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Design, Synthesis and Preliminary In-Vitro Activity of 6-Hydroxyalkyl β-Carboline Derivatives for the Development of Drug Conjugates Targeting MDM2

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The mouse-double-minute-2 (MDM2) protein, the main downregulator of the tumor suppressor p53 protein, represents a promising target for the development of novel anticancer therapies. However, the lack of selectivity and poor effectiveness in tumors bearing mutated-p53 impaired the approval of several MDM2 inhibitors for the market. In this context, the possibility of generating drug-conjugates within a MDM2 inhibitor is a growing research area aimed to overcome these drawbacks. Considering the promising MDM2 inhibition by the β-carboline-based **1**, in this work we explored the introduction of a new functionalization on it for a future conjugation while preserving its anticancer properties. Based on preliminary docking studies, we synthesized derivatives **2a–d** having linear hydroxyalkyl chains with different lengths at the 6-position of the β-carboline core, which effectively preserved the submicromolar IC_{50} on wild-type-p53-U87MG glioblastoma cell line observed with **1**. Candidates **2a**, **d** showed the functionalization was tolerated with respect of bioactivity also on mutated-p53-U138MG glioblastoma cell line, and their hydroxyl groups proved to be easily accessible when coupled to 4-pentynoic-*N,N'*-dimethylethylenediamine affording derivatives **10a**, **d** with high yields. In summary, our results led to generating novel 6-hydroxyalkyl-β-carboline compounds displaying a suitable hydroxyl-site useful to improve the efficacy and/or the tumor specificity of **1** through conjugation strategies.

Introduction

Since its discovery more than 30 years ago,[1] the mouse double minute 2 (MDM2) protein emerged as a promising target for the development of novel anticancer therapies.[2] Indeed, MDM2 shows an extensive activity in promoting tumor growth through several biological processes, and one of the most known is related to the downregulation of the tumor suppressor p53 protein. p53, also commonly referred as the "Guardian of the Genome", is a transcription factor that regulates multiple cellular processes mainly aimed to the protection of the DNA integrity.[3]

Indeed, the presence of cellular stress induces the stabilization and accumulation of the p53 in the nucleus, thereby activating the protein to signal for various target genes that initiate response mechanisms (apoptosis, DNA repair, cell cycle arrest, anti-angiogenesis and senescence), avoiding the propagation of the damaged cells.[4] More precisely, when the apoptotic pathway is activated in response to cell stress, p53 activation occurs, followed by a signalling cascade for transcriptional upregulation of target genes and expression of specific proteins involved in regulating cell death (e.g., Bcl-2 protein including Noxa, Puma and Bax). This triggers the release of mitochondrial apoptotic components, which initiate apoptosis (caspase-independent or caspase-dependent).[4d] Conversely, in presence of low levels of DNA damage, activation of p53 in response to cell stress can also initiate cell cycle arrest of damaged cells (e.g., p21), thereby being a fundamental barrier against tumor growth.[4e] In normal unstressed cell conditions, MDM2 binds to the C-terminal of the p53 protein oligomerization domain, triggering its proteasomal degradation through its E3 ligase activity.[5] In several human cancers, high levels of MDM2 oncogene, along with multiple mutations in p53 protein, is observed and thus considered a key factor in tumor development.[6] Beyond the negative effect on p53 activity, the overexpression of MDM2 is also an important contributor to enhance the tumor resistance toward, radiotherapy and to impair the immune response of the cells.[7]

Several mechanisms have been reported to assess the role of MDM2 in immuno-oncology. For instance, recent studies have shown that MDM2 inhibition can increase dendritic cell levels in tumors with wild-type p53, thereby enhancing CD8+T cell expression and improving the CD8+/Treg ratio.[8a] Furthermore, MDM2 inhibitors have demonstrated synergistic effects

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when combined with anti-PD-1 antibody-based therapies, leading to robust immune system reactivation.[8b] These findings emphasize p53's role in controlling tumor-immune cell cross-talk, providing a mechanistic rationale for the development of new immuno-oncological approaches with p53 re-activation in tumors. For these reasons, the development of MDM2 inhibitors is highly desirable for inducing innate antitumor responses, primarily by restoring p53 activity in cancer cells. To this aim, the solved crystal structure of the p53-MDM2 complex[9] revealed the key residues in the N-terminal domain of the p53 protein that interact with a hydrophobic pocket in MDM2. These amino acids served as the basis for developing a wide range of small molecules designed to bind and inhibit MDM2, thereby reactivating the tumor suppressor function of p53. Different structural cores have been investigated to build potent inhibitor, and among them the *cis*-imidazoline (*i.e.* nutlin-3a), spiro-oxindole (*i.e.* SAR405838), and piperidinones (*i.e.* AMG232) derivatives were able to reach clinical trials.[10] Unfortunately, no MDM2 inhibitors that reactivate p53 have reached the market, primarily due to unwanted side effects on normal tissues.[11] Additionally, these compounds are only effective on tumors expressing wild-type p53, while, more than 50% of tumors have mutated p53, and its activity is not restored after treatment with this first generation of MDM2 inhibitors.[12]

