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Pre-mRNA Splicing-Modulatory Pharmacophores: The Total Synthesis of Herboxidiene, a Pladienolide–Herboxidiene Hybrid Analog and Related Derivatives

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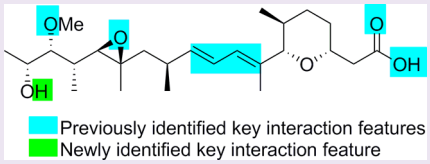
Supporting Information

ABSTRACT: Herboxidiene is a natural product that has previously been shown to exhibit antitumor activity by targeting the spliceosome. This activity makes herboxidiene a valuable starting point for the development of anticancer drugs. Here, we report an improved enantioselective synthesis of herboxidiene and the first report of its biologically active totally synthetic analog: 6-norherboxidiene. The synthesis of the tetrahydropyran moiety utilizes the novel application of inverse electron-demand Diels–Alder chemistry and the Ferrier-type rearrangement as key steps. We report, for the first time, cytotoxicity IC_{50}s for synthetic herboxidiene and analogs in human tumor cell lines. We have also demonstrated that synthetic herboxidiene and its analogs can potently modulate the alternate splicing of MDM-2 pre-mRNA.



The prominence of pre-mRNA splicing in gene expression in higher eukaryotes makes it an attractive target for therapeutic intervention, and recently this process has emerged as a potentially important therapeutic target in cancer.[1−4] The pre-mRNA splicing process involves the removal of introns (noncoding sequences) from pre-mRNA, which is followed by ligation of exons (coding sequences).[1] Alternative splicing is the mechanism by which different forms of mature mRNAs are generated from the same gene. Commonly, alternative splicing patterns determine the inclusion or exclusion of portions of the coding sequence in the mRNA, giving rise to protein isoforms that differ in their peptide sequence and hence in their chemical and biological activity.[1,5] Alternative splicing plays important roles in the development of multicellular organisms and in numerous pathologies, including cancer.[3,6] This splicing process is catalyzed by a macromolecular complex called the spliceosome, which is composed of five small nuclear ribonucleoproteins (snRNPs) (U1, U2, U4, U5, and U6) and over 150 associated proteins.

Several bacterial natural products such as herboxidiene (**1** also known as GEX1A),[7−9] pladienolide B (**2**),[10−13] FR901464[14−16] and the thailanstatins[17] have been shown to effect splicing by targeting the splicing factor 3b (SF3b) subunit, an essential component of the spliceosome. Several of these natural products also induce cell cycle arrest at the G1 and G2/M phase and show potent antitumor activity in human tumor cell lines.[11,14,18,19] Importantly, several of these natural products have also been reported to show potent *in vivo* activity in tumor xenograft models.[11,23] Ongoing synthetic work[20−27] has also provided novel synthetic spliceosome modulators such as 1-deoxy FR901464,[23] and the meayamycins[23,24] (all analogs of FR901464), as well as new structure–activity relationships for FR901464 analogs.[23] Indeed, one of the semisynthetic derivatives of **2** (E7107) has shown remarkable preclinical tumor regression efficacy and advanced to phase I clinical trials,[12] which stimulated considerable interest in the potential of splicing modulators as potential therapeutic agents for the treatment of cancer.[4,27,28] Very recently, interesting active-analogs of another natural product (FD-895), which is structurally related to **2**, have also been reported.[27]

RESULTS AND DISCUSSION

We have also been engaged in a successful effort to design new effective and highly active synthetic analogs of FR901464 and **2** by the application of a consensus pharmacophore hypothesis.[29−31] We believed that we could extend our approach by the generation of a new structurally simplified scaffold based on herboxidiene. Thus, using the guidance of our pharmacophore hypothesis, we designed a hybrid molecule (**3**) from herboxidiene (**1**) and pladienolide B (**2**), (Figure 1, Scheme 1). This hybrid **3** has the tetrahydropyran core of **1** (red) and side chain of **2** (blue). Based on our pharmacophore model, we anticipated that compound **3** could incorporate the correct molecular geometry as well as all of the key SF3B1 interaction features.[29]

For the synthesis of the hybrid analog **3** we coupled aldehyde **16b** with the Julia–Kocienski reagent **4** that has been used in the total synthesis of pladienolide, which was prepared as reported (Supporting Information, Scheme S1).[32] We were initially surprised when we tested the cytotoxicity of our hybrid molecule **3** in various human tumor cell lines and it was found

