

Communication

# Methylation of 2-Aryl-2-(3-indolyl)acetohydroxamic Acids and Evaluation of Cytotoxic Activity of the Products

Dmitrii A. Aksenov<sup>1</sup>, Alexander V. Aksenov<sup>1,\*</sup> , Lidiya A. Prityko<sup>1</sup>, Nicolai A. Aksenov<sup>1</sup> , Liliya V. Frolova<sup>2</sup> and Michael Rubin<sup>1,3,\*</sup> 

<sup>1</sup> Department of Chemistry, North Caucasus Federal University, 1a Pushkin St., 355017 Stavropol, Russia; daksenov@ncfu.ru (D.A.A.); lidiapriyko@yandex.ru (L.A.P.); naksenov@ncfu.ru (N.A.A.)

<sup>2</sup> Department of Chemistry, Purdue University, Science Building R488, 2101 East Coliseum Blvd., Fort Wayne, IN 46805, USA; lfrolova@pfw.edu

<sup>3</sup> Department of Chemistry, University of Kansas, 1567 Irving Hill Road, Lawrence, KS 66045, USA

\* Correspondence: aaksenov@ncfu.ru (A.V.A.); mrubin@ku.edu (M.R.)

**Abstract:** 2-Aryl-2-(3-indolyl)acetohydroxamic acids demonstrate promising antitumor activity, but quickly metabolize in vivo via glucuronidation of hydroxamic acid residue. In an attempt to improve their pharmacokinetics, methyl esters were synthesized via a newly developed protocol for chemoselective mono-methylation of hydroxamic acids. The cytotoxicity of these derivatives against the HeLa cell line was evaluated and found to be inferior compared to the parent lead compounds.

**Keywords:** hydroxamic acid; indoles; methylation; anti-cancer activity



**Citation:** Aksenov, D.A.; Aksenov, A.V.; Prityko, L.A.; Aksenov, N.A.; Frolova, L.V.; Rubin, M. Methylation of 2-Aryl-2-(3-indolyl)acetohydroxamic Acids and Evaluation of Cytotoxic Activity of the Products. *Molbank* **2022**, *2022*, M1307. <https://doi.org/10.3390/M1307>

Academic Editor: Hideto Miyabe

Received: 1 December 2021

Accepted: 20 December 2021

Published: 22 December 2021

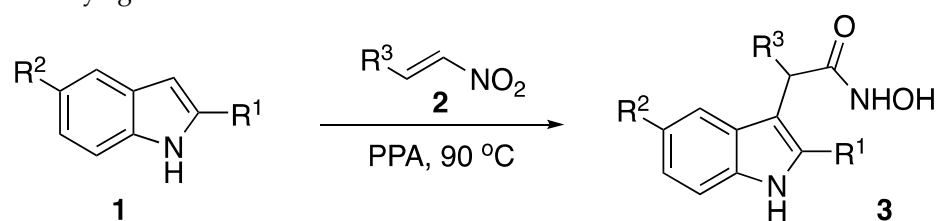
**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