In 2014 Zhang and co-workers reported the discovery of a new class of MDM2 inhibitors characterized by a β-carboline core through virtual high-throughput screening on the solved MDM2 crystal structure. After further optimization, the derivative **1** (Figure 1A), which displayed sub-micromolar IC_{50} values against several cancer cell lines and promising *in vivo* results, independent of the p53 status, emerged as the best candidate for development.[13] In addition, multiple biological assays suggested that **1** induces MDM2 degradation through autoubiquitination, which is a unique mechanism of action compared to previous MDM2 inhibitors. However, poor aqueous solubility and selectivity toward cancer cells limited its therapeutic applications. A first attempt to overcome this obstacle was performed by encapsulating the underivatized **1** in nanoparticles for improving oral delivery.[14]

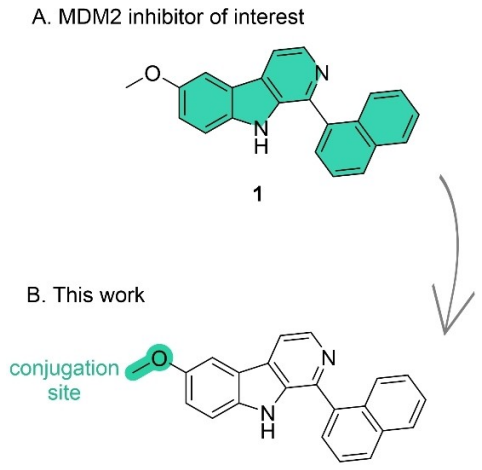


Figure 1. A) Structures of the MDM2 inhibitor **1**; B) aim of this work.

In line with our ongoing interest in developing drug-conjugates to enhance the efficacy of antitumor agents,[15] we report herein our investigations aimed at introducing new structural functionalization on compound **1**, making it suitable for future conjugation. We also explored the impact of these modifications on the drug biological activity (Figure 1B).

Indeed, the replacement of the so-called "traditional" cytotoxic agents classically used as payloads in drug-conjugates with compounds displaying the ability to trigger innate antitumor response is currently a growing research area.[16]

More specifically, we performed docking studies to carefully select an appropriate site of chemical modification on the β-carboline scaffold to preserve the affinity with MDM2. Based on these early computational studies and on the estimation of physicochemical properties, several derivatives were synthesized through an optimized synthetic pathway and tested on U87 glioblastoma cell lines displaying wild type p53. The new conjugation site was then chemically tested on two selected compounds to further introduce an alkyne moiety suitable for a classical click reaction, and finally these were additionally screened both on U87MG and on the U138-MG glioblastoma cell line displaying mutant p53 status.

Our results led to the optimization of the β-carboline compound **1** synthesis, and on the development of a synthetic approach for inserting a conjugation site at the 6-position of **1** to either generate dual-target inhibitors or for targeted strategies to deliver this promising MDM2 inhibitor directly on tumor site.

Results and Discussion

Design of Novel β-Carboline Analogues of 1

The selection of a suitable conjugation site was driven by docking studies performed on the MDM2-AM8553 complex crystal structure (PDB: 4ERE) using AutoDock Vina 1.1.2.[17] According to literature data,[2,13c,18] the β-carboline **1** lays in the same hydrophobic pocket of MDM2 where the amphipathic α-helical N-terminus of p53(15–29) naturally interacts through the hydrophobic face of the helix formed by the indole ring of Trp23, the phenyl ring of Phe19 and the hydrophobic chain of Leu26 (PDB: 1YCR).[18] For instance, the naphthyl group of **1** is buried in the clamp occupied by Leu26 of p53, whereas the methoxy group at the 6 positions of the carboline core establishes a hydrogen bond with Tyr67 (see Figure S1 of Supporting information). Additionally, substitution of the N9 positions is detrimental for antiproliferative activity, suggesting a potential hydrogen bond between the β-carboline 9-position NH and His96.[13b,19]

The observation that the methoxy group is in a solvent exposed region suggested that a further derivatization at this site may not interfere with the native interaction of the carboline core, thus not affecting the desired biological activity. Our preliminary hypothesis was also supported by the positive

binding responses of β-carboline analogues of **1** having the methoxy group replaced by fluoroethoxy or fluoropropoxy function, synthesized as ^{18}F-radiotracers for MDM2 by Chitneni and co-workers.[20]

Based on these data, and taking in account the importance of free indolic NH– in the interaction with the MDM2 protein, we designed a new class of inhibitors substituting the methoxy group at the 6-position of the β-carboline core with hydroxyethyl-(**2a**), hydroxypropyl-(**2b**), hydroxybutyl-(**2c**), and hydroxypentyl-(**2d**) chains to insert a suitable group (–OH) for further conjugation (Figure 2).

By increasing the number of sp3-hybridized atoms we expected to augment the amount of protein/ligand interactions and further enhance the potency and selectivity to the desired target.[21] To further evaluate this hypothesis, the designed hydroxyethyl derivative **2a** was further docked on MDM2 protein, showing that all the interactions previously observed with **1** were conserved, with the β-carboline scaffold laying in the same pocket.

In addition, the newly inserted hydroxyethyl chain is fully exposed on a solvent region, taking only potential distant contact with the last amino acids (e.g. Phe55, Lys51) (Figure 3).

