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Article

Selection of Natural Compounds with HMGA-Interfering Activities and Cancer Cell Cytotoxicity

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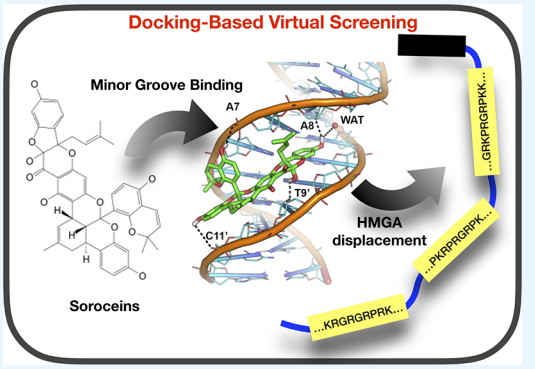
ABSTRACT: HMGA proteins are intrinsically disordered (ID) chromatin architectural factors characterized by three DNA binding domains (AT-hooks) that allow them to bind into the DNA minor groove of AT-rich stretches. HMGA are functionally involved in regulating transcription, RNA processing, DNA repair, and chromatin remodeling and dynamics. These proteins are highly expressed and play essential functions during embryonic development. They are almost undetectable in adult tissues but are re-expressed at high levels in all cancers where they are involved in neoplastic transformation and cancer progression. We focused on identifying new small molecules capable of binding into the minor groove of AT-rich DNA sequences that could compete with HMGA for DNA binding and, thus, potentially interfere with their activities. Here, a docking-based virtual screening of a unique high diversity in-house library composed of around 1000 individual natural products identified 16 natural compounds as potential minor groove binders that could inhibit the interaction between HMGA and DNA. To verify the ability of these selected compounds to compete with HMGA proteins, we screened them using electrophoretic mobility shift assays. We identified Sorocein C, a Diels−Alder (D−A)-type adducts, isolated from *Sorocea ilicifolia* and *Sorocea bonplandii* with an HMGA/DNA-displacing activity and compared its activity with that of two structurally related compounds, Sorocein A and Sorocein B. All these compounds showed a cytotoxicity effect on cancer cells, suggesting that the Sorocein-structural family may provide new and yet unexplored chemotypes for the development of minor groove binders to be evaluated as anticancer agents.



INTRODUCTION

High mobility group A (HMGA) proteins are a family of architectural nuclear factors involved in the modulation of chromatin structure and gene expression.[1,2] This family consists of three members: HMGA1a and HMGA1b, which are derived from the alternative splicing of the same gene, and the closely related HMGA2. HMGA proteins are about 100 amino acid residues long with a modular organization consisting of three strongly positively charged regions, the so-called AT-hooks that bind to the minor groove of AT-rich DNA stretches, and a C-terminal negatively charged tail.[3,4]

HMGA proteins are considered typical intrinsically disordered proteins due to the characteristic composition of their primary sequence, enriched in charged, polar, and structure-breaking amino acids and poor in bulky hydrophobic amino acids.[3] Indeed, the R, K, and P residues are clustered at the DNA-binding domain level, whereas E and D are clustered at the acidic C-terminal tail.[3]

HMGA proteins are highly interconnected hubs in the chromatin network, establishing several protein−protein interactions.[5,6] HMGA can be highly post-translationally modified by many different enzymes;[7] these features, i.e., their intrinsically disordered status and the multiplicity of post-translational modifications, could explain their multifunctionality through adaptability.[8]

The activities of HMGA proteins are explicated by two mechanisms: direct interaction with DNA and direct interaction with other proteins. These mechanisms are not mutually exclusive. In fact, HMGA can use both mechanisms to perform their functions. HMGA proteins have been studied mainly in terms of their involvement in the assembly of DNA-bound stereospecific macromolecular complexes (enhanceosomes) at the level of enhancer or promoter regions. The studies of Thanos' group have beautifully deciphered the mechanism of action of HMGA1 in the regulation of the INF-β gene

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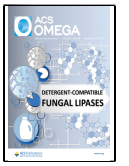

expression.[9] HMGA1 was found to be the key factor that orchestrates the formation of NF-κB-, IRFs-, and ATF-2/cJun-containing complexes through a network of protein/DNA and protein/protein interactions at the level of the enhancer of the INF-β gene, which is critical for the transcription.[10]

The expression of HMGA proteins differs in embryonic and adult tissues. In the former case, they are highly and widely expressed and play essential functions, whereas in the latter, they are generally scarce or absent. The precise spatiotemporal regulation of HMGA levels is important for proper cellular development and tissue homeostasis, and indeed, their aberrant expression has been implicated in several diseases, including cancer. Indeed, high levels of these proteins are detected in several malignancies such as breast, nervous system, thyroid, colon, lung, and prostate cancer.[11]

Importantly, the association between high HMGA protein levels and malignancies is not merely a correlation. The oncogenic role of these proteins has been demonstrated by their overexpression *in vitro* in cellular models and *in vivo* in transgenic mice. Indeed, many of their biological functions actively promote the acquisition of most cancer hallmarks and their multiple oncogenic activities have been extensively reviewed.[11−13]