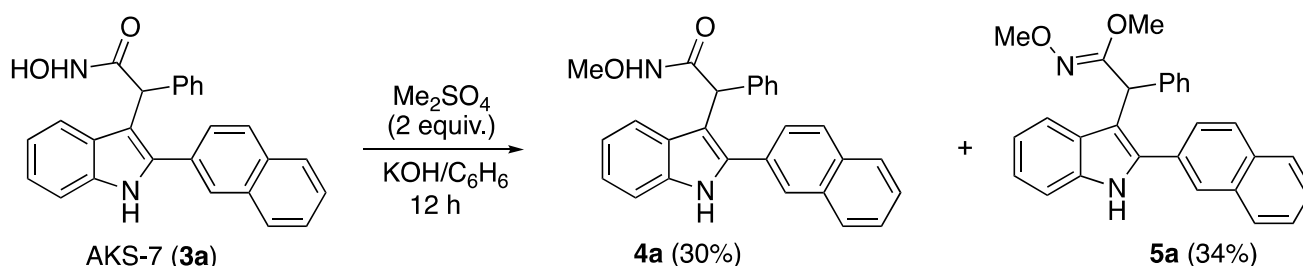
The treatment of apoptosis-resistant tumors represents a major challenge, as they often respond poorly to traditional chemotherapeutics and tend to produce metastases, typically resulting in about a 90% death toll worldwide [1–5]. For this reason, the development of novel anti-cancer drugs with alternative modes of action, suitable for treatment of such resistant cell lines, can be easily justified. Recently, we communicated a concise and highly efficient synthetic method allowing for facile preparation of 2-aryl-2-(3-indolyl)acetohydroxamic acids **3** via reaction of indole derivatives **1** with  $\beta$ -nitrostyrenes **2** in polyphosphoric acid (PPA) (Scheme 1) [6]. It was also demonstrated that these readily available compounds are active against apoptosis- and multidrug-resistant cancer cells in vitro [7]. Initial animal studies demonstrated, however, that these compounds had no effect on cancerous tumors in mice [8]. This was linked to a very unfavorable pharmacokinetic profile, as it was shown that the concentration of compound **3** in plasma was reduced below the active threshold within an hour, presumably due to facile glucuronidation of the hydroxamic acid functionality [8]. This result prompted us to search for analogs that may be more resistant to this metabolic pathway. Herein, we wish to report an attempted synthesis of 2-(1*H*-indol-3-yl)-*N*-methoxy-2-phenylacetamides **4** via methylation of the parent 2-aryl-2-(3-indolyl)acetohydroxamic acids **3** and initial evaluation of their biologic activity against the HeLa cell line.



