73 (s, 1H); ^{13}C NMR (100 MHz , DMSO) *δ*: 168.8, 140.3, 136.3, 134.9, 133.2, 132.5, 130.0, 129.2, 128.6, 128.5, 128.1, 127.9 (2C), 127.2, 126.4, 126.0 (2C), 125.7, 125.5, 125.2, 122.4, 121.1, 118.4, 111.4, 110.8, 46.2; HRMS calc`d for $C_{26}H_{20}N_2O_2Na$ $(M+Na)^+$: 415.1417, found 415.1417.

Compound 3aafa. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and (2-nitrovinyl)benzene (**2a**): 85%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and (2-nitrovinyl)benzene (**2a**): 73%; m.p. = 152-154 °C (toluene). ^{1}H NMR (500 MHz, DMSO) δ, ppm: 11.31 (br. s, 1H), 10.76 (br. s, 1H), 8.82 (br. s, 1H), 8.03-7.97 (m, 3H), 7.91 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.68 (dd, *J* = 8.5, *J* = 1.3 Hz, 1H), 7.58-7.56 (m, 2H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.29-7.17 (m, 5H), 7.09 (dd, *J* = 7.8, 7.4 Hz, 1H), 6.91 (dd, *J* = 7.8,

7.5 Hz, 1H), 5.19 (s, 1H); ^{13}C NMR (125 MHz, DMSO) δ: 168.5, 140.8, 136.4, 135.9, 132.8, 132.2, 130.0, 128.1, 128.0 (2C), 127.9, 127.8, 127.6, 127.5, 127.4 126.6, 126.4, 126.3, 126.2, 122.3, 121.3, 118.6, 110.9, 109.9, 99.9, 45.8; EA: Calcd for $C_{26}H_{20}N_2O_2$: C 79.57, H 5.14, N 7.14. Found: C 79.68, H 5.09, N 7.16; HRMS calc`d for $C_{26}H_{20}N_2O_2Na$ $(M+Na)^+$: 415.1417, found 415.1419.

Compound 3abfa. According to the method A, starting from 5-methyl-2-(2-naphthyl)-1H-indole (**3abf**) and (2-nitrovinyl)benzene (**2a**): 79%; According to the method B, starting from 4-tolyl-hydrazine (**4ab**), 2-acetylnaphthalene (**5f**) and (2-nitrovinyl)benzene (**2a**): 73%; m.p. = 133-135 °C (toluene); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.34 (br. s, 1H), 10.75 (br. s, 1H), 8.82 (br. s, 1H), 8.00-7.87 (m, 5H), 7.65 (dd, J = 8.65, 1.08 Hz, 1H), 7.60 (s, 1H), 7.57-7.54 (m, 2H), 7.29-7.17 (m, 6H), 7.92 (dd, J = 8.2, 0.9 Hz, 1H), 5.17 (s, 1H), 2.31 (s, 3H); ^{13}C NMR (100 MHz , DMSO) δ: 169.1, 141.4, 136.7, 135.3 (2C), 133.3, 132.7, 130.6, 128.6 (4C), 128.5, 128.4, 128.1, 127.9, 127.3, 127.1, 127.0, 126.8, 126.7, 123.5, 122.2, 111.2, 109.8, 46.7, 22.0; HRMS calc`d for $C_{27}H_{22}N_2O_2Na$ $(M+Na)^+$: 429.1573, found 439.1577.

Compound 3acfa. According to the method B, starting from (4-methoxyphenyl)hydrazine (**4ac**), 2-acetylnaphthalene (**5e**) and (2-nitrovinyl)benzene (**2a**): 28%; m.p. = 128-130 °C (toluene); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.31 (br. s, 1H), 10.80 (br. s, 1H), 8.85 (br. s, 1H), 8.01- 7.88 (m, 4H), 7.65 (d, J =8.59 Hz, 1H), 7.59-7.53 (m, 2H), 7.37 (s, 1H), 7.30-7.17 (m, 5H), 7.75 (dd, J = 8.7, 2.4 Hz, 1H), 5.16 (s, 1H), 3.64 (s, 3H); ^{13}C NMR (100 MHz , DMSO) δ: 168.6, 152.8, 140.8, 136.8, 132.8, 132.2, 131.7, 130.1, 128.4, 128.3, 128.1 (4C), 128.0, 127.6, 127.3, 126.6, 126.5, 126.3, 126.2, 111.5, 111.2, 109.6, 104.5, 55.2, 46.3; HRMS calc`d for $C_{27}H_{22}N_2O_3Na$ $(M+Na)^+$: 445.1523, found 445.1523

Compound 3bafc. According to the method A, starting from *N*-methyl-2-(2-naphthyl)-1H-indole (**3baf**) and 3-fluoro(2-nitrovinyl)benzene (**2c**): 54%; m.p. = 133-134 °C (toluene/petroleum ether); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 10.69 (br. s, 1H), 8.89 (br. s, 1H), 8.07-7.92 (m, 4H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.61 (m, 2H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.26-7.17 (m, 4H), 7.08 (d, *J* = 7.3 Hz, 1H), 7.0 (t, *J* = 7.3 Hz, 1H), 4.84 (s, 1H), 3.59 (s. 3H); ^{13}C NMR (100 MHz , DMSO) *δ*: 167.7, 159.8 (d, $^1J_{CF}$ = 247.2 Hz), 138.8, 136.9, 132.6, 132.5, 130.2, 129.9, 128.4 (d, $^3J_{CF}$ = 6.9 Hz), 128.2, 128.1, 127.9, 127.8, 127.6, 127.5, 126.7, 126.6, 126.5, 123.8, 121.4, 120.9, 119.2, 114.7 (d, $^2J_{CF}$ = 22.3 Hz), 109.8, 108.9, 40.4, 30.9; HRMS calc`d for $C_{27}H_{21}FN_2O_2Na$ $(M+Na)^+$: 447.1479, found 447.1493.

Compound 3bafa. According to the method A, starting from *N*-methyl-2-(2-naphthyl)-1H-indole (**3baf**) and (2-nitrovinyl)benzene (**2a**): 75%; m.p. = 114-115 °C (toluene/petroleum ether); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 10.66 (br. s, 1H), 8.84 (br. s, 1H), 8.03-7.91 (m, 4H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.62-7.60 (m, 2H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.48 (d, *J* = 8.1 Hz, 1H), 7.26-7.14 (m, 7H), 6.98 (dd, *J* = 7.8, 7.5 Hz, 1H), 4.85 (s, 1H), 3.60 (s, 3H); ^{13}C NMR (100 MHz , DMSO) *δ*: 168.5, 140.7, 138.8, 137.1, 132.6, 132.5, 130.0, 128.4, 128.2, 128.1, 127.9 (4C), 127.6, 126.7, 126.5 (2C), 126.1, 122.3, 121.3, 118.8, 110.8, 109.5, 99.5, 46.4, 30.8; EA: Calcd for $C_{27}H_{22}N_2O_2$: C 79.78, H 5.46, N 6.89. Found: C 80.03, H 5.39, N 6.81; HRMS calc`d for $C_{27}H_{22}N_2O_2Na$ $(M+Na)^+$: 429.2416, found 429.2418.