Given the biological effects of HMGA in cancer, it is clear that they could be suitable and intriguing targets to counteract its onset and/or progression. In particular, switching off or silencing HMGA expression in *in vitro* cellular models leads to remarkable antitumor effects (reviewed in refs 14 and 15). Silencing of HMGA1 impairs cancer stem self-renewal, migration, and invasiveness. Consistently, depletion of HMGA1 reduces the metastatic ability *in vivo*.[16,17] Another reason to consider HMGA proteins as potential molecular targets is that HMGA1 expression generally increases chemo- and radio-resistance in almost all cancers studied, as recently reviewed.[15]

All these considerations have led over the years to exploit different strategies to target HMGA, trying to counteract their activities (reviewed in ref 15). Minor groove binders (MGBs), small molecules capable of binding DNA in the minor groove especially in AT-rich regions, have been investigated to this end. Among the MGBs, Netropsin and Distamycin A have been extensively characterized for their ability to compete with HMGA both *in vitro* and *in vivo*.[18−20] More recently, trabectedin (ET-743 or Yondelis), an MGB used to treat advanced tissue sarcomas and patients with relapsed platinum-sensitive ovarian cancer,[21] was shown to displace HMGA1 from DNA, suggesting that its anti-oncogenic activity could be explained, at least in part, by its HMGA-interfering ability.[22]

HMGA proteins play a role in cancer initiation and development, and they have been proposed as a suitable target to interfere with neoplastic transformation.[13,15,23] Therefore, we decided to search for new small molecules that were able to bind the minor groove of AT-rich DNA sequences that could act as HMGA competitors. To this aim, a docking-based virtual screening was carried out to evaluate the ability of a proprietary in-house natural product library to target the minor groove of AT-rich DNA sequences and to compete with HMGA for the interaction with DNA. The 16 natural compounds identified *in silico* as potential MGBs were screened by electrophoretic mobility shift assays (EMSAs) to confirm their ability to compete with HMGA proteins. Interestingly, Sorocein C, a Diels−Alder (D−A)-type adduct, and two structurally related compounds, Sorocein A and Sorocein B, showed interesting HMGA/DNA-displacing activities *in vitro* and exhibited cytotoxic activity against cancer cells. These molecules share a common substructure that has not been investigated yet for the interaction with DNA, thus representing potential lead compounds for anticancer strategies based on HMGA interference.

RESULTS AND DISCUSSION

Docking-Based Virtual Screening of the In-House Library of Natural Compounds. A proprietary high diversity library composed of around 1,000 individual natural products, isolated mainly from indigenous plants collected in biodiversity-rich countries, especially in tropical and subtropical areas, and enlarged with their semi-synthetic and synthetic derivatives, was used as a unique source of compounds for screening purposes. The library is available at the Organic Chemistry Laboratory of the Department of Chemistry and Technology of Drugs of Sapienza University of Rome, Italy,[24] and consists of natural products belonging to different classes that have been fully characterized. Over the past 10 years, the in-house library has represented a valid source of hits/leads for different pharmacological activities, and it has been successfully screened *in silico* and *in vitro* for the identification of hit and lead compounds in previous early-stage drug discovery projects.[24−28]

The 3D structure of HMGA has not been solved yet, most likely due to challenges in structural determination posed by the intrinsically disordered nature of these proteins. Nevertheless, atomistic details of the interaction between DNA and one AT-hook of HMGA1 are available by X-ray crystallography,[29] allowing the structure-based search for putative MGBs that could compete with HMGA1. To this aim, molecular docking of the compounds represented in the in-house library was carried out with AutoDock 4.2.[30] Coordinates of the HMGA1 peptide were removed from the DNA minor groove, while the water molecule bridging Arg38 of HMGA1 to DT6 of DNA was explicitly included in the receptor structure. Compounds of the in-house library were then ranked based on the free energy of binding estimated by AutoDock 4.2, while chemical diversity was assessed by cluster analysis carried out using a combination of fingerprints and substructure search.[31,32] Specifically, docked compounds were grouped based on their chemical and structural similarity, and the groups were sorted based on the docking score of the best compound included in each of them. The aim of this operation was to select compounds endowed with the least structural and chemical redundancy in the test set for experimental studies and to formally explore the largest space of the in-house library in terms of substructures and pharmacophores. Finally, coupling the energy score with chemical diversity and the inspection of binding poses led to selecting 16 natural products (**1**−**16**) as putative MGBs that were potentially able to inhibit the interaction between DNA and HMGA1 (Table 1 and Supplementary Table S1).