Having in mind the flat aromatic structure of **1** and its poor aqueous solubility as reported in literature,[14] the physicochemical properties of the hydroxyalkyl-β-carboline derivatives **2a–d** were evaluated through the free web tool run by the Swiss Institute of Bioinformatics – *SwissADME*.[22] As expected, the size, solubility and lipophilicity remained comparable with the reference compound, while the polarity, flexibility and carbon bond saturation were increased with the alkyl chain prolongation. Finally, all compounds obeyed the Lipinski rule-of-five and obtained the bioavailability score of 0.55. The bioavailability score confirms that all the investigated compounds have good absorption, with an estimated minimal oral bioavailability of 10% in rats.[22b] This semi-quantitative rule-based method indicates that hydroxyalkyl-derivatives **2a–d** hold a promise as potential candidates in drug discovery (see Figure S2 of Supporting information). On the other hand, the performed *in silico* ADME studies are not fully applicable for the second generation of β-carboline derivatives **10a**, **d** with an uncleavable linker, as their pharmacokinetics are dictated by plasma stability and targeted delivery mechanisms.[23]

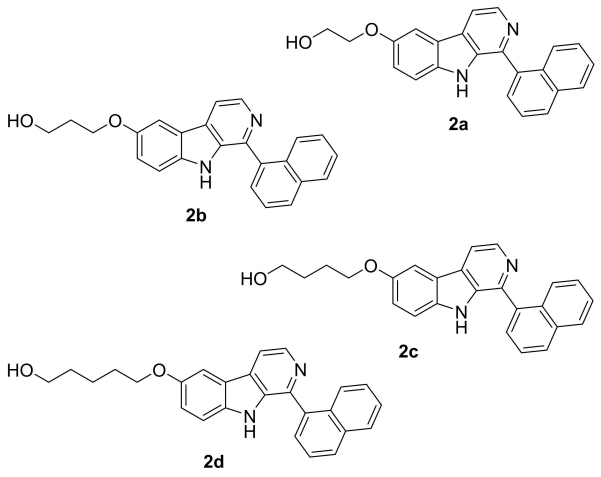


Figure 2. Structures of the hydroxyethyl-(**2a**), hydroxypropyl-(**2b**), hydroxybutyl-(**2c**) and hydroxypentyl-(**2d**) β-carboline derivatives of **1**.

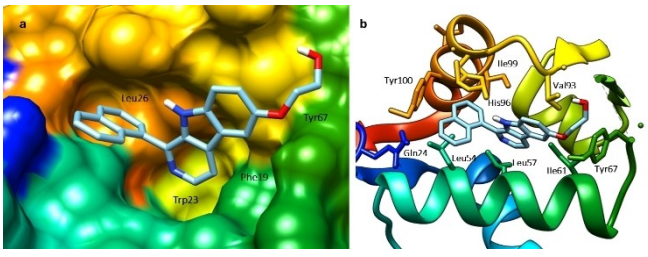
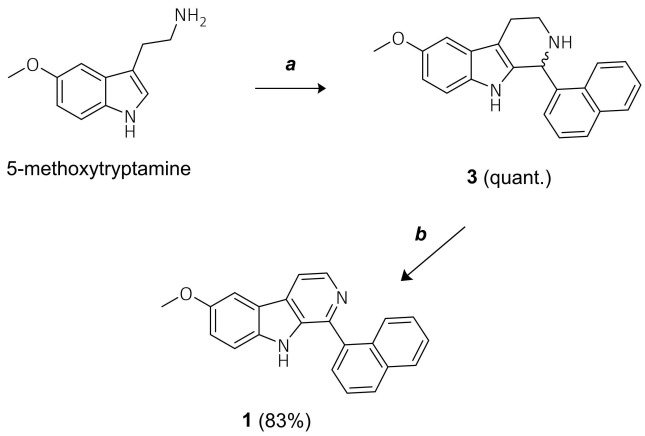


Figure 3. a) The binding pocket of the hydroxyethyl β-carboline **2a** in interaction with MDM2 (PDB: 4ere). The protein is depicted as a surface while the carboline derivative is coloured in light blue (red: oxygen, blue: nitrogen). b) A model of the interactions of the hydroxyethyl β-carboline **2a** with MDM2, rendered as cartoon, while the residues in binding with the carboline derivative are depicted as sticks.

Chemical Synthesis of Hydroxyalkyl-β-Carboline Derivatives 2a–d

The β-carboline-based MDM2 inhibitor **1** is the main precursor for the preparation of the new hydroxyalkyl derivatives. To provide a reliable and robust methodology for a gram-scale synthesis, the previously reported synthesis,[13c] comprising a straightforward 2-steps procedure with a 33% overall yield, was optimized (Scheme 1). In particular, the Pictet-Spengler reaction between the commercially available 5-methoxytryptamine and 1-naphthylaldheyde was significantly improved by working in a protic environment such as 2-propanol instead of the previously reported dichloromethane (DCM). Indeed, these conditions facilitate the proton transfer step and allow to achieve full conversion of the starting material to compound **3** with quantitative yield.[24]

The subsequent aromatization of the tetrahydro-β-carboline **3** was optimized by using 2,3-dichloro-5,6-dicyano quinone



Scheme 1. Optimized two-steps synthesis of **1**. *a.* 1-Naphtylaldehyde, TFA, 2-PrOH, reflux, 24 h; *b.* DDQ, 1,4-dioxane, reflux, 6 h.