Compound 3bafd. According to the method A, starting from *N*-methyl-2-(2-naphthyl)-1H-indole (**3baf**) and 2-bromo(2-nitrovinyl)benzene (**2d**): 36%; m.p. = 109-113 °C (toluene/petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.91 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 7.00 Hz, 1H), 7.69-7.64 (m, 1H), 7.59-7.49 (m, 5H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 7.17-7.13 (m, 2H), 7.09-7.06 (m, 1H), 5.43 (s, 1H), 3.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 167.6, 139.8, 139.0, 137.0, 132.5, 132.4, 132.3, 130.9, 129.8, 128.4, 128.2, 128.1, 127.8, 127.6, 127.5,

127.2, 126.8, 126.6, 126.4, 123.9, 121.4, 120.2, 119.4, 110.0, 109.5, 47.1, 31.0; HRMS calc`d for $C_{27}H_{21}BrN_2O_2Na$ $(M+Na)^+$: 507.0679, found 507.0677.

Compound 3aafe. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 4-isopropyl(2-nitrovinyl)benzene (**2a**): 73%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 4-isopropyl(2-nitrovinyl)benzene (**2e**): 61%; m.p. = 147-148 °C (toluene/petroleum ether). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.44 (br. s, 1H), 10.76 (br. s, 1H), 8.82 (br. s, 1H), 8.02-7.9 (m, 4H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.66 (dd, *J* = 8.5, 1.59 Hz, 1H) 7.60-7.53 (m, 2H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.17-7.06 (m, 5H), 6.92 (ddd, *J* = 15.0, 7.5, 0.5 Hz, 1H), 5.16 (s, 1H), 2.86-2.76 (m, 1H), 1.14 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 168.8, 146.2, 138.1, 136.4, 136.0, 132.8, 132.2, 130.1, 128.1, 128.0 (2C), 127.9, 127.6, 127.5, 126.7, 126.5, 126.3, 125.9 (2C), 122.4, 121.3, 118.6, 111.0, 110.0, 45.9, 39.9, 33.0, 23.9 (2C); Calc`d for $C_{29}H_{26}N_2O_2$: C 80.16, H 6.03, N 6.45. Found: C 80.31, H 5.95, N 6.36; HRMS calc`d for $C_{29}H_{26}N_2O_2Na$ $(M+Na)^+$: 457.1884, found 457.1887.

Compound 3aaff. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 3,4-dimethoxy(2-nitrovinyl)benzene (**2f**): 60%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 3,4-dimethoxy(2-nitrovinyl)benzene (**2f**): 56%; m.p. = 143-144 °C (toluene); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.44 (br. s, 1H), 10.70 (br. s, 1H), 8.81 (br. s, 1H), 8.02-7.89 (m, 4H), 7.85 (d, *J* = 8.1 Hz, 1H), 7.67 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.59-7.54 (m, 2H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.09 (t, *J* = 7.2 Hz, 1H), 6.94 (t, *J* = 7.3 Hz, 1H), 6.85-6.83 (m, 2H), 7.76 (dd, *J* = 8.4, 1.5 Hz, 1H), 5.12 (s, 1H), 3.68 (s, 3H), 3.59 (s, 3H); ^{13}C NMR (100 MHz , DMSO) *δ*: 168.8, 148.3, 147.4, 136.4, 136.0, 133.1, 132.8, 132.2, 130.1, 128.1, 127.9, 127.8, 127.6, 127.5, 126.7, 126.5, 126.3,

122.2, 121.3, 120.4, 118.6, 112.4, 111.6, 111.0, 110.2, 55.5, 55.4, 45.9; HRMS calc`d for $C_{28}H_{24}N_2O_4Na$ $(M+Na)^+$: 475.1628, found 475.1635

Compound 3aafg. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 4-fluoro(2-nitrovinyl)benzene (**2g**): 76%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 4-fluoro(2-nitrovinyl)benzene (**2g**): 64%; m.p. = 138-139°C (toluene/petroleum ether); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.50 (br. s, 1H), 10.80 (br. s, 1H), 8.88 (br. s, 1H), 8.04-7.97 (m, 4H), 7.92 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.67 (dd, *J* = 8.5, *J* = 1.3 Hz, 1H), 7.59-7.55 (m, 2H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.27-7.23 (m, 2H), 7.13-7.08 (m, 3H), 6.93 (dd, *J* = 7.8, 7.5 Hz, 1H), 5.18 (s, 1H); ^{13}C NMR (100 MHz , DMSO) *δ*: 168.4, 160.7 (d, $^1J_{CF}$ = 242.5 Hz), 136.9 (d, $^4J_{CF}$ = 3.0 Hz), 136.4, 136.2, 132.8, 132.2, 130.0, 129.8 (d, $^3J_{CF}$ = 8.1 Hz, 2C), 128.1, 128.0, 127.7, 127.6, 127.5, 126.6, 126.5, 126.4, 122.0, 121.4, 118.8, 114.8 (d, $^2J_{CF}$ = 21.6 Hz, 2C), 111.0, 109.7, 45.5; EA: Calcd for $C_{26}H_{19}FN_2O_2$: C 76.08, H 4.67, N 6.83. Found: C 76.23, H 4.62, N 6.76; HRMS calc`d for $C_{26}H_{19}FN_2O_2Na$ $(M+Na)^+$: 432.1244, found 432.2432.

Compound 3aafh. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 2-chloro(2-nitrovinyl)benzene (**2h**): 84%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 2-chloro(2-nitrovinyl)benzene (**2h**): 72%; m.p. = 164-166 °C (toluene/petroleum ether). ^{1}H NMR (500 MHz, DMSO) δ, ppm: 11.59 (br. s, 1H), 10.67 (br. s, 1H), 8.80(br. s, 1H), 8.01-7.94 (m, 2H), 7.79 (d, *J* = 8.6 Hz, 2H), 7.60-7.54 (m, 4H), 7.45 (t, *J* = 8.2 Hz, 2H), 7.39(dd, *J* = 1.6, 5.5 Hz, 2H), 7.29-7.23 (m, 3H), 7.14 (t, *J* = 7.3 Hz, 1H), 6.85(t, *J* = 7.6 Hz, 1H), 5.46 (s, 1H); ^{13}C NMR (125 MHz , DMSO) *δ*: 167.5, 138.4, 136.3, 136.1, 133.0, 132.8, 132.2, 131.0, 129.9, 129.1, 128.4, 128.1, 128.0, 127.9, 127.6, 127.0, 126.6, 126.4, 126.2, 121.5, 120.8, 119.2, 111.3,

108.8, 99.5, 44.6; EA: Calcd for $C_{26}H_{19}ClN_2O_2$: C 73.15, H 4.49, N 6.56. Found: C 73.26, H 4.42, N 6.61; HRMS calc`d for $C_{26}H_{19}ClN_2O_2Na$ $(M+Na)^+$: 449.1027, found 449.1012.