It is not surprising that a considerable part of these compounds belongs to the chemical class of alkaloids (**1**−**11**), given their positive charge in physiological conditions that nicely matches the negative charge of the DNA phosphate backbone. Alkaloids are one of the largest and most intriguing families of natural compounds, characterized by vast structural diversity with no uniform classification. Another compound was a triterpene (**12**), which belongs to a subclass of terpenes consisting of six isoprene units. Several candidate hits belong to the chemical class of polyphenols (**13**−**16**). Among the flavonoid phenol subclass containing a basic skeleton of

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Table 1. Natural Products Selected from the In-House Library

cluster	common name	M.W.	chemical class
1	Pseudobrucine (BBN180)	410.47	alkaloids
2	Sempervirine·HCl (BBN176)	227.35, 308.81 (+HCl)	
3	Yohimbine·HCl (BBN174)	354.45, 390.91 (+HCl)	
4	Veratrine·HCl (BBN173)	591.74, 628.20 (+HCl)	
5	Berberine·H_2SO_4 (BBN125)	336.37, 434.44 (+H_2SO_4)	
6	Sanguinarine·HNO_3 (BBN126)	332.33, 395.35 (+HNO_3)	
7	Dicentrine (BBN172)	339.39	
8	Boldine (BBN178)	327.38	
9	Atropine·H_2SO_4 (BBN177)	289.38, 387.45 (+H_2SO_4)	
10	4-OH-quinazoline (BBN179)	146.15	
11	Tubocurarine (BBN181)	610.75	
12	Betulin (BBN84)	442.73	terpenes
13	Cudraflavone B (BBN175)	420.46	flavonoids
14	6-Chromane-*O*-7-flavanone (BBN108)	308.38	
15	Phloretin (BBN101)	274.27	dihydro-chalcone
16	Sorocein C (BBN114)	756.80	Diels–Alder-type adduct
17	Sorocein A (BBN249)	630.69	
18	Sorocein B (BBN113)	658.7	

diphenylpropane, namely, two benzene rings (ring A and B) linked by a three-carbon chain that forms a closed pyran ring (the C ring) with a benzenic A ring, three compounds were selected for further investigation: two flavones (**13** and **14**), featuring a double bond between positions 2 and 3 and a ketone in position 4 of the C ring, and a dihydro-chalcone (**15**). Among the non-flavonoid phenols, a Diels–Alder (D–A)-type adduct (**16**), biosynthetically derived from [4 + 2]-cycloaddition of chalcones and dehydroprenylphenols, was chosen.

***In Vitro* Evaluation of the HMGA/DNA-Displacing Activity of the Selected MGBs.** EMSAs were performed to test whether the compounds selected using the *in silico* molecular docking screening described above were effective in interfering with HMGA/DNA complex formation. We decided to evaluate two different double-stranded DNA probes with different lengths and spacing of AT-rich sequences: one from the promoter region of the insulin receptor gene (E3) and another one from the enhancer of the interferon-β gene (PRDII). HMGA proteins have been demonstrated to bind both *in vitro* and *in vivo* to these cis-regulatory sequences, participating in the gene expression regulation of these two genes.[9,33] The conditions for the EMSAs were optimized to visualize both unbound DNA and HMGA/DNA complexes to eventually detect either interfering or enhancing effects on HMGA/DNA complexes. All compounds were analyzed for both HMGA proteins (HMGA1 and HMGA2) and probes (E3 and PRDII) at 100 μM. Netropsin (N) was selected as a positive control, since it is an MGB that can displace HMGA proteins from AT-rich sequences, while chloramphenicol (CHL) was used as a negative control. Figure 1 shows a representative experiment. Compounds **16**, **13**, and **8** were able to hamper the formation of complexes for both proteins using the E3 probe, since the signal corresponding to the HMGA/DNA complex disappeared (compound **16**) or was present at a lower intensity (compounds **13** and **8**). It is also interesting to note that compound **2** had a slight enhancing effect on the formation of the HMGA/DNA complex, which is more evident in the EMSA analysis performed with HMGA1. EMSA analyses performed with the PRDII probe confirmed the HMGA/DNA complex-displacing activity of compound **16**, whereas that of compounds **13** and **8** was barely detectable for HMGA1 and undetectable for HMGA2 (Supplementary Figure S1). Compound **4** interferes with the formation of the HMGA1/DNA complex but not with the HMGA2/DNA one using two different probes, E3 and PRDII (Figure 1 and Supplementary Figure S1). Only compound **16** was able to displace both proteins from their DNA target sequences; therefore, we focused our attention only on this compound that corresponds to Sorocein C, a Diels–Alder (D–A)-type adduct, isolated from two plants belonging to the family Moraceae, *Sorocea ilicifolia* and *Sorocea bonplandii* that are both used in Brazilian traditional medicine (Table 1 and Supplementary Table S1).[34−36]

Diels–Alder (D–A)-type adducts are a class of structurally rare and complex polyphenolic natural compounds featuring a