**Scheme 1.** Preparation of 2-aryl-2-(3-indolyl)acetohydroxamic acids.

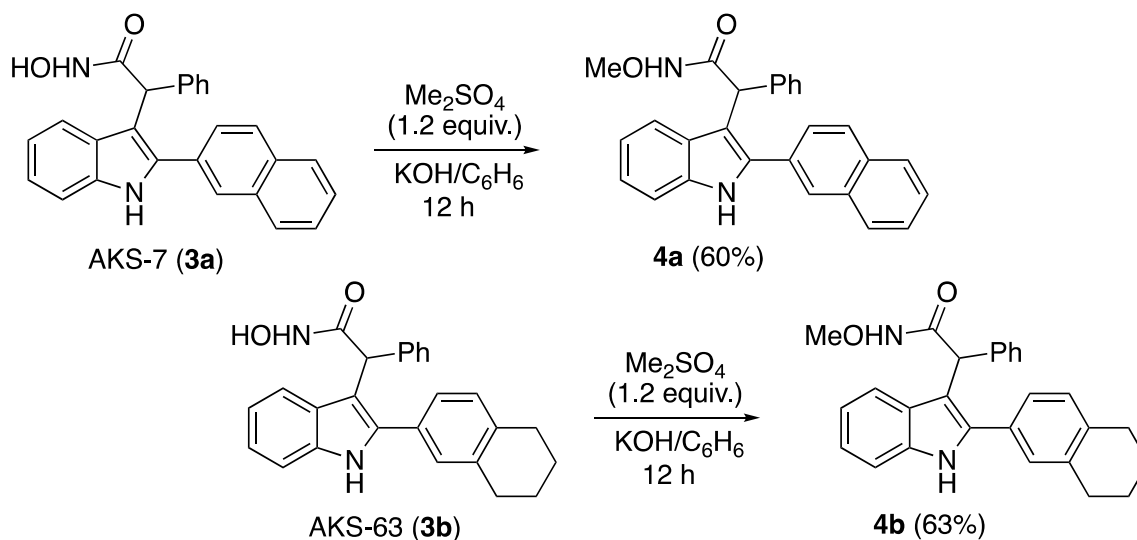
## 2. Results and Discussion

Initial attempts at methylation involved the treatment of hydroxamic acid AKS-7 (**3a**) with dimethyl sulfate (1.0 equiv.) in aqueous KOH according to the published procedure [9]. This method, however, did not produce any methylation, as **3a** remained unchanged. We speculated that the lack of reactivity might be due to a poor solubility of the starting material in aqueous media. To address this issue, we decided to treat **3a** with excess dimethyl sulfate (2.0 equiv.) in a biphasic system consisting of aqueous KOH and non-polar organic solvents such as benzene. This modification proved more productive, affording the desired 2-(1*H*-indol-3-yl)-*N*-methoxyacetamide **4a** in moderate yield, along with a comparable amount of methyl 2-(1*H*-indol-3-yl)-*N*-methoxyacetimidate **5a** resulting from double-fold methylation (Scheme 2).



**Scheme 2.** Non-selective methylation of 2-phenyl-2-(3-indolyl)acetohydroxamic acid **3a**.

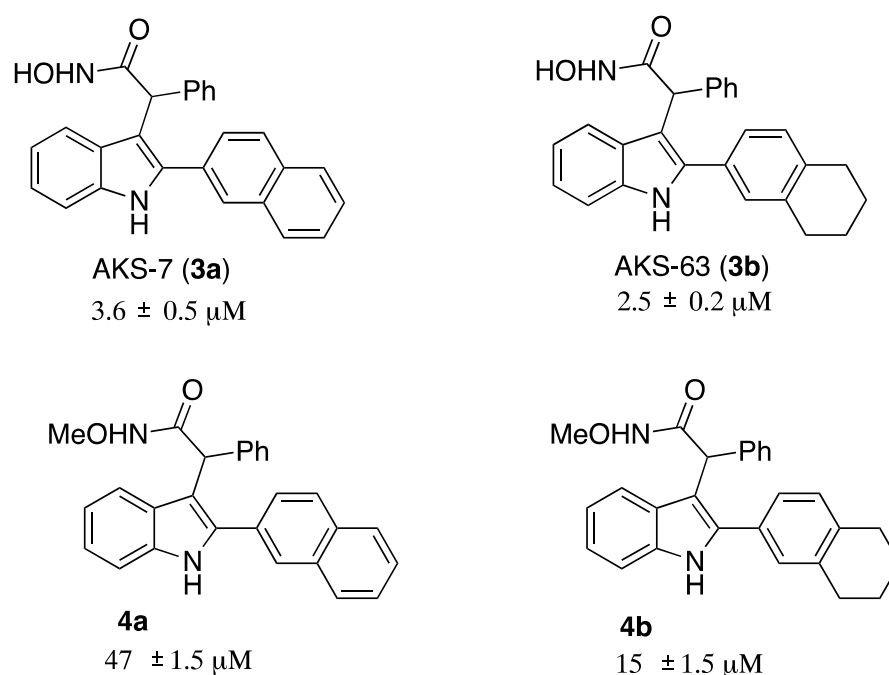
In order to improve the chemoselectivity towards mono-methylation, the next set of optimization test reactions was performed in the presence of 1.2 equiv. of dimethyl sulfate. Optimal results were obtained when the reaction of **3a** was carried out at room temperature for 12–15 h. This allowed the isolation of **4a** with 60% yield (Scheme 3). The compound AKS-63 (**3b**) also reacted smoothly, affording the corresponding *O*-methylation product **4b** with 63% yield. We did not detect the formation of *N*-methylated indole derivatives [10].



**Scheme 3.** Chemoselective methylation of 2-phenyl-2-(3-indolyl)acetohydroxamic acids **3a,b**.

The evaluation of the anti-cancer activity of the synthesized compounds **4a,b** was performed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay against the human HeLa cervical adenocarcinoma cell line. The results are presented in Figure 1, along with data obtained for the parent compounds **3a,b**. It should be pointed out that in both cases, methoxy-protected hydroxamic acids **4** demonstrated inferior cytotoxic activities, as their  $\text{IC}_{50}$  values were about an order of magnitude higher than those obtained for non-protected analogs. Evidently, hydrophilic interaction at the

hydroxamic acid functionality is important for the bioactivity, and therefore the featured protection cannot be used in the design of better anti-cancer drugs based on this scaffold.