Compound 3aafi. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 3,4-dichloro(2-nitrovinyl)benzene (**2i**): 45%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 3,4-dichloro(2-nitrovinyl)benzene (**2i**): 43%; m.p. = 144-150 °C (toluene/petroleum ether). ^{1}H NMR (500MHz, DMSO) δ, ppm: 11.58 (br. s, 1H), 10.85 (br. s, 1H), 8.97 (br. s, 1H), 8.01 (s, 1H), 7.98-7.91 (m, 2H), 7.73 (d, J = 8.1 Hz,1H), 7.65 (d, J = 8.3 Hz, 2H), 7.58-7.52 (m, 3H), 7.43-7.36 (m, 2H), 7.18-7.11 (m, 2H), 6.97 (t, J = 7.4 Hz, 1H), 5.21 (s, 1H); ^{13}C NMR (125 MHz , DMSO) δ: 167.8, 141.8, 136.6, 136.4, 132.8, 132.3, 130.7, 130.4, 129.9, 129.7, 129.0, 128.6, 128.3, 128.1, 127.7, 127.6, 127.5, 126.6 (2C), 126.5, 121.6, 121.6, 119.1, 111.3, 108.6, 45.5; EA: Calcd for $C_{26}H_{18}Cl_2N_2O_2$: C 67.69, H 3.93, N 6.07. Found: C 67.83, H 3.87, N 6.15; HRMS calc`d for $C_{26}H_{18}Cl_2N_2O_2Na$ $(M+Na)^+$: 483.0638, found 483.0643, 485.0662.

Compound 3aafc. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 3-fluoro(2-nitrovinyl)benzene (**2c**): 75%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 3-fluoro(2-nitrovinyl)benzene (**2c**): 64%; m.p. = 126-127 °C (toluene /petroleum ether). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.54 (br. s, 1H), 10.86 (br. s., 1H), 8.92 (br. s., 1H), 8.03-7.90 (m, 4H), 7.74 (d, J= 8.1 Hz, 1H), 7.65 (dd, J= 8.4, 1.7 Hz, 1H), 7.60-7.54 (m, 2H), 7.41-7.28 (m, 2H), 7.25 (dd, J= 5.1, 1.4 Hz, 2H), 7.16 (d, J= 7.5 Hz, 1H), 7.11 (ddd, J= 7.5, 7.5, 0.8 Hz, 1H), 6.95 (ddd, J= 11.4, 7.6, 0.6 Hz, 1H), 5.19 (s., 1H); ^{13}C NMR (100 MHz , DMSO) δ: 168.1, 162.0 ($^1J_{CF}$ = 242.0 Hz) 143.7 (d, $^3J_{CF}$ = 6.0 Hz), 136.4, 132.8, 132.3, 130.0, 129.9, 129.8, 128.0 (2C), 127.7, 127.6 (2C), 126.6, 126.5, 126.4, 124.2, 121.9, 121.4, 118.8, 114.7 (d, $^2J_{CF}$ = 21.8 Hz),

113.1 (d, $^{2}J_{CF}$ = 20.7 Hz), 111.1, 109.2, 46.0; HRMS calc`d for $C_{26}H_{19}FN_2O_2Na$ $(M+Na)^+$: 433.1323, found 433.1337.

Compound 3aafj. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 4-(trifluoromethoxy)(2-nitrovinyl)benzene (**2j**): 56%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 4-(trifluoromethoxy)(2-nitrovinyl)benzene (**2j**): 52%; m.p. = 136-137°C (toluene/petroleum ether).^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.52 (br. s., 1H), 10.83 (br. s., 1H), 8.9 (br. s., 1H), 8.02-7.90 (m, 4H), 7.75 (d, *J* = 8.08 Hz, 1H), 7.65 (dd, *J* = 9.2, 1.2 Hz, 1H), 7.59-7.54 (m, 2H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.33 (d, *J*= 8.8 Hz, 2H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.10 (ddd, *J* = 7.6, 7.5, 0.5 Hz, 1H), 6.94 (t, *J* = 7.4 Hz, 1H), 5.22 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 168.2, 146.7, 140.1, 136.4 (2C),, 136.3, 132.8, 132.2, 129.8 (2C), 128.0 (3C), 127.6 (2C), 126.6, 126.5, 126.4, 121.9, 121.4, 120.7 (2C), 120.0 (q, $^{1}J_{CF}$ = 255.5 Hz), 118.8, 111.1, 109.3, 45.6; HRMS calc`d for $C_{27}H_{19}F_3N_2O_2Na$ $(M+Na)^+$: 499.1240, found 499.1232

Compound 3aafk. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 3,4-dimethyl(2-nitrovinyl)benzene (**2k**): 70%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 3,4-dimethyl(2-nitrovinyl)benzene (**2k**): 59%; m.p. = 144-147 °C (toluene/petroleum ether). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.43 (br. s, 1H), 10.73 (br. s, 1H), 8.81 (br. s, 1H), 8.03-7.97 (m, 3H), 7.92-7.90 (m, 1H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.65 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.60-7.54 (m, 2H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.10-7.06 (m, 1H), 7.02-7.01 (m, 2H), 6.94-6.89 (m, 2H), 5.11 (s, 1H), 2.15 (s, 3H), 2.13 (s, 3H);); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 168.8, 138.1, 136.4, 136.0, 135.5, 133.9, 132.8, 132.2, 130.1, 129.1 (2C), 128.1, 128.0, 127.9, 127.6, 127.5, 126.7, 126.5, 126.3, 125.5, 122.4, 121.3, 118.5, 110.9, 110.1, 45.9, 19.6, 18.9; Calc`d for $C_{28}H_{24}N_2O_2$: C

79.98, H 5.75, N 6.66. Found: C 80.09, H 5.69, N 6.69; HRMS calc`d for $C_{28}H_{24}N_2O_2Na$ $(M+Na)^+$: 443.1730, found 443.1732.

Compound 3aafd. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 2-bromo(2-nitrovinyl)benzene (**2d**): 57%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 2-bromo(2-nitrovinyl)benzene (**2d**): 55%; m.p. = 134-135 °C (toluene/petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.45 (br. s, 1H), 7.88-7.86 (m, 3H), 7.71 (s, 1H), 7.59 (d, *J* = 7.7 Hz, 2H), 7.55-7.50 (m, 3H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.22-7.17 (m, 2H), 7. 13 (ddd, *J* = 7.9, 7.6, 0.1 Hz, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 5.65 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 169.8, 137.7, 137.6, 136.3, 133.5, 133.4, 133.1, 131.1, 129.4, 129.1, 129.0, 128.5, 128.0, 127.9, 127.8, 127.7, 126.9, 126.8, 125.6, 125.3, 123.0, 121.1, 120.3, 111.4, 108.0, 48.1; HRMS calc`d for $C_{24}H_{21}BrN_2O_2Na$ $(M+Na)^+$: 471.0679, found 471.0692.

Compound 3aafl. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 4-ethoxy(2-nitrovinyl)benzene (**2l**): 81%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 4-ethoxy(2-nitrovinyl)benzene (**2l**): 72%; m.p. = 157-161 °C (toluene/petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.36 (br. s, 1H), 8.35 (br. s, 1H),7.86-7.77 (m, 4H), 7.55-7.49 (m, 4H), 7.39 (t, *J* = 3.9 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 2H), 7.16 (dd, *J* = 7.4, 7.6 Hz, 1H), 7.04 (dd, *J* = 7.5, 7.6 Hz, 1H), 6.81 (d, *J* = 8.6 Hz, 2H), 5.29 (s, 1H), 3.99 (q, *J* = 7.0 Hz, 2H), 1.40 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz , $CDCl_3$) δ, ppm: 170.8, 158.3, 137.1, 136.3 (2C), 133.4, 133.0, 130.0 (2C) 129.4, 128.9, 128.3, 127.9 (2C) 127.6, 126.9, 126.8, 126.0, 122.9, 120.8, 120.7, 114.9 (2C), 111.3, 109.7, 63.6, 47.0, 15.0; EA: Calcd for $C_{28}H_{24}N_2O_3$: C 77.04, H 5.54, N 6.42. Found: C 77.23, H 5.48, N 6.32; HRMS calc`d for $C_{28}H_{24}N_2O_3Na$ $(M+Na)^+$: 459.1679, found 459.1673.