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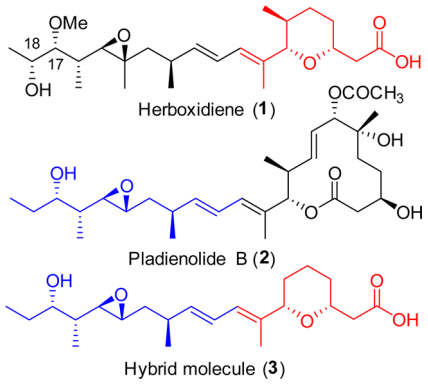
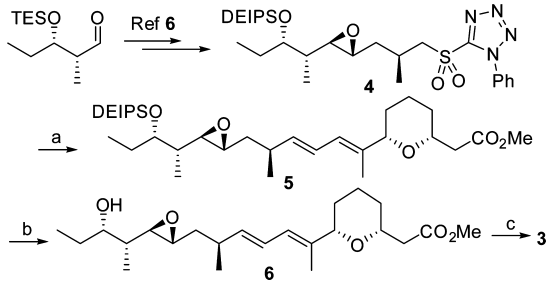


Figure 1. Structures of splicing modulators: herboxidiene (**1**), pladienolide (**2**), and our synthetic hybrid molecule (**3**).

Scheme 1. Synthesis of the Pladienolide–Herboxidiene Hybrid Molecule 3[a]



[a]Reagents and conditions: (a) **16b**, KHMDS, THF, −78 °C, 1.5 h, 46%; (b) TBAF, THF, 76%; (c) $KOSiMe_3$, THF, rt, 79%.

inactive (IC_{50} > 20 μM). In these assays, we used natural **1** (prepared by bacterial fermentation, purchased from Cfm Oskar Tropitzsch e.K) as a standard. For comparison, we also referred to the reported activity of **2** (IC_{50}s in the nanomolar range).[10,33] Based on these data we hypothesized that the hydrogen-bond donor (OH) at C18 represents an additional new pharmacophore feature in herboxidiene and that this key functionality is required in order to effectively interact with SF3B1 (see Figure 2). To investigate our hypothesis, we next turned our attention toward the synthesis of herboxidiene and its analogs that maintain this hydrogen-bond donor feature.

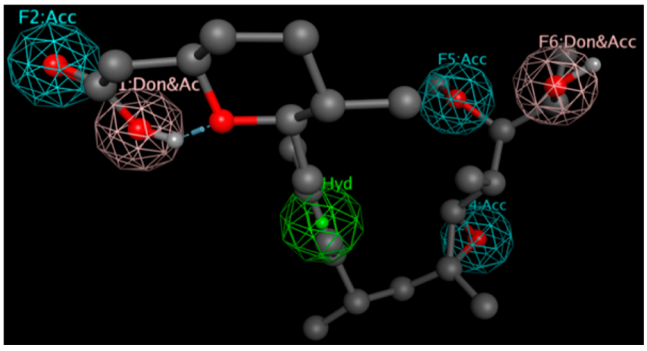


Figure 2. Hypothetical pharmacophore features for herboxidiene.[4] The features are F1, Donor (Don) and acceptor (Acc); F2, Acc; F3, Hydrophobic (Hyd); F4, Acc (epoxide); F5, Acc; and F6, Don and Acc. The pharmacophore is calculated using the Molecular Operating System 2011.10 from the Chemical Computing Group, Inc.

Herboxidiene (**1**) is a bacterial secondary metabolite, originally isolated from the *Streptomyces chromofuscus* strain A7847 by Isaac et al. and initially evaluated as a herbicide.[7] Subsequently, Edmunds et al. determined the absolute configuration of **1**,[8] which was further confirmed by the first total synthesis by Blakemore and Kocienski.[34] Importantly, a study by Horiguchi et al. also reported the *in vivo* antitumor activity of **1** in a murine tumor model.[23] Of particular interest to our lab was the recent photoaffinity-labeling study, which found that the SF3b1 (SAP155) protein is also the major target of **1**.[9] Because **1** has a simpler structure, when compared to the other known splicing modulators, it is an especially attractive starting point for the development of structure–activity relationship (SAR) studies. Therefore, **1** represents a unique opportunity for the discovery of drug-like synthetic agents for SF3b modulation.