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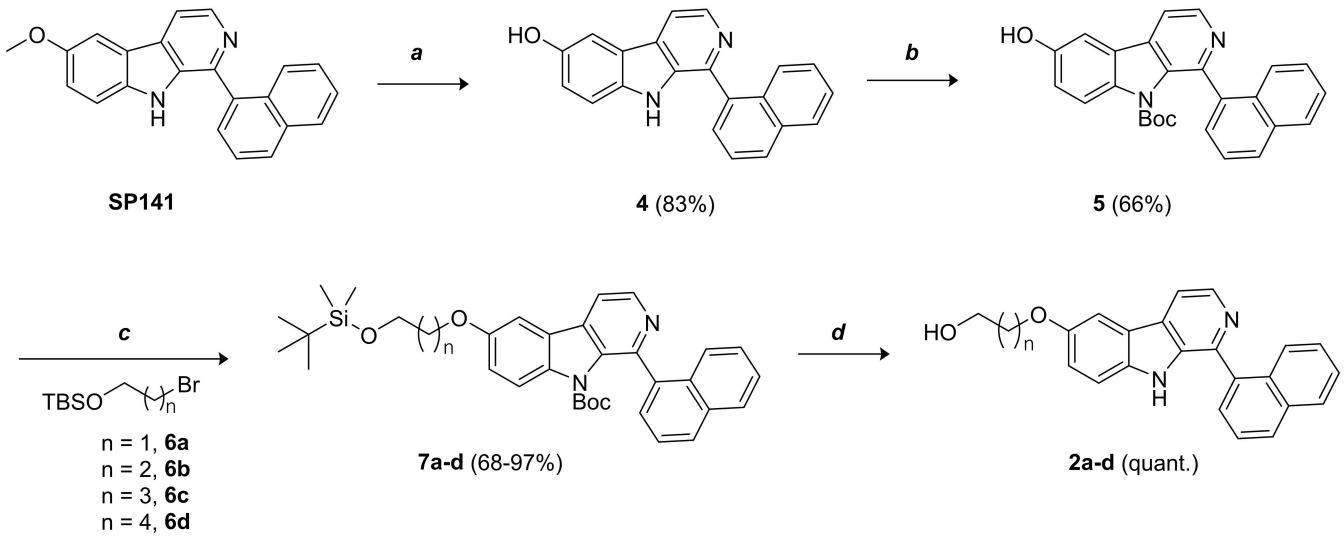


Scheme 2. Synthesis of the final hydroxyalkyl derivatives **2a**–**d**. a. BBr_3, DCM, −78 °C to r.t., 72 h; b. i) TBS–Cl, imidazole, DMF, 2 h, r.t.; ii) Boc_2O, DMAP, TEA, THF, o.n., r.t.; iii) TBAF, THF, 0.5h, r.t. c. TBSO-alkyl bromide, Cs_2CO_3, DMF, 50 °C, 6 h; d. HCl 4 M in 1,4-dioxane/MeOH, r.t., 72 h; n = 1,2,3,4.

(DDQ) as oxidant in 1,4-dioxane as solvent under reflux for 6 hours,[25] obtaining the desired compound **1** in 83% yield.

The procedure for further derivatization of the MDM2 inhibitor at position 6 is shown in Scheme 2. The methoxy group was cleaved by treatment with a boron tribromide solution in DCM, affording the 6-hydroxy-β-carboline **4** in high yield, as reported previously.[20]

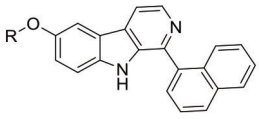
Early attempts to alkylate compound **4** at position 6, through deprotonation of the phenolic OH with Cs_2CO_3 and subsequent treatment with the suitable O-TBDMS bromide derivatives (**6a**–**d**), obtained by *O*-protection of the commercially available 1-hydroxyalkylbromides following a reported procedure,[26] failed to give reasonable yields of the desired *O*-hydroxyalkylated products. The main reason was the very poor regioselectivity of the reaction, which was demonstrated by the isolation of the *N*-alkylated compound at the indolic NH in an almost 1 to 1 ratio (see Scheme S1 of supporting information). For this reason, protection of the NH group was considered. Previous attempts to introduce the Boc group directly on **1** failed due to the acidic environment generated by the demethylation step, which led to the removal of the carbamate moiety. The desired product was finally achieved by introducing the Boc group through a one-pot-three step procedure on compound **4**.[27] The phenolic OH was at first selectively protected with a silyl-based protecting group and, to follow, the Boc group at the indolic NH was introduced. The final deprotection of the OH with TBAF allowed to achieve the pure *N*-Boc-protected intermediate **5** with an overall yield of 66%. This compound was successively reacted with **6a**–**d** to obtain TBDMS-protected alkoxy-β-carboline derivatives **7a**–**d** in optimal yields as shown in Scheme 2. To obtain the desired β-carboline hydroxyalkyl derivatives **2a**–**d**, compounds **7a**–**d** were finally treated with HCl 4 M in 1,4-dioxane/MeOH at room temperature for 72 hours for simultaneously removing the Boc and the TBDMS protecting groups with full conversion in all cases (Scheme 2).