Compound 3aafm. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 4-methyl(2-nitrovinyl)benzene (**2m**): 80%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 4-methyl(2-nitrovinyl)benzene (**2m**): 72%; m.p. = 135-140 °C (toluene/petroleum ether). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.46 (br. s, 1H), 10.79 (br. s, 1H), 8.85 (br. s, 1H), 8.03-7.90 (m, 4H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.67 (dd, *J* = 8.6, 1.4 Hz, 1H), 7.59-7.54 (m, 2H), 7.39-7.36 (m, 2H), 7.29-7.17 (m, 3H), 7.08 (ddd, *J* = 7.5, 7.5, 1.0 Hz, 1H), 6.9 (ddd, *J* = 7.5, 7.5, 0.8 Hz,1H), 5.19 (s, 1H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 168.6, 140.8, 136.4, 136.1, 132.8, 132.2, 130.0 (2C), 128.1 (2C), 128.0 (3C), 127.9, 127.6, 127.5, 126.6, 126.5, 126.3, 126.2, 122.3, 121.3, 118.6, 111.0, 109.9, 46.2, 35.8; Calc`d for $C_{27}H_{22}N_2O_2$: C 79.78, H 5.46, N 6.89. Found: C 79.91, H 5.40, N 6.94; HRMS calc`d for $C_{27}H_{22}N_2O_2Na$ $(M+Na)^+$: 429.1573, found 429.1703.

Compound 3aafn. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 3-methyl(2-nitrovinyl)benzene (**2n**): 76%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 3-methyl(2-nitrovinyl)benzene (**2n**): 69%; m.p. = 155-156 °C (toluene/petroleum ether). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.45 (br. s, 1H), 10.76 (br. s, 1H), 8.83 (br. s, 1H), 8.02-7.89 (m, 4H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.66 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.59-7.54 (m, 2H), 7.39-7.37 (m, 2H), 7.30-7.06 (m, 3H), 7.03-6.98 (m, 1H), 6.93-6.89 (m, 1H), 5.15 (s, 1H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 168.6, 140.7, 137.0, 136.4, 136.1, 132.8, 132.2, 130.1, 128.6, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 126.9, 126.7, 126.5, 126.4, 125.2, 122.3, 121.3, 118.6, 111.0, 109.9, 108.3, 46.2; Calc`d for $C_{27}H_{22}N_2O_2$: C 79.78, H 5.46, N 6.89. Found: C 79.91, H 5.39, N 6.96; HRMS calc`d for $C_{27}H_{22}N_2O_2Na$ $(M+Na)^+$: 429.1573, found 429.1569.

Compound 3aafo. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 4-(*N*,*N*-diethylamino)(2-nitrovinyl)benzene (**2o**): 53%; According to the method B, starting from phenyl-

hydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 4-(*N*,*N*-diethylamino)(2-nitrovinyl)benzene (**2o**): 50%; m.p. = 168-170 °C (chloroform). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.40 (br. s, 1H), 10.65 (br. s, 1H), 8.76 (br. s, 1H), 8.01 (d, *J* = 9.6 Hz, 2H), 7.98-7.90 (m, 2H), 7.85 (d, *J* = 8.1 Hz, 1H), 7.67 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.59-7.53 (m, 2H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.07 (ddd, *J* = 7.6, 7.1, 1.1 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 2H), 6.91 (ddd, *J* = 7.3, 7.1, 0.9 Hz, 1H), 6.55 (d, *J* = 8.9 Hz, 2H), 5.05 (s, 1H), 3.26 (q, *J* = 7.0 Hz, 4H), 1.03 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 169.3, 145.9, 136.4, 135.7, 132.9, 132.2, 130.3, 128.9 (2C), 128.1, 128.0 (2C), 127.6, 127.5, 127.1, 126.7, 126.5, 126.3, 122.7, 121.3, 118.5, 111.3 (2C), 110.9, 110.8, 45.4, 43.6 (2C), 12.4 (2C); Calc`d for $C_{30}H_{29}N_3O_2$: C 77.73, H 6.31, N 9.06. Found: C 77.85, H 6.27, N 8.99; HRMS calc`d for $C_{30}H_{29}N_3O_2Na$ $(M+Na)^+$: 486.2152, found 486.2159.

Compound 3aafp. According to the method A, starting from 2-(2-naphthyl)-1*H*-indole (**3aaf**) and 4-(*N*,*N*-dimethylamino)(2-nitrovinyl)benzene (**2p**): 45%; According to the method B, starting from phenylhydrazine (**4aa**), 2-acetylnaphthalene (**5f**) and 4-(*N*,*N*-diethylamino)(2-nitrovinyl)benzene (**2p**): 43%; m.p. = 168-167 °C (toluene/petroleum ether). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.40 (br. s, 1H), 10.67 (br. s, 1H), 7.77 (br. s, 1H), 8.03-7.90 (m, 4H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.67 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.60-7.53 (m, 2H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.09-7.04 (m, 3H), 6.9 (ddd, *J* = 8.1, 7.1, 1.0 Hz, 1H), 6.63 (dt, *J* = 8.9, 2.4 Hz, 2H), 5.07 (s, 1H), 2.82 (s, 6H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 169.2, 149.0, 136.4, 135.8, 132.9, 132.2, 130.2, 128.6 (2C), 128.3, 128.2, 128.1, 128.0, 127.6, 127.4, 126.7, 126.5, 126.3, 122.7, 121.3, 118.5, 112.2 (2C), 110.9, 110.7, 45.4, 40.25 (2C); Calc`d for $C_{28}H_{25}N_3O_2$: C 77.22, H 5.79, N 9.65. Found: C 77.39, H 5.71, N 9.59; HRMS calc`d for $C_{28}H_{25}N_3O_2Na$ $(M+Na)^+$: 458.1839, found 458.1846.

Compound 3cafa. According to the method A, starting from *N*-butyl-2-(2-naphthyl)-1*H*-indole (**3caf**) and (2-nitrovinyl)benzene (**2a**): 83%; m.p. = 110-112 °C (carbon tetrachloride). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 10.63 (br. s, 1H), 8.83 (br. s, 1H), 8.06-7.97 (m, 4H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.63-7.59 (m, 2H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.23-7.12 (m, 7H), 6.95 (t, *J* = 7.5 Hz, 1H), 4.77 (s, 1H), 4.05 (m, 2H), 1.49 (m, 2H), 1.02 (q, *J* = 7.37 Hz, 2H), 0.62 (t, *J* = 7.29 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 168.5, 140.7, 138.6, 136.3, 132.7, 132.6, 128.7, 128.0, 127.9 (4C), 127.8 (2C), 127.7, 126.7 (2C), 126.5 (2C), 126.12, 122.5, 121.3, 118.8, 111.1, 109.0, 46.3, 43.0, 31.5, 19.2, 13.4 ; HRMS calc`d for $C_{30}H_{28}N_2O_2Na$ $(M+Na)^+$: 471.2043, found 417.2054.