In the realm of herboxidiene total synthesis, impressive progress has been made recently.[35−40] However, to the best of our knowledge, no study on the evaluation of the biological activity of synthetic **1** and its analogs in human cancer cell lines has been reported to date. A recent publication by Koide and co-workers provides the only report on the biological activity of synthetic **1** and this is in a nontumor (HEK293) cell line.[41] Therefore, as a first step in exploring totally synthetic analogs of **1**, as potential antitumor therapeutics, we embarked on the total synthesis of the natural product herboxidiene and its close analogs.

Our assembly of **1** and its analogs took advantage of a convergent strategy in which tetrahydropyran cores **16a**/**16b** would be coupled with a herboxidiene side-chain precursor through the versatile Julia–Kocienski olefination reaction. We envisioned the synthesis of aldehydes **16a**/**16b** from **14a**/**14b,** which in turn could be derived via Ferrier-type rearrangement of compounds **9a**/**9b.** We planned to construct dihydropyranones **9a**/**9b** using an inverse electron-demand Diels–Alder reaction between (*S*)-2-(benzyloxy)propanal (**7**) and diene **8a** or **8b**. The sulfone **19** can be derived from alcohol **18**, which is conveniently prepared by an efficient method reported by Urpi and co-workers.[35]

Our tetrahydropyran synthesis commenced with the preparation of diene **8a**/**8b** (as a 10:1 mixture of *Z* and *E* isomers) using a method reported by Li et al.[43] The $MgBr_2$ mediated inverse electron-demand Diels–Alder reaction of diene **8a** or **8b** and (*S*)-2-(benzyloxy)propanal (**7**) then provided dihydropyranones **9a** and **9b** as single diastereomers.[42] The reduction of the ketone moiety was achieved under Luche conditions to selectively provide the alcohols **10a** and **10b**. The latter, in turn, were acetylated with Ac_2O and Et_3N to provide the corresponding acetates **11a** and **11b**. In an effort to obtain the tetrahydropyran core, we initially investigated application of the Ireland–Claisen rearrangement of **11a**, which has been used in the construction of analogous tetrahydropyrans.[44] We anticipated that Ireland–Claisen conditions, followed by esterification with CH_2N_2 would lead to **12a**. Unfortunately, in our hands this reaction sequence failed to provide the desired products in acceptable yields. We therefore pursued the alternative route to these intermediates that installed the desired stereochemistry and ester moiety in one step via a Ferrier-type rearrangement using a silylketene acetal.[45−47] The stereochemical outcome of this reaction has previously been shown to be highly dependent on the conditions employed.[49] We developed conditions for this transformation that provided a 2:1 mixture of *cis* and *trans* fused

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substituted dihydropyrans **12a** (in 72% yield) and **12b** (in 70% yield). This ratio is identical to that reported in a related C-glycolsylation reaction of this type.[47] We found that *cis* and *trans* isomers of **12a** and **12b** were readily separated by flash chromatography to give the stereochemically pure products. The relative stereochemistry of both isomers was established by NOESY experiments and by single crystal X-ray structures of 4-nitrophenyl ester derivatives (see Figure 3 and Supporting

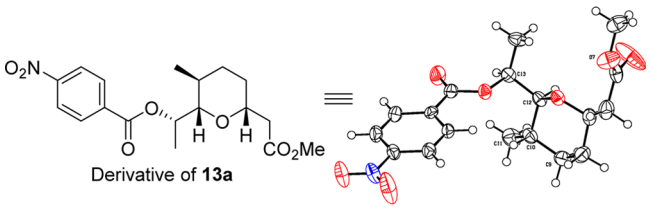


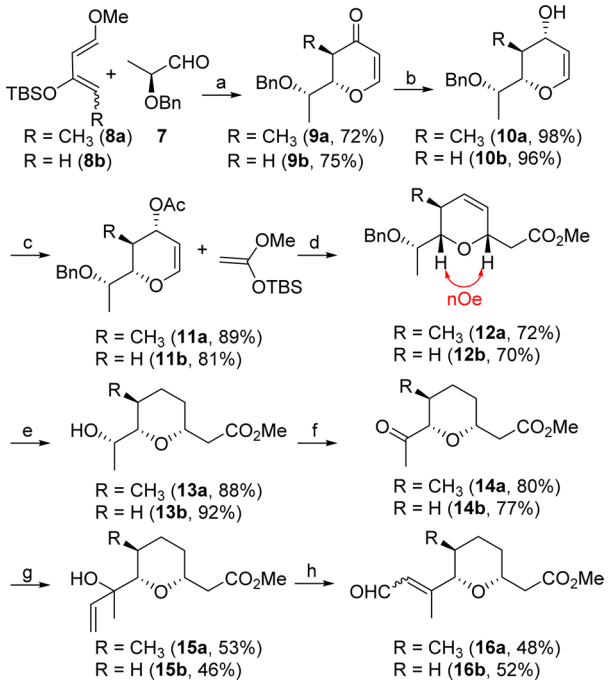
Figure 3. X-ray structure of nitrobenzoyl derivative of **13a**.