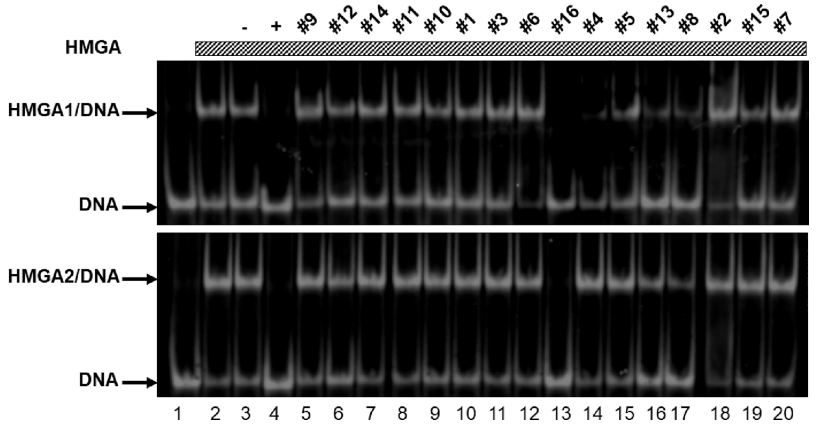

Figure 1. Compounds **16**, **13**, and **8** modulate both HMGA1/DNA and HMGA2/DNA complex formation. EMSA (T = 7%) is performed with constant amounts of HMGA1 protein (1.56 pmoles), HMGA2 protein (0.78 pmoles), drug concentration (100 μM), and ATTO 680-labeled DNA probes E3 (0.05 pmoles). Lane 1: DNA probe alone. Lane 2: HMGA1/HMGA2 alone. Lane 3: HMGA1/HMGA2 with CHL (−, 100 μM) as negative control. Lane 4: HMGA1/HMGA2 with Netropsin (+, 10 μM) as positive control. Lanes 5–20: putative MGB compounds. Detection is performed by IR fluorescence.

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central cyclohexene ring endowed with different stereocenters, biosynthetically derived from [4 + 2]-cycloaddition between two partners among flavonoids, chalcones, stilbenes, and 2-arylbenzofurans as precursors.[37,38] Fascinatingly, Sorocein C (**16**) consists of a complex tricyclic acetal moiety derived from intramolecular acetalization of the carbonyl group of the D−A-type adduct with two phenolic side groups.

Docking simulations showed that Sorocein C can bind in the minor groove of DNA and establish H-bond interactions with nucleotides (Figure 2). Specifically, Sorocein C is H-bonded to

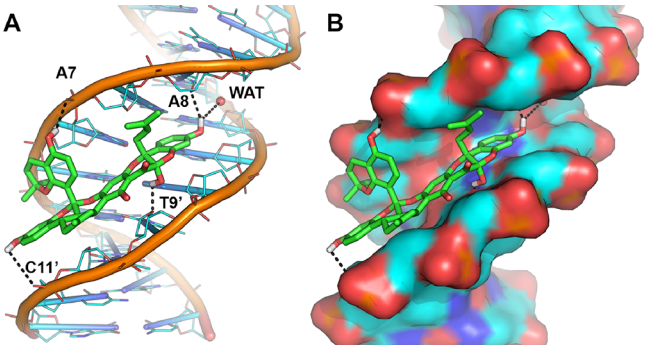

Figure 2. Docking-based binding mode of Sorocein C within the minor groove of DNA. The crystallographic structure of a DNA dodecamer duplex (PDB: 3UXW) is shown as lines and cartoon (panel A) or surface (panel B). Sorocein C is shown as green sticks, while H-bond interactions are highlighted by black dashed lines.

the crystallographic water molecule as well as the sugar moiety of A8 from one strand and T9′ from the complementary strand. In addition, it is H-bonded to the phosphate backbone of A7 from one strand and C11′ from the complementary strand (Figure 2). Notably, in its docking pose, Sorocein C nicely overlaps with the crystallographic binding mode of HMGA1 AT-hook (Supplementary Figure S2), strongly suggesting that Sorocein C might compete with HMGA1 for the binding to DNA, in agreement with experimental evidence provided in this work.

To thoroughly investigate the biological activity of this unique and complex architecture, we also tested two other structurally related compounds, i.e., Sorocein A (**17**) and Sorocein B (**18**), which have been isolated from the same plants.[34−36]

These three Sorocein family members (A, B, and C) were tested for their ability to displace HMGA proteins from DNA. Since they completely displaced HMGA proteins from DNA at the initial concentration of 100 μM (data not shown), we decided to perform EMSA titration assays to better characterize the efficiency of these three compounds using Netropsin as a reference. The results showed that they efficiently disrupted HMGA1/DNA complexes down to the lower μM concentration range (Figure 3) but not as efficiently as Netropsin, which was in the 10 nM range. This evidence is strongly correlated with the results of molecular docking simulations (Table 2), suggesting that Netropsin is a stronger binder to the target DNA sequence compared to D-A-type adducts investigated herein, as well as that Soroceins have a similar ability in binding the target AT-rich DNA sequence used for the docking-based virtual screening and, thus, the same potential HMGA/DNA complex formation-interfering activity. Taken together, these results suggest an intriguing inhibitory effect of this family of molecules on HMGA1/DNA complex formation.