Compound 3dafa. According to the method A, starting from *N*-(*sec*-butyl)-2-(2-naphthyl)-1*H*-indole (**3daf**) and (2-nitrovinyl)benzene (**2a**): 80%; m.p. = 131-133 °C (carbon tetrachloride). ^{1}H NMR (400 MHz, DMSO) *δ*, ppm: 10.64 (br. s, 1H), 8.83 (br. s, 1H), 8.09-7.88 (m, 4H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.62-7.56 (m, 2H), 7.51 (d, *J* = 8.3 Hz, 1H), 7.53-7.11 (m, 7H), 6.94 (t, *J* = 7.6 Hz, 1H), 4.77 (s, 1H), 3.99-3.90 (m, 2H), 1.88-1.87 (m, 1H),0.56-0.54 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 168.6, 140.7, 138.8, 136.7, 132.7, 132.5, 128.8, 128.0, 127.9 (4C), 127.8 (2C), 127.7, 126.7, 126.6, 126.5, 126.1, 122.4, 121.2 (2C), 118.7, 111.2, 110.3, 50.5, 46.3, 28.4, 19.8 (2C); HRMS calc`d for $C_{30}H_{28}N_2O_2Na$ $(M+Na)^+$: 471.2043, found 417.2048.

Compound 3cafe. According to the method A, starting from *N*-butyl-2-(2-naphthyl)-1*H*-indole (**3caf**) and 4-isopropyl(2-nitrovinyl)benzene (**2e**): 68%; m.p. = 132-134 °C (carbon tetrachloride). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 10.60 (br. s, 1H), 8.05-7.94 (m, 4H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.62-7.58 (m, 2H), 7.48 (d, *J* = 8.2 Hz, 2H), 7.14 (t, *J* = 7.3 Hz, 1H), 7.08-7.02 (m, 4H), 6.96 (t, *J* = 7.5 Hz, 1H), 4.74 (s, 1H), 4.04-4.03 (m, 2H), 2.82-2.74 (m, 1H), 1.52-1.45 (m, 2H), 1.12 (d, *J* = 6.9 Hz, 6H), 1.02 (q, *J* = 7.38 Hz, 2H), 0.62 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 168.7, 146.0,

138.5, 138.0, 136.3, 132.7, 132.5, 130.0, 128.7, 128.3, 128.1, 128.0, 127.8 (2C), 127.7, 126.7 (2C), 126.5, 125.8 (2C), 122.5, 121.2, 118.7, 111.3, 109.8, 46.0, 43.0, 32.9, 31.5, 23.8 (2C), 19.2, 13.4; HRMS calc`d for $C_{33}H_{34}N_2O_2Na$ $(M+Na)^+$: 513.2512, found 513.2521.

Compound 3eafa. According to the method A, starting from *N*-benzyl-2-(2-naphthyl)-1*H*-indole (**3eaf**) and (2-nitrovinyl)benzene (**2a**): 75%; m.p. = 118-120 °C (carbon tetrachloride). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 10.68 (br.s, 1H), 8.86 (br. s, 1H), 7.99-7.87 (m, 4H), 7.77 (d, 1H), 7.43-7.34 (m, 3H), 7.26-7.15 (m, 8H), 7.08 (t, 1H), 6.95 (t, 1H), 6.85 (d, 1H), 5.32 (m, 2H), 4.83 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 168.5, 140.6, 138.9, 138.2, 136.6, 132.6 (2C), 128.4 (2C), 128.0 (4C), 127.8 (2C), 127.7, 127.0, 126.9, 126.8, 126.6, 126.2, 126.0 (3C), 122.6, 121.6, 119.2, 111.7, 110.3, 46.8, 46.4; HRMS calc`d for $C_{33}H_{26}N_2O_2Na$ $(M+Na)^+$: 505.1883, found 505.1886.

Synthesis of compound 6. A solution of **3aaaa** (390 mg, 0.99 mmol) and PCl_3 (140 mg, 1.02 mmol) in EtOAc is refluxed for 2 h. After the reaction mixture is allowed to cool down to rt, it is washed with $NaHCO_3$ (15 mL) and water (2x15 mL). The solvent is then removed on the rotary evaporator and the residue is recrystallized from toluene to afford 266 mg (0.74 mmol, 75%) of nitrile **6**. m.p. = 146-147 °C (toluene); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.85 (br.s, 1H), 8.09-7.94 (m, 4H), 7.7 (dd, *J* = 8.5, 1.8 Hz, 1H) 7.61-7.57 (m, 2H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.39-7.29 (m, 5H), 7.19 (ddd, *J* = 8.1, 7.1, 1.07 Hz, 1H), 7.05 (ddd, *J* = 8.0, 7.1, 0.9 Hz, 1H), 6.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 136.6, 136.4, 136.3, 132.8, 132.5, 129.0 (2C), 128.8, 128.6, 128.2, 127.7, 127.5, 126.8 (5C), 126.4, 126.1, 122.3, 120.0, 119.8, 118.8, 111.9, 105.0, 32.7; HRMS calc`d for $C_{26}H_{18}N_2Na$ $(M+Na)^+$: 381.1362, found 381.1362.

Synthesis of compound 7. A solution of nitrile **6** (360 mg, 1.00 mmol) is stirred in 80% PPA (3 g) for 1 h at 80 ºC. The reaction mixture is then allowed to cool down to rt, poured in water (15 mL) and neutralized with NH_4OH. The obtained precipitate is collected by filtration and recrystallized from EtOAc to yield 369 mg (0.98 mmol, 98%) of amide **7**. m.p. = 333-335 °C (EtOAc); ^{1}H NMR (400 MHz, DMSO) δ, ppm: 11.49 (br. s., 1H), 8.03-7.87 (m, 4H), 7.69 (dd, *J* = 8.6, 1.3 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.63-7.59 (m, 2H), 7.41-7.36 (m, 2H), 7.29-7.16 (m, 5H), 7.09 (t, *J* = 7.8 Hz, 1H), 6.91 (t, 7.3 Hz, 1H), 5.28 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ, ppm: 173.6, 141.2, 136.5, 136.1, 132.8, 132.2, 130.1, 128.5 (2C), 128.1 (3C), 127.9, 127.7, 127.4 (3C), 126.6, 126.4, 126.2, 121.5, 121.4, 118.8, 111.2, 110.4, 49.1; HRMS calc`d for $C_{26}H_{20}N_2ONa$ $(M+Na)^+$: 399.1468, found 399.1478.