**Figure 1.** Antiproliferative activity ( $\text{IC}_{50}$ ) of the synthesized compounds against HeLa cervical adenocarcinoma.

### 3. Materials and Methods

Human cervical adenocarcinoma HeLa cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), and cultured in DMEM supplemented with 10% fetal bovine serum (FBS). The antiproliferative properties of the synthesized compounds were evaluated using the MTT assay [11]. All compounds were dissolved in DMSO at a concentration of either 100 or 50 mM prior to cell treatment. The cells were trypsinized and seeded at  $4 \times 10^3$  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  $\mu\text{M}$ , and incubated for 48 h in 200  $\mu\text{L}$  of medium. Then, 20  $\mu\text{L}$  of MTT reagent in serum-free medium (5 mg/mL) was added to each well, and the cells were incubated for a further 2 h. The medium was removed, and the resulting formazan crystals were resolubilized in 200  $\mu\text{L}$  of DMSO. A490 was measured using a Molecular Devices THERMOMax plate reader. The experiments were performed in quadruplicate and repeated at least twice for each compound. Cells treated with 0.1% DMSO were used as a negative control; 1  $\mu\text{M}$  phenyl arsine oxide (PAO) was used as a positive control.

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  $\mu\text{m}$ ) silica gel 60 F254 glass-backed plates (EMD Chemicals Inc., Gibbstown, NJ, USA). Visualization was accomplished with UV light. Flash chromatography was performed using silica gel (32–63  $\mu\text{m}$ , 60  $\text{\AA}$  pore size).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on Bruker DRX-400 and DRX-500 spectrometers. Chemical shifts ( $\delta$ ) are reported in ppm relative to the TMS internal standard. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br. (broad). See Supporting Information for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral charts.

*N*-Hydroxy-2-phenyl-2-(2-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-indol-3-yl)acetamide (AK S-63, **3b**): A mixture of phenylhydrazine (108 mg, 1.00 mmol), 1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethan-1-one (174 mg, 1.00 mmol), and 80% PPA (2.0 g) was vigorously stirred at

100–110 °C. The reaction progress was monitored via TLC. After complete consumption of the starting materials, the mixture was cooled to 65–70 °C, and 2-nitrostyrene (179 mg, 1.20 mmol) was added in a single portion. The mixture was stirred for 1 h, while the reaction progress was again monitored via TLC. When all intermediate indole was consumed, the mixture was quenched with cold water (50 mL) and neutralized with aqueous ammonia to pH 8. The formed precipitate was collected and recrystallized from benzene/petroleum ether. The title compound was obtained as colorless crystals, mp 131–134 °C (benzene/petroleum ether). The yield was 297 mg (0.75 mmol, 75%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.22 (s, 1H), 10.73 (s, 1H), 8.81 (s, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.37 (s, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.29–7.12 (m, 8H), 7.03 (t, *J* = 7.5 Hz, 1H), 6.85 (t, *J* = 7.5 Hz, 1H), 5.07 (s, 1H), 2.76 (s, 4H), 1.77 (s, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 168.7, 141.0, 136.8, 136.4, 136.3, 136.1, 129.7, 129.2, 129.1, 128.3, 128.0 (4C), 127.9, 126.2, 125.7, 122.2, 121.0, 118.4, 110.8, 108.9, 46.2, 28.8, 28.6, 22.7, 22.7; HRMS (ESI-TOF, *m/z*): Calculated for C<sub>26</sub>H<sub>24</sub>N<sub>2</sub>NaO<sub>2</sub> (M + Na)<sup>+</sup>, 419.1730; found 419.1731.