Antiproliferation Assay on wt-p53U87MG Glioblastoma Cell Line

The β-carboline **1** was reported to exhibit sub-micromolar cytotoxicity on a large panel of glioblastoma cell lines.[13a] To evaluate if the hydroxyalkyl derivatives **2a**–**d** showed the same effect, the synthesized compounds were assayed first against U87MG glioblastoma cell line bearing wild type-p53 (wt-p53), having **1** and the additional compound **4** as controls. As indicated in Table 1, except for compound **4**, which was insoluble, all hydroxyalkyl-β-carboline derivatives **2a**–**d** retained cell growth inhibition activity in the sub-micromolar range, similar to the reference molecule **1**.

Interestingly, the cytotoxic activity slightly increased ranging from the hydroxyethyl-derivative **2a** to the hydroxypentyl-derivative **2d**, suggesting that the solvent exposed area

Table 1. Cytotoxicity of the first-class hydroxyalkyl- β-carboline derivatives 2a-d against wt-p53U87MG glioblastoma cell line.



Compound	Alkyl chain (R)	IC_{50} (μM) at 72 h on U87MG[a]
1	–CH_3	0.106 ± 0.42
4	–H	> 1000
2a	HO	0.903 ± 4.15
2b	HO	0.620 ± 1.30
2c	HO	0.548 ± 1.65
2d	HO	0.416 ± 1.05

[a] Each compound was tested in triplicates; the data are presented as mean ± SD.

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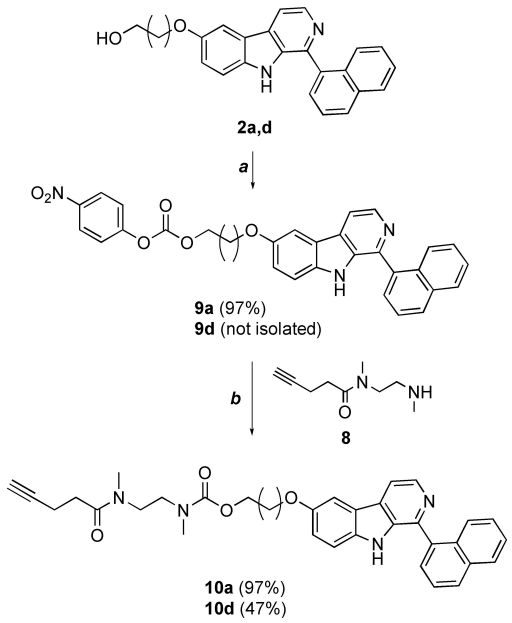
observed with our preliminary docking studies could host and presumably interact better with longer carbon chains through hydrophobic interactions. Additionally, the longer alkyl chain enhances lipophilicity, a property that generally improves passive diffusion through biological membranes, should facilitate drug transport across barriers. In turn, this might accelerate intracellular drug accumulation at MDM2 target sites.[28]

Second Generation of β-Carboline Derivatives for Drug Conjugation

With the aim of widening the possibilities of chemical conjugation and intrigued by the conserved biological activity of compounds **2a**–**d**, we decided to insert an alkyne moiety suitable for a click reaction with an azide group, which would allow the generation of uncleavable drug-conjugates. Indeed, these types of conjugates hold great importance since they exhibit high plasma stability and limited off-target toxicity.[29] After evaluating the preliminary *in-vitro* activity, docking studies and ADME properties of **2a**–**d**, the shortest and the longest hydroxyalkyl chain derivatives (compounds **2a** and **2d**) were selected for this purpose. Compound **2a** has the most similar ADME profile to the reference molecule **1**, with improved flexibility and polarity but maintaining unfavorable unsaturation. On the other side compound **2d** deviated the most from **1** profile, exhibiting a desirable lower unsaturation factor, keeping other properties within the desired range (see Figure S2 of Supporting information).

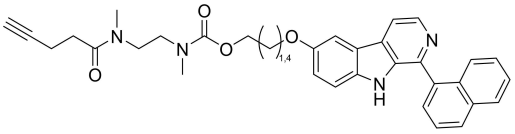
The introduction of a terminal alkyne group could potentially be achieved through several chemical strategies. For instance, any terminal alkyne with a leaving group attached to the opposite end of its carbon chain can serve as an electrophilic partner in the nucleophilic substitution reaction with compound **5**, leading to terminal alkyne derivatives of **2a**–**d**. Alternatively, an effective approach for generating uncleavable conjugates involves the use of an alkyne moiety like propargylamine or related alkynyl-amines to generate stable carbamate-containing building blocks.[30] Similarly to this strategy, we decided to introduce a terminal alkyne group into the **2a**–**d** through the formation of a carbamate moiety between the secondary amine of compound **8** and the hydroxyl group of carboline derivatives. The intermediate **8**, which derives from the reaction between 4-pentynoic acid and the *N*,*N*'-dimethylethylenediamine, was previously utilized in our studies to synthesize uncleavable conjugates, avoiding the inclusion of hydrolyzable ester bonds in the final products (Scheme 3).[15c] Our second class of β-carboline analogues was obtained by activating the hydroxyalkyl derivatives **2a** and **2d** with 4-nitrophenyl chloroformate generating the compound **9a**, which was isolated with 97% yield, and **9d** with full conversion but without isolation due to instability issues. The obtainment of the desired final alkyne-derivatives **10a** and **10d** in 97% and 47% yield, respectively, through a coupling mediated by di*iso*propylethylamine (DIPEA) and dimethylaminopirydine (DMAP), confirmed the possibility to use the hydroxyl group as accessible conjugation site (Scheme 3).