Cell culture

Human cancer cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), the European Collection of Cell Culture (ECACC, Salisbury, UK) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). Human cervical adenocarcinoma HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). Human mammary carcinoma MCF-7 cells were cultured in RPMI supplemented with 10% FBS. The U87 cells (ATCC HTB-14) were cultured in DMEM culture medium, while the A549 cells (DSMZ ACC107) were cultured in RPMI culture medium supplemented with 10% heat-inactivated FBS. The glioblastoma multiforme Hs683 (ATCC HTB-138) and the T98G (ATCC CRL-1690) cell lines were cultivated in DMEM supplemented with 10% FBS. The Human uterine sarcoma MES-SA and MES-SA/Dx5 cells were cultured in RPMI-1640 medium supplemented with 10% FBS with MES SA/Dx5 maintained in the presence of 500 nM Doxorubicin (Sigma). SKMEL-28 cells (ATCC HTB72) and U373 glioblastoma cells (ECACC 08061901) were cultured in RPMI culture medium supplemented with 10% heat-inactivated FBS. Cell culture media were supplemented with 4

mM glutamine (Lonza code BE17-605E), 100 μg/mL gentamicin (Lonza code 17-5182), and penicillin-streptomycin (200 units/ml and 200 μg/ml) (Lonza code 17-602E). Neurosphere culture GBM 031810 was established using known methods[47] and maintained in Neurobasal medium (Invitrogen Carlsbad, CA) with B27 supplement (20ul/ml; Invitrogen), Glutamax (10ul/ml; Invitrogen), fibroblast growth factor-2 (20 ng/ml; Peprotech, Rocky Hill, NJ, USA), epidermal growth factor (20 ng/ml; Peprotech), heparin (32 IE/ml; Sigma Aldrich, St. Louis, MO), and penicillin-streptomycin (1X, Invitrogen). Growth factors and heparin were renewed twice weekly. NHDF (code CC-2509) and NHLF (code CC-2512) cells lines were purchased from Lonza and were cultivated in FGM™-2 BulletKit™ culture medium (Lonza). All cell lines were cultured in T25 flasks, maintained and grown at 37° C, 95% humidity, 5% CO_2.

Antiproliferative Properties

Antiproliferative properties of the synthesized compounds were evaluated by MTT assay was used. All compounds were dissolved in DMSO at a concentration of either 100 mM or 50 mM prior to cell treatment. The cells were trypsinized and seeded at 4 x 10^3 cells per well into 96-well plates. The cells were grown for 24 h, treated with compounds at concentrations ranging from 0.001 to 100 μM and incubated for 48 h in 200 μL media. 20 μL of MTT reagent in serum free medium (5 mg/mL) was added to each well and incubated further for 2 h. Media was removed and the resulting formazan crystals were re-solubilized in 200 μL of DMSO. A_{490} was measured using a Molecular Devices Thermomax plate reader. The experiments were performed in quadruplicate and repeated at least twice for each compound per cell line. Cells treated with 0.1% DMSO were used as a negative control; 1 μM phenyl arsine oxide (PAO) was used as a positive control.

Selection of Doxorubicin Resistant Cells

Selection of Doxorubicin Resistant Cells. Selection of the MES-SA/Dx5 cell line was done according to Harker et al.[48] The cells were split and allowed to adhere overnight. The next day cells were initially exposed to a DOX concentration of 100 nM, which represented the GI_{50} concentration. The cells were maintained at this DOX concentration until their growth rate reached that of the untreated cells. The DOX concentration was then increased in two-fold increments following the same growth criteria at each concentration to a final DOX concentration of 500 nM. Each new DOX concentration required approximately 2 passages to reach the growth rate of the untreated cells.

Quantitative videomicroscopy

The effects of **3aafa** on the viability of human U373 glioblastoma and SKMEL melanoma cells

were characterized in vitro using computer-assisted phase contrast video microscopy, as described elsewhere.[49]

Redifferentiation of malignant U87 cells to an astrocytic phenotype.

U87 cells were plated at a density of 5 x 10^4 cells per well in 24-well plate in DMEM supplemented with 10% FBS. The following day, the cells in each well were re-fed with 1 mL of fresh DMEM/10% FBS, and treated with **3aafa** to a final concentration between 15 and 5 μM. Cells were placed into the CO_2 incubator and media not replaced for the duration of the experiment.

LogP calculations

The log P values were determined theoretically using three different programs and the data was then used to find the mean log P and standard deviation. These programs included ChemAxon's Marvin sketch[50,51] the Molinspiration software[52] and VCCLAB's ALOGPS software.[53,54]

Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Abbreviations Used. ATCC, American Type Culture Collection; DAPI, 4',6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DSMZ, Deutsche Sammlung von Mikroorganismen and Zellkulturen; DOX, doxorubicin; ECACC, European Collection of Cell Culture; ESI, electrospray ionization; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; GGR, global growth ratio; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; MDR, multidrug resistance; MGMT, O^6-methylguanine-DNA-methyltransferase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NSCLC, non-small-cell lung cancer; PAO, phenyl arsine oxide; NMR, nuclear magnetic resonance; P-gp, P-glycoprotein; SAR, structure-activity relationship; PODO, podophyllotoxin; PPA, polyphosphoric acid; SD, standard deviation; TLC, thin layer chromatography; TMS, tetramethylsilane.

References

1. Kaufmann, S. H.; Earnshaw, W. C. Induction of Apoptosis by Cancer Chemotherapy. *Exp. Cell Res.* **2000**, *256*, 42-49.
2. Kornienko, A.; Mathieu, V.; Rastogi, S.; Lefranc, F.; Kiss, R. Therapeutic Agents Triggering Non-Apoptotic Cancer Cell Death. *J. Med. Chem.* **2013**, *56*, 4823-4839.
3. Brenner, H. Long-term survival rates of cancer patients achieved by the end of the 20th century: a period analysis. *The Lancet*, **2002**, *360*, 1131-1135.

4. Kleihues, P.; Cavenee, W. K. Pathology and Genetics of Tumors of the Nervous System, International Agency for the Research on Cancer (IARC) and WHO Health Organization. Oxford, UK: *Oxford Press*. **2000.**
5. Lefranc, F.; Sadeghi, N.; Camby, I.; Metens, T.; De Witte, O.; Kiss, R. Present and potential future issues in glioblastoma treatment. *Expert Rev. Anticancer Ther*. **2006,** *6*, 719-732.
6. Stupp, R.; Hegi, M.E.; Mason, W.P.; van den Bent, M.J.; Taphoorn, M.J.; Janzer, R.C.; Ludwin, S.K.; Allgeier, A.; Fisher, B.; Belanger, K.; Hau, P.; Brandes, A.A.; Gijtenbeek, J.; Marosi, C.; Vecht, C. J.; Mokhtari, K.; Wesseling, P.; Villa, S.; Eisenhauer, E.; Gorlia, T.; Weller, M.; Lacombe, D.; Cairncross G.; Mirimanof, R. -O. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial *Lancet Oncol*. **2009**, *10*, 459-466.
7. Giese, A.; Bjerkvig, R.; Berens, M. E.; Westphal, M. Cost of migration: Invasion of malignant gliomas and implications for treatment *J. Clin. Oncol*. **2003,** *21*, 1624-1636.
8. Simpson, C. D.; Anyiwe, K.; Schimmer, A. D. Anoikis resistance and tumor metastasis. *Cancer Lett*. **2008**, *272*, 177-185.
9. Savage, P.; Stebbing, J.; Bower, M.; Crook, T. Why does cytotoxic chemotherapy cure only some cancers? *Nat. Clin. Pract. Oncol*. **2009**, *6*, 43-52.
10. Wilson, T. R.; Johnston, P. G.; Longley, D. B. Anti-Apoptotic Mechanisms of Drug Resistance in Cancer. *Curr. Cancer Drug Targets* **2009**, *9*, 307-319.
11. Lamoral-Theys, D.; Pottier, L.; Dufrasne, F.; Nève, J.; Dubois, J.; Kornienko, A.; Kiss, R.; Ingrassia, L. Natural polyhenols that display anticancer properties through inhibition of kinase activity. *Curr. Med. Chem*. **2010**, *17*, 812-815.