*N*-Methoxy-2-(2-(naphthalen-2-yl)-1H-indol-3-yl)-2-phenylacetamide (**4a**): *N*-Hydroxy-2-(2-(naphthalen-2-yl)-1H-indol-3-yl)-2-phenylacetamide (AKS-7, **3a**) [7] (392 mg, 1.00 mmol) was dissolved in aqueous potassium hydroxide (78 mg, 2.00 mmol in 0.78 mL of water). This solution was cooled in an ice bath and crushed ice was added (0.15 g) followed by dimethyl sulfate (163 mg, 1.20 mmol) and benzene (7.5 mL). The mixture was stirred for 12 h at room temperature, and the reaction progress was monitored via TLC. After complete consumption of the starting material, the mixture was quenched with water (10 mL) and basified with aqueous ammonia to pH 8–9. The formed solution was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were concentrated under vacuum and the residual solid was purified by flash column chromatography on silica gel, eluting with a mixture of benzene and ethyl acetate, 6:1. The title compound was obtained as yellowish crystalline solid, mp 204–207 °C (EtOAc), *R*<sub>f</sub> 0.29 (C<sub>6</sub>H<sub>6</sub>/EtOAc, 6:1). Yield 244 mg (0.60 mmol, 60%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.53 (s, 1H), 11.36 (s, 1H), 8.04–7.97 (m, 4H), 7.89 (d, *J* = 6.4 Hz, 1H), 7.69 (t, *J* = 9.7 Hz, 2H), 7.58–7.56 (m, 2H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.31–7.20 (m, 6H), 7.10 (t, *J* = 7.4 Hz, 1H), 6.93 (t, *J* = 7.6 Hz, 1H), 5.14 (s, 1H), 3.58 (s, 3H); <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 168.5, 140.4, 136.4, 136.2, 132.8, 132.3, 129.9, 128.2 (3C), 128.1 (4C), 128.0, 127.7, 127.6, 127.5, 126.6, 126.4, 121.7, 121.4, 118.8, 111.1, 109.4, 63.1, 46.2; FT IR, cm<sup>-1</sup>: 3200, 3362 (NH), 1643 (CONH). HRMS (ESI-TOF, *m/z*): Calculated for C<sub>27</sub>H<sub>22</sub>N<sub>2</sub>NaO<sub>2</sub> (M + Na)<sup>+</sup>, 429.1573; found 429.1576.

*N*-Methoxy-2-phenyl-2-(2-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-indol-3-yl)acetamide (**4b**): This material was obtained from *N*-hydroxy-2-phenyl-2-(2-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-indol-3-yl)acetamide (AKS-63, **3b**) (396 mg, 1.00 mmol) according to the procedure described above for preparation of compound **4a**. The title compound was obtained as light grey crystalline solid, mp 181–184 °C, *R*<sub>f</sub> 0.37 (C<sub>6</sub>H<sub>6</sub>/EtOAc, 6:1). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.30 (br. s, 1H), 11.29 (br. s, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.31–7.23 (m, 2H), 7.23–7.13 (m, 6H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.88 (t, *J* = 7.5 Hz, 1H), 5.01 (s, 1H), 3.56 (s, 3H), 2.76 (m, 4H), 1.77 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 168.6, 140.6, 136.8, 136.6, 136.5, 136.1, 129.6, 129.2 (2C), 128.2 (2C), 128.1 (2C), 127.7, 126.3, 125.7, 121.6, 121.1, 118.6, 111.0, 108.4, 63.1, 46.3, 28.8, 28.6, 22.7, 22.6; FT IR, cm<sup>-1</sup>: 3351 (NH), 1678 (CONH); HRMS (ESI-TOF, *m/z*): Calculated for C<sub>27</sub>H<sub>26</sub>N<sub>2</sub>NaO<sub>2</sub> (M + Na)<sup>+</sup>, 433.1886; found 433.1883.

#### 4. Conclusions

A chemoselective method for mono-methylation of 2-aryl-2-(3-indolyl)acetohydroxamic acids **3** was developed and employed to prepare samples of 2-(1H-indol-3-yl)-*N*-methoxy-2-phenylacetamides **4**. These were used to assess the importance of the OH group for the cytotoxic activity of indole-containing derivatives of hydroxamic acid. Protection of this group would potentially allow the improvement of the unfavorable pharmacokinetics of acetohydroxamic acids **3** used as anti-cancer agents in vivo. It was found, however, that the

free OH group is critically important for the high cytotoxicity of hydroxamic acid derivatives, and the protection approach should not be considered in further SAR investigations.