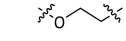
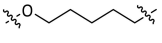


Scheme 3. Synthesis of the second generation of β-carboline derivatives **10a** and **10d**. *a.* 4-nitrophenyl chloroformate, pyridine, DCM, 0 °C to r.t., 6 h; *b.* Pentynammido-dimethylethylendiammine, DIPEA, DMAP, DMF, 0 °C to r.t., 24 h; n = 1,4.

The antiproliferative activity of these two novel compounds was tested on the U87MG glioblastoma cell line for 72 hours to evaluate the effect of further chain elongation on MDM2 binding. Table 2 shows that the potency of **10a** slightly increases compared to the parent hydroxyethyl compound **2a**, likely due to more potential interactions with the MDM2 protein afforded by the longer chain. In contrast, the potency of **10d** decreases compared to the hydroxypentyl compound **2d**, indicating that an excessively long chain can impair the overall activity (Table 2).

Table 2. Cytotoxicity of second-class hydroxyalkyl-β-carboline derivatives 10a,d against wt-p53U87MG glioblastoma cell line.



Compound	Alkyl chain	IC_{50} (μM) at 72 h on U87MG[a]
10a		0.454 ± 0.92
10d		0.617 ± 1.03

[a] Each compound was tested in triplicates; the data are presented as mean ± SD.

Antiproliferation Assay on Mutated-p53U138MG Glioblastoma Cell Line Assay

Accounting the reported MDM2 inhibition activities of **1** regardless of p53 status, we finally evaluated the two selected hydroxyalkyl derivatives **2a** and **2d** and the related alkyne derivatives **10a** and **10d** on the U138MG glioblastoma cell line displaying mutated p53.[32] In the case of the hydroalkyl derivatives **2a**, **d**, both compounds exhibit cytotoxic activity on U138 MG cell line in the sub-micromolar range, comparable to the reference compound (**1**) and confirming the conservation of the cytotoxicity in mutated p53 cancer cell lines (Table 3).

Analogous to the U87MG cell line, the hydroxypentyl-β-carboline derivative (compound **2d**) showed greater potency than the hydroxyethyl-derivative **2a**. By contrast, both alkyne **10a** and **10d** were found to be less cytotoxic, but in the same range as their hydroxyalkyl- analogues (compounds **2a** and **2d**, respectively).

Conclusions

In summary, in this work we investigated the potential to introduce a suitable conjugation site in the β-carboline core of the promising MDM2 inhibitor **1** without negatively affecting its original antitumor activity. Based on preliminary docking studies, the design and the synthesis of novel β-carboline analogues of **1** was performed substituting the methoxy group at the 6-position with linear hydroxyalkyl chains (compounds **2a**–**d**), displaying the hydroxyl group as functionalizable moiety. The chemical synthesis of all derivatives was accomplished with high yields, and the antiproliferative activities on U87MG glioblastoma cancer cell line bearing wt-p53 confirmed the tolerability of structure modifications at the selected site of the heterocycle core. *In silico* ADME studies identified **2a** and **2d** as the most promising candidates, which were similarly active on the U138MG glioblastoma cell line displaying mutated-p53 protein, confirming the unique feature of **1** compared to other classes of MDM2 inhibitors. The increased polarity of these derivatives might also help improve their solubility. The potential to exploit hydroxyl function as conjugation site was validated by successfully obtaining 4-pentynoic-*N,N'*-dimethylethylenediamide derivatives, in which a terminal alkyne group could undergo a click chemistry azide-alkyne cycloaddition. In future perspective, the successful incorporation of the amine-carbamate self-immolative spacer *N,N'*-dimethylethylenediamine could also enable the development of cleavable conjugates. In such systems, the hydroxyalkyl-β-carbolines **2a**,**d** could be selectively released within or in proximity of tumor cells upon specific external stimuli, such as environmental conditions or proteolytic enzymes. Additionally, the primary alcohol group of **2a**–**d** might also offer flexibility in release kinetics; indeed, depending on the design, it could result in either slow[31a] or rapid release rates,[31b] making these conjugates adaptable to a variety of drug delivery technologies.

Finally, the β-carboline analogues **10a**, **d** were easily obtained in high yields even without the protection of the indolic NH, and both showed IC_{50} in the same sub-micromolar range of the parent compounds.

In conclusion, we established a new synthetic route to obtain 6-hydroxyalkyl-β-carboline compounds as MDM2 inhibitors providing a suitable site for further conjugation. Indeed, with a growing number of cases worldwide, innovative therapeutic strategies are currently under investigation to overcome the several drawbacks of traditional antitumor therapies. In this context, conjugating an MDM2 inhibitor agent with a different chemical moiety capable of improving the efficacy and/or the tumor specificity may lead to more effective treatments while helping to avoid drug resistance, thus positively impacting the recovery of the patients.

Table 3. Cytotoxicity of compounds **2a**, **2d**, **10a** and **10d** against mutated p53U138MG glioblastoma cell line.