12. Van Goietsenoven, G.; Andolfi, A.; Lallemand, B.; Cimmino, A.; Lamoral-Theys, D.; Gras, T.; Abou-Donia, A.; Dubois, J.; Lefranc, F.; Mathieu, V.; Kornienko, A.; Kiss, R.; Evidente, A. *J*. Amaryllidaceae Alkaloids Belonging to Different Structural Subgroups Display Activity against Apoptosis-Resistant Cancer Cells. *J*. *Nat*. *Prod*. **2010**, *73*, 1223-1227.

13. Lamoral-Theys, D.; Andolfi, A.; Van Goietsenoven, G.; Cimmino, A.; Le Calvé, B.; Wauthoz, N.; Mégalizzi, V.; Gras, T.; Bruyère, C.; Dubois, J.; Mathieu, V.; Kornienko, A.; Kiss, R.; Evidente, A. Lycorine, the Main Phenanthridine Amaryllidaceae Alkaloid, Exhibits Significant Anti-Tumor Activity in Cancer Cells that Display Resistance to Proapoptotic Stimuli: an Investigation of Structure-Activity Relationship and Mechanistic Insight. *J*. *Med*. *Chem*. **2009**, *52*, 6244-6256.

14. Evdokimov, N.; Lamoral-Theys, D.; Mathieu, V.; Andolfi, A.; Pelly, S.; van Otterlo, W.; Magedov, I.; Kiss, R.; Evidente, A.; Kornienko, A. In search of a cytostatic agent derived from the alkaloid lycorine: Synthesis and growth inhibitory properties of lycorine derivatives. *Bioorg*. *Med*. *Chem*. **2011**, *19*, 7252-7261.

15. Luchetti, G.; Johnston, R.; Mathieu, V.; Lefranc, F.; Hayden, K.; Andolfi, A.; Lamoral-Theys, D.; Reisenauer, M. R.; Champion, C.; Pelly, S. C.; van Otterlo, W. A. L.; Magedov, I. V.; Kiss, R.; Evidente, A.; Rogelj, S.; Kornienko, A. Bulbispermine: A Crinine-Type Amaryllidaceae Alkaloid Exhibiting Cytostatic Activity toward Apoptosis-Resistant Glioma Cells. *ChemMedChem*, **2012**, *7*, 815-822.

16. Gottesman, M. M.; Fojo, T.; Bates, S. E. Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nat*. *Rev*. *Cancer* **2002**, *2*, 48-58.

17. Saraswathy, M.; Gong, S. Q. Different strategies to overcome multidrug resistance in cancer. *Biotechnol*. *Adv*. **2013**, *31*, 1397-1407.

18. Chen, G. K.; Duran, G. E.; Mangili, A., Beketic-Oreskovic, L.; Sikic, B. I. MDR1 activation is the predominant resistance mechanism selected by vinblastine in MES-SA cells *Br. J. Cancer*. **2000**, *83*, 892-898.

19. Geney, R., Ungureanu, M., Li, D.; Ojima, I. Overcoming multidrug resistance in taxane chemotherapy. *Clinical Chem. Lab. Med*. **2002**, *40*, 918-925.

20. Aksenov, A. V.; Smirnov, A. N.; Aksenov, N. A.; Aksenova, I. V.; Frolova, L. V.; Kornienko, A.; Magedov, I. V.; Rubin, M. Metal-FreeTransannulation Reaction of Indoles with Nitrostyrenes: A Simple Practical Synthesis of 3-Substituted 2-Quinolones. *Chem. Commun*. **2013**, *49*, 9305-9307.

21. KrennHrubec, K.; Marshall, B. L.; Hedgin, M.; Verdin, E.; Ulrich, S. M. Design and evaluation of "Linkerless" hydroxamic acids as selective HDAC inhibitors. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 2874-2878.

22. Tessier, P.; Smil, D. V.; Wanhab, A.; Leit, S.; Rahil, J.; Li, Z.; Deziel, R.; Besterman, J. M. Diphenylmethylene hydroxamic acids as selective class IIa histone deacetylase inhibitors. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 5684-5688.

23. Mosmann, T. Rapid colorimetric assay for cellular growth and survival - application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55-63.

24. Dasari, R.; Banuls, L. M. Y.; Masi, M.; Pelly, S. C.; Mathieu, V.; Green, I. R.; van Otterlo, W. A. L.; Evidente, A.; Kiss, R.; Kornienko, A. C1,C2-Ether Derivatives of the Amaryllidaceae Alkaloid Lycorine: Retention of Activity of Highly Lipophilic Analogues Against Apoptosis-Resistant Cancer Cells. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 923-927.

25. Magedov, I. V.; Lefranc, F.; Frolova, L. V.; Banuls, L. M. Y.; Peretti, A. S.; Rogelj, S.; Mathieu, V. ; Kiss, R.; Kornienko, A. Antiproliferative Activity of 2,3-Disubstituted Indoles Toward Apoptosis-Resistant Cancers Cells. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 3277-3282.

26. Bury, M.; Girault, A.; Mégalizzi, V.; Spiegl-Kreinecker, S.; Mathieu, V.; Berger, W.; Evidente, A.: Kornienko, A.; Gailly, P.; Vandier, C.; Kiss, R. Ophiobolin A induces paraptosis-like cell death in human glioblastoma cells by decreasing BKCa channel activity. *Cell Death Dis*. **2013**, *4*, e569.

27. Van Goietsenoven, G. ; Mathieu, V. ; Lefranc, F. ; Kornienko, A.; Evidente, A. ; Kiss, R. Narciclasine as well as other Amaryllidaceae Isocarbostyrils are Promising GTPase-Targeting Agents against Brain Cancers. *Med. Res. Rev*. **2013**, *33*, 439-466.

28. Branle, F.; Lefranc, F.; Camby, I.; Jeuken, J.; Geurts-Moespot, A.; Sprenger, S.; Sweep, F.; Kiss, R.; Salmon, I. Evaluation of the efficiency of chemotherapy in in vivo orthotopic models of human glioma cells with and without 1p19q deletions and in C6 orthotopic allografts serving for the evaluation of surgery combined with chemotherapy. *Cancer* **2002**, *95*, 641-655.

29. Li, J. ; Hu, W. ; Lan, Q. *J*. The apoptosis-resistance in t-AUCB-treated glioblastoma cells depends on activation of Hsp27 *Neurooncol*. **2012**, *110*, 187-194.

30. Mathieu, A. ; Remmelink, M. ; D'Haene, N. ; Penant, S. ; Gaussin, J.F. ; Van Ginckel, R. ; Darro, F.; Kiss, R.; Salmon, I. Development of a chemoresistant orthotopic human nonsmall cell lung carcinoma model in nude mice: analyses of tumor heterogenity in relation to the immunohistochemical levels of expression of cyclooxygenase-2, ornithine decarboxylase, lung-related resistance protein, prostaglandin E synthetase, and glutathione-S-transferase-alpha (GST)-alpha, GST-mu, and GST-pi. *Cancer* **2004**, *101*, 1908-1918.