**Supplementary Materials:** The following are available online:  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral charts.

**Author Contributions:** D.A.A.—investigation; A.V.A.—conceptualization, supervision, and funding acquisition; L.A.P.—investigation; N.A.A.—methodology and formal analysis; L.V.F.—investigation and formal analysis; M.R.—conceptualization, supervision, and writing (original draft, review, and editing). All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Russian Science Foundation (grant #21-73-00044, <https://rscf.ru/project/21-73-00044/> (accessed on 10 December 2021)).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** We are grateful to the Department of Biology, New Mexico Tech. for help in the testing of the antiproliferative properties of compounds.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. 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. [[CrossRef](#)]
2. Lamoral-Theys, D.; Pottier, L.; Dufrasne, F.; Neve, J.; Dubois, J.; Kornienko, A.; Kiss, R.; Ingrassia, L. Natural polyphenols that display anticancer properties through inhibition of kinase activity. *Curr. Med. Chem.* **2010**, *17*, 812–825. [[CrossRef](#)] [[PubMed](#)]
3. Savage, P.; Stebbing, J.; Bower, M.; Crook, T. Why does cytotoxic chemotherapy cure only some cancers? *Nat. Clin. Pract. Oncol.* **2009**, *6*, 43–52. [[CrossRef](#)] [[PubMed](#)]
4. Simpson, C.D.; Anyiwe, K.; Schimmer, A.D. Anoikis resistance and tumor metastasis. *Cancer Lett.* **2008**, *272*, 177–185. [[CrossRef](#)] [[PubMed](#)]
5. 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. [[CrossRef](#)] [[PubMed](#)]
6. Aksenov, A.V.; Smirnov, A.N.; Aksenov, N.A.; Aksenova, I.V.; Frolova, L.V.; Kornienko, A.; Magedov, I.V.; Rubin, M. Metal-free transannulation reaction of indoles with nitrostyrenes: A simple practical synthesis of 3-substituted 2-quinolones. *Chem. Commun.* **2013**, *49*, 9305–9307. [[CrossRef](#)] [[PubMed](#)]
7. Aksenov, A.V.; Smirnov, A.N.; Magedov, I.V.; Reisenauer, M.R.; Aksenov, N.A.; Aksenova, I.V.; Pendleton, A.L.; Nguyen, G.; Johnston, R.K.; Rubin, M.; et al. Activity of 2-aryl-2-(3-indolyl)acetohydroxamates against drug-resistant cancer cells. *J. Med. Chem.* **2015**, *58*, 2206–2220. [[CrossRef](#)] [[PubMed](#)]
8. Segat, G.C.; Moreira, C.G.; Santos, E.C.; Heller, M.; Schwanke, R.C.; Aksenov, A.V.; Aksenov, N.A.; Aksenov, D.A.; Kornienko, A.; Marcon, R.; et al. A new series of acetohydroxamates shows in vitro and in vivo anticancer activity against melanoma. *Investig. New Drugs* **2020**, *38*, 977–989. [[CrossRef](#)] [[PubMed](#)]
9. Johnson, J.E.; Ghafouripour, A.; Haug, Y.K.; Cordes, A.W.; Pennington, W.T.; Exner, O. Synthesis and configurations of O-substituted hydroximoyl chlorides. Stereochemistry and mechanism of alkoxide ion substitution at the carbon-nitrogen double bond. *J. Org. Chem.* **1985**, *50*, 993–997. [[CrossRef](#)]
10. Ottoni, O.; Cruz, R.; Alves, R. Efficient and simple methods for the introduction of the sulfonyl, acyl and alkyl protecting groups on the nitrogen of indole and its derivatives. *Tetrahedron* **1998**, *54*, 13915–13928. [[CrossRef](#)]
11. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [[CrossRef](#)]