Information (SI)). At this point, the simultaneous reduction of the alkene moiety and benzyl ether cleavage of **12a** and **12b** was accomplished by catalytic hydrogenation. Next, the oxidation of the resulting alcohols **13a** and **13b** yielded the corresponding ketones **14a** and **14b**. Grignard reaction of **14a** and **14b** with vinylmagnesium bromide then furnished the tertiary alcohols **15a** and **15b** as a mixture of diastereomers. Finally, PCC oxidation of **15a** or **15b** afforded the desired aldehydes **16a** and **16b** as inseparable mixtures of *E*:*Z* isomers (7:2). (We found, however, that the final products could be separated to give pure *E* derivatives, see Scheme 3 and discussion below). This concise route toward the tetrahydropyran core of **1** represents a significant advancement in the syntheses of the key intermediate **14a**.[35−39]

We found that the method shown in Scheme 2 could provide compound **14b** in 6 steps in an overall yield of 25% (see SI). Our improved route shown in Scheme 2 was also more efficient for the synthesis of aldehyde **16b**. In addition to the route shown in Scheme 2, we also successfully implemented a known approach to **14b** via oxa-Michael cyclization followed by functionalization from the resulting *cis* fused pyran using a published procedure (see SI, Scheme S2).[48] We were also able to confirm the relative and absolute stereochemistry of **14a** and **14b** using single crystal X-ray crystallography of derivatives (see SI: Schemes S3 and S4, Figure S1, and Figure S2).

We then turned our attention to the acyclic side chain of **1**. Recently, Urpi and co-workers reported an efficient method for the synthesis of alcohol **10** starting from the commercially available ester **17** in nine linear steps.[23] Using this procedure, with some minor modifications, we were able to synthesize compound **18** on a multigram scale (see SI). The resulting substrate **18** was subjected to a Mitsunobu reaction with 1-phenyl-1H-tetrazole-5-thiol, followed by ammonium molybdate-catalyzed oxidation, to provide sulfone **19**. With sulfone **19** and aldehyde **16a** and **16b** in hand, we next focused on the convergent coupling to provide **20a** and **20b**. These products were obtained in moderate yields in the presence of KHMDS via the Julia−Kocienski olefination. Removal of the *tert*-butyldimethylsilyl group in **20a** and **20b** with HCl, followed by C18 hydroxyl directed selective epoxidation of alcohols **21a** and **21b** with catalytic $VO(acac)_2$ provided the pure diastereomers **22a** and **22b** in good yields, as expected based on the literature precedence.[35−38] The purification step at this point also readily removed the minor C9,C10-Z isomer as

Scheme 2. Synthesis of Tetrahydropyran 16a/16b[a]



[a]*Reagents and conditions*: (a) $MgBr_2$, THF, rt, 12 h; (b) $CeCl_3 \cdot 7H_2O$, $NaBH_4$, MeOH, 30 min; (c) Ac_2O, Et_3N, CH2Cl2, 0 °C, 1 h; (d) Ti(*i*-OPr)$_2Cl_2$, toluene, −78 °C to rt, 16 h, (*cis*:*trans* = 2:1); (e) H_2, Pd/C, EtOAc, 1 h; (f) Dess−Martin periodinane (DMP), CH_2Cl_2, 30 min; (g) vinylmagnesium bromide, CH_2Cl_2, −78 °C, 24 (h) Pyridinium chlorochromate (PCC), CH_2Cl_2, 40 °C, 18 h, *E*:*Z* = 7:2.

shown in Scheme 3 (see SI), which had been carried over from **16a/16b**. Hydrolysis of the methyl esters **22a** and **22b** under basic conditions afforded pure herboxidiene (**1**) and C6-norherboxidiene (**23**). This series of reactions also provided important analogs for SAR studies, such as **21a**, **22a**, and **22b**. Additionally we prepared the C18 ketone derivative **24** by oxidation of **22b** with DMP, in order to confirm the importance of the C18 hydrogen-bond donor feature in the herboxidiene pharmacophore.