Cytotoxicity to Cancer Cells. Based on the HMGA/DNA-displacing activity of the MGBs shown above, we investigated

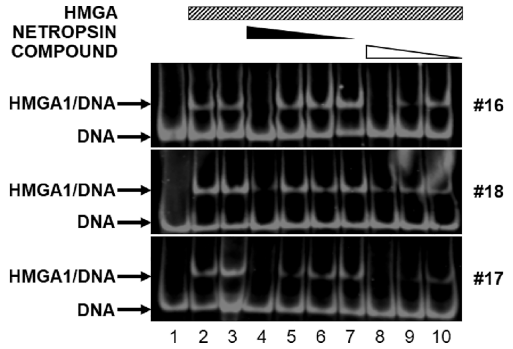

Figure 3. MGBs disrupt HMGA1/DNA complexes with different efficacies. EMSA (T = 7%) is performed with constant amounts of HMGA1 protein (1.56 pmoles) and ATTO 680-labeled DNA probes E3 (0.05 pmoles). Lane 1: DNA probe alone. Lanes 2 and 3: HMGA1 alone. Lanes 4−7: decreasing concentration (16, 3.2, 0.64, and 0.128 nM) of Netropsin. Lanes 8−10: decreasing concentration of Sorocein C (**16**) (12.5, 6.25, and 3.125 μM), Sorocein B (**18**) (3.12, 1.56, and 0.78 μM), and Sorocein A (**17**) (6.25, 3.125, and 1.56 μM).

Table 2. Docking Scores of Netropsin and Sorocein Derivatives Investigated in This Work

compound	estimated free energy of binding (AutoDock4.2) kcal/mol
Netropsin	−10.89
Sorocein A	−8.62
Sorocein B	−8.91
Sorocein C	−9.04

whether these compounds have a cytotoxic effect on cancer cells. The IC_{50} values calculated after 72 h exposure of MDA-MB-231 breast cancer cells to Soroceins (B, C, and A) are approximately between 29 and 34 μM (Figure 4). It was not possible to calculate IC_{50} values for Netropsin, because the cell viability was higher than 95% of the relative controls at all concentrations tested, i.e., IC_{50} values were higher than the highest concentration tested (i.e., 30 μM) (Table 3).

There is a big discrepancy between the efficacy range of Netropsin *in vivo* and *in vitro*: nM efficacy *in vitro* vs a much lower (μM range) efficacy *in vivo*. The main reason of this discrepancy could reasonably be attributed to Netropsin's cell membrane permeability. Netropsin has a net positive charge that strongly impairs its ability to pass across the cell membrane.

Indeed, there are several works that (i) report high IC_{50} values with respect to cell viability,[39−41] (ii) discuss the low cell permeability of Netropsin, and (iii) explore alternative strategies to improve the cellular internalization of this drug.[42,43] Moreover, higher Netropsin concentration levels are required to perform *in vivo* HMGA1-interfering activities[44−46] in comparison with those needed when used in *in vitro* experiments that, in agreement with our results, are usually in the nM range.[18,47] Soroceins, on the contrary, have a molecular structure quite different from that of Netropsin, and their hydrophobicity is higher. The fact that Soroceins display both cytotoxicity and HMGA-displacing activity in the same μM concentration range let us hypothesize that these two effects could be correlated, even though, to definitely prove this, further experiments are needed.

All the three Sorocein family members display comparable IC_{50} values; despite the fact that we investigated one cell line, this is an important information regarding the structure/function link of these compounds and suggests that they could

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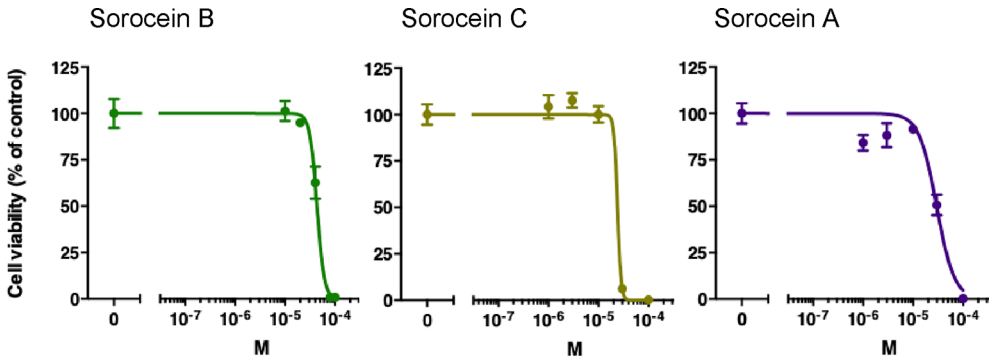

Figure 4. IC_{50} values of Sorocein B, C, and A. IC_{50} values of Soroceins B, C, and A were calculated in MDA-MB-231 cells after 72 h exposure. M corresponds to molarity.