31. Lefranc, F.; Nuzzo, G.; Hamdy, N. A.; Fakhr, I.; Banuls, L. M. Y.; van Goietsenoven, G. V.; Villani, G.; Mathieu, V.; van Soest, R.; Kiss, R.; Ciavatta, M. L. In Vitro Pharmacological and Toxicological Effects of Norterpene Peroxides Isolated from the Red Sea Sponge Diacarnus erythraeanus on Normal and Cancer Cells. *J. Nat. Prod*. **2013,** *76*, 1541-1547.

32. Mathieu, V. ; Pirker, C.; Martin de Lasalle, E. ; Vernier, M. ; Mijatovic, T. ; De Neve, N. ;

Gaussin, J.F. ; Dehoux, M. ; Lefranc, F. ; Berger, W. ; Kiss, R. The sodium pump alpha-1 subunit: A disease progression-related target for metastatic melanoma treatment. *J. Cell. Mol. Med*. **2009**, 13, 3960-3972.

33. Dong, Y.; Han, Q.; Zou, Y.; Deng, Z.; Lu, X.; Wang, X.; Zhang, W.; Jin, H.; Su, J.; Jiang, T.; Ren, H. Long-term exposure to imatinib reduced cancer stem cell ability through induction of cell differentiation via activation of MAPK signaling in glioblastoma cells. *Mol. Cell Biochem*. **2012**, *370*, 89-102.
34. Zhang, L.; Li, P.; Hsu, T.; Aguilar, H. R.; Frantz, D. E.; Schneider, J. W.; Bachoo, R. M.; Hsieh, J. Small-molecule blocks malignant astrocyte proliferation and induces neuronal gene expression. *Differentiation* **2011**, *81*, 233-242.
35. Harker, W. G.; Sikic, B. I. Multidrug (pleiotropic) resistance in doxorubicin-selected variants of the human sarcoma cell line MES-SA. *Cancer Res*. **1985**, *45*, 4091-4096.
36. Singh, S. K.; Hawkins, C.; Clarke, I. D.; Squire, J. A.; Bayani, J.; Hide, T.; Henkelman, R. M.; Cusimano, M. D.; Dirks, B. P. Identification of human brain tumour initiating cells. *Nature* **2004**, *432*, 396–401.
37. Yuan, X.; Curtin, J.; Xiong Y.; Liu, G.; Waschsmann-Hogiu, S.; Black, K. L.; Yu, J. S. Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* **2004**, 23, 9392–9400.
38. Galli, R.; Binda, E.; Orfanelli, U.; Cipelletti, B.; Gritti, A.; De Vitis, S.; Fiocco, R.; Foroni, C.; Dimeco, F.; Vescovi, A. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res*. **2004**, *64*, 7011–7021.
39. Lee, J.; Kotliarova, S.; Kotliarov, Y.; Li, A.; Su, Q.; Donin, N. M.; Pastorino, S.; Purow, B. W.; Christopher, N.; Zhang, W.; Park, J. K.; Fine, H. A. Tumor stem cells derived from

glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell* **2006**, *9*, 391–403.

40. Bao, S.; Wu, Q.; McLendon, R. E.; Hao, Y.; Shi, Q.; Hjelmeland, A. B.; Dewhirst, M. W.; Bigner, D. D.; Rich, J. M. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **2006**, *444*, 756–760.
41. Liu, G.; Yuan, X.; Zeng, Z.; Tunici, P.; Ng, H.; Abdulkadir, I. R.; Lu, L.; Irvin, D.; Black, K. L.; Yu, J. S. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol. Cancer* **2006**, *5*, 67.
42. Johannessen, T. C.; Bjerkvig, R.; Tysnes, B. B. DNA repair and cancer stemlike cells - Potential partners in glioma drug resistance? *Cancer Treat Rev*. **2008**, *34*, 558–567.
43. Ma, S.; Lee, T. K.; Zheng, B. J.; Chan, K. W.; Guan, X. Y. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. *Oncogene* **2008**, *27*, 1749–1758.
44. Weller, M.; Stupp, R.; Reifenberger, G.; Brandes, A. A.; van den Bent, M. J.; Wick, W.; Hegi, M. E. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? *Nat. Rev. Neurol*. **2010**, *6*, 39-51.
45. Worrall D. E. The action of ammonia and aromatic Amines on 4-methylnitrostyrene and related compounds. *J. Am. Chem. Soc*. **1938**, *60*, 2841-2844.
46. Kurihara, T.; Kanbara, H.; Kubodera, H.; Matsumoto, S.; Kaino, T. Third-order nonlinear optical properties of DEANST: a new material for nonlinear optics. *Opt. Commun*. **1991**, *84*, 149-154.
47. Gunther, H. S.; Schmidt, N. O.; Philips, H. S.; Kemming, D.; Kharbanda, S.; Soriano, R.; Modrusan, Z.; Meissner, H.; Westphal, M.; Lamszus, K. Glioblastoma-derived stem cell-

enriched cultures form distinct subgroups according to molecular and phenotypic criteria. Oncogene **2008**, *27*, 2897-2909.

48. For a recent example, see: Regina, G.; Bai, R.; Rensen, W. M.; Di Cesare, E.; Coluccia, A.; Piscitelli, F.; Famiglini, V.; Reggio, A.; Nalli, M.; Pelliccia, S.; Da Pozzo, E.; Costa, B.; Granata, I.; Porta, A.; Maresca, B.; Soriani, A.; Iannitto, M. L.; Santoni, A.; Li, J.; Cona, M. M.; Chen, F.; Ni, Y.; Brancale, A.; Dondio, G.; Vultaggio, S.; Varasi, M; Mercurio, C.; Martini, C.; Hamel, E.; Lavia, P; Novellino, E.; Silvestri, R. Toward Highly Potent Cancer Agents by Modulating the C-2 Group of the Arylthioindole Class of Tubulin Polymerization Inhibitors. *J. Med. Chem.* **2013**, *56*, 123−149.
49. Debeir, O.; Megalizzi V.; Warze N.; Kiss, R.; Decaestecker C. Videomicroscopic extraction of specific information on cell proliferation and migration in vitro. *Exp. Cell Res.* **2008**, *314*, 2985-2998.
50. MarvinSketch: Marvin 6.3.0, 2014, ChemAxon (http://www.chemaxon.com).
51. Klopman, G.; Li, J. -Y.; Wang, S.; Dimayuga, M. Computer Automated log P Calculations Based on an Extended Group Contribution Approach. *J. Chem. Inf. Comput. Sci.*, **1994**, *34*, 752-781.
52. Molinspiration Cheminformatics, Interactive log P Calculator, www.molinspiration.com, 2014.
53. Tetko, I. V.; Gasteiger, J.; Todeschini, R.; Mauri, A.; Livingstone, D.; Ertl, P.; Palyulin, V. A.; Radchenko, E. V.; Zefirov, N. S.; Makarenko, A. S.; Tanchuk, V. Y.; Prokopenko, V. V.: *J. Comput. Aid. Mol. Des.*, **2005**, *19*, 453-463.
54. VCCLAB, Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005.

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