Next, we evaluated the cytotoxicity of both natural and synthetic **1** and its analogs on six human cancer cell lines (Table 1). The IC_{50} values of synthetic **1** were found to be in the low nanomolar range (4.5−22.4 nM), which was comparable to the results with the natural **1** (4.3−46.3 nM). Surprisingly, the methyl ester precursor of **1** (compound **22a**) also displayed highly potent cytotoxicity (IC_{50} = 6.2−15.8 nM). However, a great loss in activity (>200-fold) was observed for triene **21a** as compared to its epoxide derivative **22a**. This result is consistent with the early reports of the herbicidal activity dependence on an intact epoxide group in several semisynthetic analogs of herboxidiene.[7] C6-Norherboxidiene (**23**) showed a loss in activity (∼10-fold) indicating that this methyl group is contributing to the potency of **1**. Most importantly, when the ketone analog **24** was evaluated, it was found to be essentially inactive, with IC_{50} values in micromolar range (>5−25 μM). This low activity of **24** clearly supports our hypothesis that the hydrogen-bond donor (OH) interaction feature at C18 is required for the potency of this class of compounds in addition to the other features previously described (Figure 2).[4,29] This result is also consistent with the lack of activity seen in the hybrid molecule **3**.

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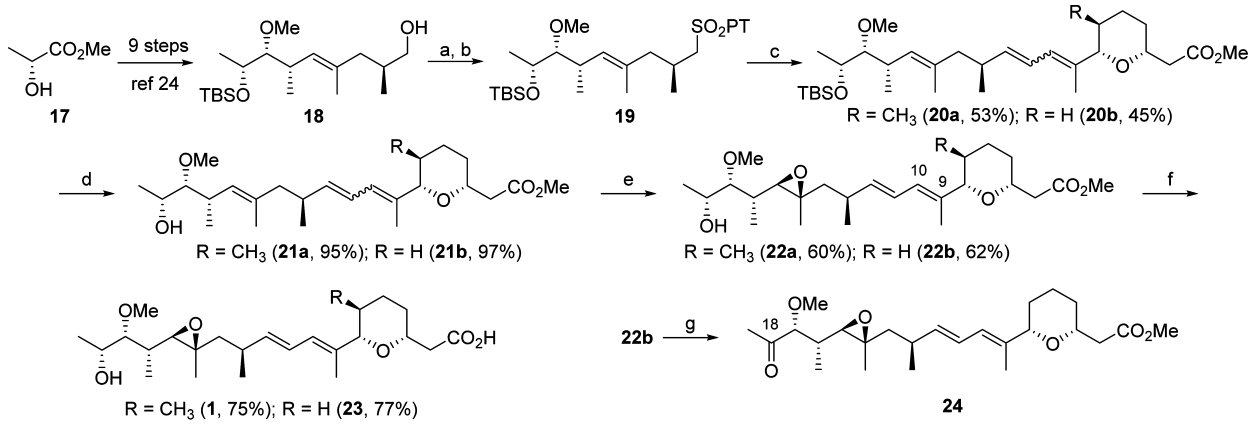
Scheme 3. Synthesis of Herboxidiene (1) and Its Analogs[a]



[a]*Reagents and conditions*: (a) Diisopropyl azodicarboxylate (DIAD), 5-mercapto-1-phenyltetrazole, PPh_3, THF, rt, 3 h, 89%; (b) $(NH_4)_6Mo_7O_{24}$, 30% H_2O_2, EtOH, rt, 24 h, 88%; (c) KHMDS, **16a or 16b**, THF, −78 °C, 1.5 h, (*E*:*Z* = 7:2); (d) 0.16 M HCl, MeOH, (e) $VO(acac)_2$, *t*-BuOOH, CH_2Cl_2; (f) $KOSiMe_3$, THF; (g) Dess−Martin periodinane (DMP), CH_2Cl_2, 55%. PT = phenyltetrazole.