Table 3. IC_{50} Values of Soroceins B, C, and A in MDA-MB-231 Breast Cancer Cells after 72 h of Treatment[a]

	IC_{50} [μM]			
	Sorocein B	Sorocein C	Sorocein A	Netropsin
MDA-MB-231	33.8 ± 13.9	29.0 ± 6.8	32.8 ± 6.8	>30

[a]IC_{50} values are the mean ± S.D. of at least three experiments, each performed in quadruplicate.

represent potential leading compounds to be further optimized with respect to their minor groove-displacing activity perturbing the DNA-mediated activities of HMGA proteins that are usually expressed at very high level in almost all cancer cells and considered as potential molecular targets.[12,15,23,48,49]

In conclusion, in this work, we have identified Soroceins A, B, and C as a group of structurally related compounds that are able to interfere with the binding of HMGA1 and HMGA2 to AT-rich DNA sequences, which exhibit cytotoxic activity toward cancer cells. These findings are promising and may be further explored for the development of a new group of MGBs to be evaluated as potential anticancer drugs.

METHODS

Chemistry. All the tested compounds are known structures belonging to the in-house library of natural products available from the Organic Chemistry Laboratory of the Department of Chemistry and Technology of Drugs of Sapienza University of Rome, Italy. The chemical identity of non-commercial compounds was assessed by re-running NMR experiments and proved to be in agreement with the literature data.

Compound **1** (Pseudobrucine or 5a-hydroxy-10,11-dimethoxy-2,4a,4a1,5,5a,7,8,8a1,15,15a-decahydro-14*H*-4,6-methanoindolo[3,2,1-*ij*]oxepino[2,3,4-de]pyrrolo[2,3-*h*]-quinolin-14-one) was purchased from THE BioTeK (CAS: 560-30-5, Don Julian Road City of Industry, CA, USA) and used without further purification.

Compound **2** (Sempervirine hydrochloride or 1,2,3,4-tetrahydroindolo[2′,3′:3,4]pyrido[1,2-*b*]isoquinolin-6-ium-13-ide hydrochloride) showed NMR spectra identical to those reported in the literature.[50]

Compound **3** (Yohimbine hydrochloride or (1R,2S,4aR,13bS,14aS)-methyl 2-hydroxy-1,2,3,4,4a, 5,7,8,13,13b,14,14a-dodecahydroindolo[20,30:3,4]pyrido[1,2-*b*]isoquinoline-1-carboxylate hydrochloride) was purchased from Sigma-Aldrich (CAS: 65-19-0, St. Louis, MO, USA) and used without further purification.

Compound **4** (Veratrine hydrochloride or (3S,4S,4aS,6aS,6bR,8S,8aS,9R,9aS,12S,15aS,15bR,16aR,16bS)-4,6b,8,8a,9,15b-hexahydroxy-9,12,16b-trimethyldocosahydro-2*H*-4,16a-epoxybenzo[4,5]indeno[1,2-*h*]pyrido[1,2-*b*]-isoquinolin-3-yl (*E*)-2-methylbut-2-enoate hydrochloride) was purchased from Sigma-Aldrich (CAS: 17666-25-0, St. Louis, MO, USA) and used without further purification.

Compound **5** (Berberine sulfate or 9,10-dimethoxy-5,6-dihydro-[1,3]dioxolo[4,5-*g*]isoquinolino[3,2-*a*]isoquinolin-7-ium sulfate) was purchased from Adooq Bioscience (CAS: 633-66-9, Barranca Parkway, Suite 250 Irvine, CA, USA) and used without further purification.

Compound **6** (Sanguinarine nitrate or 13-methyl-[1,3]-dioxolo[4′,5′:4,5]benzo[1,2-*c*][1,3]dioxolo[4,5-*i*]-phenanthridin-13-ium) was purchased from BocSciences (CAS: 4752-86-7, 45−16 Ramsey Rd., Shirley, NY 11967, USA) and used without further purification.

Compound **7** (Dicentrine or (*S*)-10,11-dimethoxy-7-methyl-6,7,7a,8-tetrahydro-5*H*-[1,3]dioxolo[4′,5′:4,5]benzo[1,2,3-*de*]-benzo[*g*]quinoline) was purchased from Biosynth (CAS: 28832-07-7, Nobelova, P.O., Bratislava, Slovakia) and used without further purification.

Compound **8** (Boldine or 1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de*,*g*]quinoline-2,9-diol) was purchased from Sigma-Aldrich (CAS: 476-70-0, St. Louis, MO, USA) and used without further purification.

Compound **9** (Atropine sulfate or 8-methyl-8-azabicyclo[3.2.1]octan-3-yl 3-hydroxy-2-phenylpropanoate sulfate) was purchased from Sigma-Aldrich (CAS: 55−48-1, St. Louis, MO, USA) and used without further purification.

Compound **10** (4-OH-quinazoline or quinazolin-4-ol) was purchased from Parchem (CAS: 491-36-1, 415 Huguenot Street, New Rochelle, New York) and used without further purification.

Compound **11** (Tubocurarine or (11*S*,51*R*)-36,57-dihydroxy-16,56-dimethoxy-12,52,52-trimethyl-11,12,13,14,51,52,53,54-octahydro-2,6-dioxa-1(7,1),5(1,8)-diisoquinolin-2-iuma-3(1,3),7(1,4)-dibenzenacyclooctaphane-12,52-diium) showed NMR spectra identical to those reported in the literature.[51]

Compound **12** (Betulin or (1R,3aS,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1*H*-cyclopenta[*a*]chrysen-9-ol) was purchased from Sigma-Aldrich (CAS: 473-98-3, St. Louis, MO, USA) and used without further purification.