Compound	IC_{50} (μM) at 72 h on U138MG[a]
1	0.130 ± 0.30
2a	0.467 ± 1.94
2d	0.352 ± 1.50
10a	0.611 ± 2.15
10d	0.812 ± 5.04

[a] Each compound was tested in triplicates; the data are presented as mean ± SD.

Acknowledgements

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Antitumor Agents · Drug Delivery · Protein-Protein Interactions · Carboline

[1] L. Cahilly-Snyder, T. Yang-Feng, U. Francke, D. L. George, *Somatic Cell Mol. Genet.* **1987**, *13*, 235.
[2] W. Wang, N. Albadari, Y. Du, J. F. Fowler, H. T. Sang, W. Xian, F. McKeon, W. Li, J. Zhou, R. Zhang, *Pharmacol. Rev.* **2024**, *76*, 414.
[3] D. P. Lane, *Nature* **1992**, *358*, 15.

[4] a) K. H. Vousden, C. Prives, *Cell* **2009**, *137*, 413; b) M. B. Kastan, C. E. Canman, C. J. Leonard, *Cancer Metastasis Rev.* **1995**, *14*, 3; c) E. R. Kastenhuber, S. W. Lowe, *Cell* **2017**, *170*, 1062; d) M. Hemann, S. Lowe, *Cell Death Differ.* **2006**, *13*, 1256–1259; e) K. Engeland, *Cell Death Differ.* **2022**, *29*, 946–960.
[5] a) C. G. Maki, *J. Biol. Chem.* **1999**, *274*,16531; b) A. J. Levine, *Nat. Rev. Cancer* **2020**, *20*, 471–480.
[6] a) P. A. Muller, K. H. Vousden, *Nat. Cell Biol.* **2013**, *15*, 2; b) M. Wade, Y. C. Li, G. M. Wahl, *Nat. Rev. Cancer* **2013**, *13*, 83.
[7] H. Hou, D. Sun, X. Zhang, *Cancer Cell Int.* **2019**, *19*, 216.
[8] a) H. Q. Wang, I. J. Mulford, F. Sharp, J. Liang, S. Kurtulus, G. Trabucco, D. S. Quinn, T. A. Longmire, N. Patel, R. Patil, M. D. Shirley, Y. Chen, H. Wang, D. A. Rudd, C. Fabre, J. A. Williams, P. S. Hammerman, J. Mataraza, B. Platzer, E. Halilovic, *Cancer Res.* **2021**, *81* (11), 3079–3091; b) W. Wang, N. Albadari, Y. Du, J. F. Fowler, H. T. Sang, W. Xian, F. McKeon, W. Li, J. Zhou, R. Zhang, *Pharmacol. Rev.* **2024**, *76* (3), 414–453.
[9] P. H. Kussie, S. Gorina, V. Marecha, B. Elenbaas, J. Moreau, A. J. Levine, N. P. Pavletich, *Science* **1996**, *274*, 948.
[10] Y. Yao, Q. Zhang, Z. Li, H. Zhang, *Cancer Cell Int.* **2024**, *24*, 170.
[11] a) H. Zhu, H. Gao, Y. Ji, Q. Zhou, Z. Du, L. Tian, Y. Jiang, K. Yao, Z. Zhou, *J. Hematol. Oncol.* **2022**, *15*, 91; b) L. Haronikova, O. Bonczek, P. Zatloukalova, F. Kokas-Zavadil, M. Kucerikova, P. J. Coates, R. Fahraeus, B. Vojtesek, *Cell. Mol. Biol. Lett.* **2021**, *26*, 53.
[12] X. Chen, T. Zhang, W. Su, Z. Dou, D. Zhao, X. Jin, H. Lei, J. Wang, X. Xie, B. Cheng, Q. Li, H. Zhang, C. Di, *Cell Death Dis.* **2022**. *13*, 974.
[13] a) S. R. Punganuru, V. Artula, W. Zhao, M. Rajaei, H. Deokar, R. Zhang, J. K. Buolamwini, K. S. Srivenugopal, W. Wang, *Cells.* **2020**, *9*, 1592; b) S. A. Patil, J. K. Addo, H. Deokar, S. Sun, J. Wang, W. Li, D. P. Suttle, W. Wang, R. Zhang, J. K. Buolamwini, *Drug Des.* **2017**, *6*, 143; c) W. Wang, J. J. Qin, S. Voruganti, K. S. Srivenugopal, S. Nag, S. Patil, H. Sharma, M. H. Wang, H. Wang, J. K. Buolamwini, R. Zhang, *Nat. Commun.* **2014**, *5*, 5086.
[14] J. J. Qin, W. Wang, S. Sarkar, R. Zhang, *J. Controlled Release* **2016**, *237*, 101.
[15] a) M. Zambra, I. Ranđelović, F. Talarico, A. Borbély, L. Svajda, J Tóvári, G. Mező, L. Bodero, S. Colombo, F. Arrigoni, E. Fasola, S. Gazzola, U. Piarulli, *Front. Pharmacol.* **2023**, *14*, 1215694; b) L. Bodero, S. Parente, F. Arrigoni, A. Klimpel, I. Neundorf, S. Gazzola, U. Piarulli, *Eur. J. Org. Chem.* **2021**, *2021* (17), 2383; c) A. R. M. Dias, L. Bodero, A. Martins, D. Arosio, S. Gazzola, L. Belvisi, L. Pignataro, C. Steinkühler, A. Dal Corso, C. Gennari, U. Piarulli, *ChemMedChem* **2019**, *14*, 938; d) L. Feni, S. Parente, C. Robert, S. Gazzola, D. Arosio, U. Piarulli, I. Neundorf, *Bioconjugate Chem.* **2019**, *30*, 2011.