Table 1. Cytotoxicity of Herboxidiene and Its Analogs in Various Human Cancer Cell Lines

		IC_{50} (nM)[b]						
entry	cell Lines[a]	natural **1**	synthetic **1**	**21a**	**22a**	**22b**	**23**	**24**
1	JeKo-1	4.3 ± 1.5	4.5 ± 0.7	1306 ± 660	12.0 ± 1.8	310 ± 57	55.2 ± 7.2	>4700
2	HeLa	14.7 ± 2.5	6.8 ± 1.4	903 ± 137	11.0 ± 2.3	310 ± 37	74.7 ± 3.8	>7600
3	PC-3	46.3 ± 5.2	22.4 ± 4.2	2022 ± 562	18.9 ± 1.9	266 ± 41	256 ± 35.0	>28000
4	SK-MEL-2	34.0 ± 32.0	14.0 ± 11.0	>1800	20.0 ± 18	1243 ± 187	301 ± 293	>240000
5	SK-N-AS	12.7 ± 2.3	6.5 ± 0.9	859 ± 165	14.7 ± 2.0	646 ± 286	90.5 ± 8.9	>5000
6	WiDr	12.0 ± 1.9	7.2 ± 0.3	1271 ± 594	8.1 ± 1.2	246 ± 42	122 ± 20	>5400

[a]Cancer Type: JeKo-1 (Mantle cell lymphoma); HeLa (Cervical adenocarcinoma); PC-3 (Prostate adenocarcinoma); SK-MEL-2 (Malignant melanoma); SK-N-AS (Neuroblastoma); WiDr (Colorectal adenocarinoma). [b]Cells were treated continuously with the compound for 72 h. Cytotoxicity was determined as the IC_{50} values calculated from the percentage of viable cells remaining at 72 h, measured with CellTiter-Glo reagent. The IC_{50} values represent the average of three independent determinations ± SE.

Previously, we have reported the use of an assay for the evaluation of the efficacy of alternative splicing modulator drugs.[31,49] This assay depends on our observation that the splicing modulator drugs that we have evaluated induce exon skipping in *MDM2* pre-mRNA, which results in the formation of shorter isoforms that can readily be detected (see SI, Figure S3). In addition to our investigation of the cytotoxicity of these compounds we also performed this *MDM2* alternative splicing assay for compounds **1**, **22a**, and **23**, in order to determine whether these compounds modulate the splicing machinery. This assay was performed as previously described.[49] The results shown in Figure 4 demonstrate for the first time that both natural and synthetic **1** potently induce *MDM2* alternate splicing of *MDM2* pre-mRNA. Synthetic **1** appears to be slightly more potent in this induction (Figure 4), we believe this is due to the higher purity of the synthetic material when compared to the commercially available fermentation product (~92% pure). Although **22a** and **23** are less potent than **1**, it is clear that these two compounds are effective in the induction of alternative splicing of *MDM2* pre-mRNA and that the potency of **22a** is superior to sudemycin C1. All of these results are consistent with the pharmacophore features shown in Figure 2.

In conclusion, the flexible and concise syntheses of herboxidiene and its previously unexplored analogs were accomplished by the orchestration of synthetic schemes that build on published chemistry, which include the novel application of the inverse electron-demand Diels−Alder reaction, a Ferrier-type rearrangement as well as the established

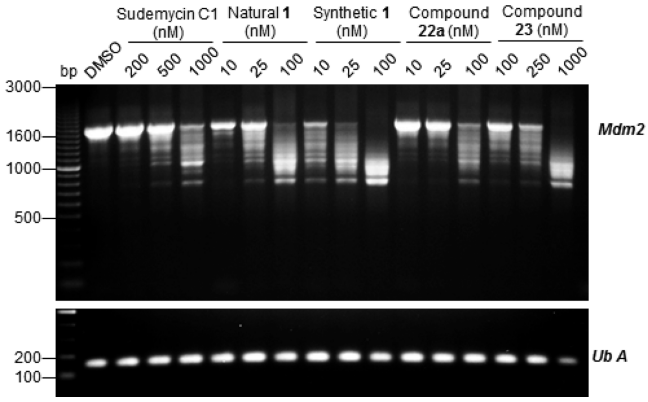


Figure 4. Herboxidiene and its analogs modulate alternate splicing of *MDM2* in Rh18 cells. The cells are exposed to drug for 8 h in these experiments (see SI for assay details).[49]

Julia−Kocienski reaction, as key steps. Initial cytotoxicity assays of the synthetic compounds provided a good initial insight into the pharmacophore features for this class of compounds. We have also demonstrated that synthetic herboxidiene and its potent analogs efficiently modulate the alternative mRNA splicing. This study provides strong evidence that synthetic herboxidiene analogs may serve as good anticancer drug lead candidates in the discovery of new synthetic agents targeting the pre-mRNA splicing process.

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METHODS

The methods are reported in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and methods, ORTEP images for X-ray structures, full characterization data and copies of spectra. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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