Compound **13** (Cudraflavone B or 8-(2,4-dihydroxyphenyl)-5-hydroxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-2*H*,6*H*-

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pyrano[3,2-*g*]chromen-6-one) was purchased from ChemFaces (CAS: 19275-49-1, CheCheng Rd., WETDZ, Wuhan, Hubei, PRC) and used without further purification.

Compound **14** (6-Chromane-*O*-7-flavanone or 5,7-dihydroxy-2-(4-hydroxyphenyl)-3-methoxy-4*H*-chromen-4-one) showed NMR spectra identical to those reported in the literature.[52]

Compound **15** (Phloretin or 3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one) was purchased from Sigma-Aldrich (CAS: 60-82-2, St. Louis, MO, USA) and used without further purification.

Compound **16** (Sorocein C) showed NMR spectra identical to those reported in the literature.[36]

Compound **17** (Sorocein A or (3aS,3a1R,8aR,13bS)-6-((*E*)-2,4-dihydroxystyryl)-8a-(5-hydroxy-2,2-dimethyl-2*H*-chromen-8-yl)-2-methyl-1,3a1,8a,13b-tetrahydro-3aH-benzo[3,4]-isochromeno[1,8-*bc*]chromene-4,11-diol) was purchased from THE BioTeK (CAS: 137460-77-6, Don Julian Road City of Industry, CA, USA) and used without further purification.

Compound **18** (Sorocein B or (*E*)-1-((3aS,3a1R,13bS)-4,11-dihydroxy-8a-(5-hydroxy-2,2-dimethyl-2*H*-chromen-8-yl)-2-methyl-1,3a1,8a,13b-tetrahydro-3aH benzo[3,4]isochromeno-[1,8-*bc*]chromen-5-yl)-3-(2,4-dihydroxyphenyl)prop-2-en-1-one) showed NMR spectra identical to those reported in the literature.[35]

Molecular Docking. Docking simulations were carried out with AutoDock 4.2.[30] The DNA dodecamer duplex coded by PDB 3UXW was taken as a rigid receptor[29] upon removal of the coordinates of the HMGA1 AT-hook. The binding site was centered within the T6-A7′ Watson−Crick base pairing and had a dimension of 50, 60, and 38 points in the *xyz* Cartesian system (grid center coordinates: 35.38, 27.33, 29.11) using the default spacing of 0.375 Å between points. Ten runs of the genetic algorithm for each docked compound were stored and visually inspected. Ligand cluster analysis was carried out as described previously.[32]

HMGA1 and HMGA2 Recombinant Proteins. Full-length recombinant human HMGA1a and HMGA2 were expressed in *Escherichia coli* as previously described,[53] extracted by 5% PCA (w/v), purified by RP-HPLC, and checked for their purity and molecular masses by both SDS-PAGE and MS. Purified proteins were quantified according to a modified Waddell method.[53]

Electrophoretic Mobility Shift Assay (EMSA). ATTO-labeled oligonucleotides (0.1 pmoles) were incubated with 0.78−25 pmoles of recombinant HMGA proteins in the presence of 180 mM NaCl, 1 mM $MgCl_2$, 0.01% w/v BSA, 8% v/v glycerol, 0.015 U of poly(dG/dC), and 10 mM Tris/HCl (pH 7.9; 20 μL final volume). Drugs dissolved in DMSO or DMSO alone were added (10% DMSO final concentration). HMGA/DNA complexes were initially formed (30 min), and then drugs were added (30 min) prior to gel loading. All incubations were performed at 4 °C. Ten microliters of samples was loaded onto native 7 or 10% polyacrylamide gel (for E3 and PRDII respectively) in Tris-borate-EDTA buffer (45 mM Tris, 45 mM boric acid, 1.25 mM EDTA, pH 8.3 TBE 0.5×). Anode and cathode buffers were TBE 0.5×. Gels were subjected to a pre-run of 1 h at 15 V/cm. DNA/protein complexes were electrophoresed at 4 °C at 15 V/cm, and at the end of the run, gels were dried on 3MM paper. ATTO 680 fluorescence was detected using an Odyssey CLx (LI-COR Biosciences, Lincoln, Nebraska, U.S.). The following probes were used in EMSA experiments: E3, 5′-AGAAAAACTCCATC-TAAAAAAAAAAAAAAAAAAAAAAAAAACA-3′; PRDII, 5′-GGGAAATTCCGTGGGAAATTCCGAGCT-3′.

Cell Cultures. Human MDA-MB-231 breast cancer cells were maintained in Dulbecco's modified Eagle's medium (DMEM, EuroClone, Devon, UK) supplemented with 10% FBS (fetal bovine serum, Gibco, Invitrogen, Paisley, Scotland, UK), 2 mM L-glutamine (EuroClone), 1% nonessential amino acids, and 100 IU/mL penicillin and 100 μg/mL streptomycin (EuroClone). The cell line was maintained in an incubator with 5% CO_2 and 100% relative humidity at 37 °C. The cells of a confluent monolayer were removed from the flasks with a trypsin−EDTA solution. Cell viability was determined using the trypan blue exclusion assay. For experimental purposes, cells were seeded in multiwell culture clusters.