[16] H. P. Gerber, P. Sapra, F. Loganzo, C. May, *Biochem. Pharmacol.* **2016**, *102*, 1e6.
[17] O. Trott, A. J. Olson, *J. Comp. Chem* **2010**, *31*, 455.
[18] H. Deokar, M. Deokar, W. Wang, R. Zhang, J. K. Buolamwini, *Med. Chem. Res.* **2018**, *27*, 2466–2481.
[19] P. H. Kussie, S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A. J. Levine, N. P. Pavletich, *Science* **1996**, *274*, 948.
[20] S. K. Chitneni, Z. Zhengyuan, B. E. Watts, M. R. Zalutsky, *Pharmaceuticals* **2021**, *4*, 358.
[21] F. Lovering, J. Bikker, C. Humblet, *Med. Chem.* **2009**, *52* (21), 6752–6.
[22] a) SwissADME. (n.d.). "Molecular Modeling Group of the SIB | Swiss Institute of Bioinformatics." can be found under: http://www.swissadme.ch/ **2024**; b) A. Daina, O. Michielin, V. Zoete, *Sci. Rep.* **2017**, *7*, 42717.
[23] a) E. Kraynov, A. V. Kamath, M. Walles, E. Tarcsa, A. Deslandes, R. A. Iyer, A. Datta-Mannan, P. Sriraman, M. Bairlein, J. J. Yang, M. Barfield, G. Xiao, E. Escandon, W. Wang, D. A. Rock, N. V. Chemuturi, D. J. Moore, *Drug Metab. Dispos.* **2016**, *44* (5), 617–623; b) M. Dorywalska, P. Strop, J. A. Melton-Witt, A. Hasa-Moreno, S. E. Farias, M. G. Casas, K. Delaria, V. Lui, K. Todd Poulsen, J. Sutton, G. L. Bolton, D. Zhou, L. Moine, R. Dushin, T.-T. Tran, S.-H. Liu, M. Rickert, D. Foletti, D. L. Shelton, J. Pons, A. Rajpal, *PLoS One* **2015**, *10* (7), e0132282.
[24] a) S. Kumar, Y.-H. Wang, P.-J. Chen, Y.-C. Chang, H. K. Kashyap, Y.-C Shen, H.-P. Yu, T.-L. Hwang, *Bioorg. Chem.* **2021**, *111*, 104846; b) L.-N. Wang, S.-L. Shena, J. Qu, *RSC Adv.* **2014**, *4*, 30733.
[25] a) C. A. Foley, Y. A. Al-Issa, K. P. Hiller, S. P. Mulcahy, *ACS Omega* **2019**, *4*, 9807; b) T. J. Hagen, K. Narayanan, J. Names, J. M. Cook, *J. Org. Chem.* **1989**, *54*, 2170.
[26] M. A. Christiansen, M. B. Andrus, *Tetrahedron Lett.* **2012**, *53* (36), 4805.
[27] a) D. Svestka, J. Otevrel, P. Bobal, *Adv. Synth. Catal.* **2022**, *364*, 2174; b) A. Purtsas, M. Rosenkranz, E Dmitrieva, O Kataeva, H–J Knölker *Chem. Eur. J.* **2022**, *28*, e202104292.
[28] a) A. Mandal, M. Patel, Y. Sheng, A. K. Mitra *Curr. Drug Targets.* **2016**, *17* (15), 1773–1798; b) S. Han, L. Mei, T. Quach, C. Porter, N. Trevaskis *Pharm. Res.* **2021**, *38* (9), 1497–1518.
[29] L. Gong, H. Zhao, Y. Liu, H. Wu, C. Liu, S. Chang, L. Chen, M. Jin, Q. Wang, Z. Gao, W. Huang, *Acta Pharm. Sin. B* **2023**, *13*, 3659.
[30] R. Walther, M. Park, N. Ashman, M. Welch, J. S. Carroll, D. R. Spring *Chem. Commun.* **2024**, *60* (55), 7025.
[31] a) M. Mason, L. Bisbal Lopez, F. Bashiri, A. Herrero, A. Baron, R. Bucci, L. Pignataro, C. Gennari, A. Dal Corso, *ChemBioChem* **2024**, *25*, e202400174; b) R. V. Kolakowski, K. T. Haelsig, K. K. Emmerton, C. I. Leiske, J. B. Miyamoto, J. H. Cochran, R. P. Lyon, P. D. Senter, S. C. Jeffrey, *Angew. Chem. Int. Ed.* **2016**, *55*, 7948.
[32] M. M. Pedrote, M. F. Motta, G. D. S. Ferretti, D. R. Norberto, T. C. L. S. Spohr, F. R. S. Lima, E. Gratton, J. L. Silva, G. A. P. De Oliveira, *iScience.* **2020**, *23* (2), 100820.
[33] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923.

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