Cytotoxicity Test. Cells were seeded in 96-well plates at a density of 4000/100 μL complete medium per well and allowed to grow for 24 h. They were then incubated for 72 h with 1−300 μM solutions of compounds #18, #16, and #17 obtained by serial dilution of 10 mM in DMSO stock solution with complete medium. Netropsin was used as a known competitor of HMGA1 and HMGA2 for DNA binding at concentrations of 1−30 μM obtained by serial dilutions in complete medium of 1 mM in DMSO stock solution. Complete medium or complete medium containing DMSO at a final concentration of 1% was added to the negative controls. Cell cytotoxicity analysis by MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) was performed after 72 h of incubation. Briefly, MTT dissolved in phosphate-buffered saline (PBS, 5 mg/mL) was added to all wells (10 μL per 100 μL medium), and plates were incubated at 37 °C with 5% CO_2 and 100% relative humidity for 4 h. At this time, the medium was discarded and 200 μL of DMSO was added to each well according to the method of Alley et al.[54] Absorbance units were measured at λ = 570 nm using a spectrophotometer (FLUOstar Omega, BMG LABTECH). IC_{50} values were calculated from concentration−response curves, and they are the mean ± S.D. of at least three separate experiments, each performed in quadruplicate. The fitting procedure used is nonlinear regression performed with GraphPad Prism 6 for Mac OS X (version 6.0b, GraphPad Software, San Diego, CA, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02043.

EMSAs (Supplementary Figure S1); structural model (Supplementary Figure S2) (PDF)

Chemical characteristics of the selected compounds (Supplementary Table S1) (XLSX)

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Author Contributions

M.M. performed the virtual screening. F.G., D.Q., I.R., and B.B. provided the compounds. A.B., L.S., and R.S. performed the EMSA experiments. M.G. and A.B. performed the cell culture experiments. R.S., A.B., S.C., and G.M. analyzed and interpreted the data. M.M., R.S., and G.M. supervised the study. M.M., F.G., R.S., and G.M. wrote the first draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

MGBs;minor groove binders; HMGA;high mobility group A; EMSA;electrophoretic mobility shift assay; CHL;chloramphenicol; N;Netropsin; PDB;Protein Data Bank; IC_{50};half maximal inhibitory concentration; PCA;perchloric acid; RP-HPLC;reverse-phase high-performance liquid chromatography; SDS-PAGE;sodium dodecyl−sulfate polyacrylamide gel electrophoresis; MS;mass spectrometry; BSA;bovine serum albumin; DMSO;dimethyl sulfoxide; TBE;Tris-borate-EDTA; MTT;((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS;phosphate-buffered saline

■ REFERENCES

(1) Sgarra, R.; et al. High Mobility Group A (HMGA): Chromatin Nodes Controlled by a Knotty miRNA Network. *Int. J. Mol. Sci.* **2020**, *21*, 717.
(2) Reeves, R. Nuclear functions of the HMG proteins. *Biochim. Biophys. Acta* **2010**, *1799*, 3−14.
(3) Sgarra, R.; et al. Nuclear phosphoproteins HMGA and their relationship with chromatin structure and cancer. *FEBS Lett.* **2004**, *574*, 1−8.
(4) Reeves, R. Molecular biology of HMGA proteins: hubs of nuclear function. *Gene* **2001**, *277*, 63−81.
(5) Sgarra, R.; et al. Discovering high mobility group A molecular partners in tumour cells. *Proteomics* **2005**, *5*, 1494−1506.
(6) Sgarra, R.; et al. Interaction proteomics of the HMGA chromatin architectural factors. *Proteomics* **2008**, *8*, 4721−4732.
(7) Zhang, Q.; Wang, Y. High mobility group proteins and their post-translational modifications. *Biochim. Biophys. Acta* **2008**, *1784*, 1159−1166.
(8) Sgarra, R.; et al. HMGA molecular network: From transcriptional regulation to chromatin remodeling. *Biochim. Biophys. Acta* **2010**, *1799*, 37−47.
(9) Munshi, N.; et al. Coordination of a transcriptional switch by HMGI(Y) acetylation. *Science* **2001**, *293*, 1133−1136.
(10) Yie, J.; Merika, M.; Munshi, N.; Chen, G.; Thanos, D. The role of HMG I(Y) in the assembly and function of the IFN-beta enhanceosome. *EMBO J.* **1999**, *18*, 3074−3089.
(11) Fusco, A.; Fedele, M. Roles of HMGA proteins in cancer. *Nat. Rev. Cancer* **2007**, *7*, 899−910.
(12) Sgarra, R.; et al. High Mobility Group A (HMGA) proteins: Molecular instigators of breast cancer onset and progression. *Biochim. Biophys. Acta Rev. Cancer* **2018**, *1869*, 216−229.
(13) Wang, L.; et al. High Mobility Group A1 (HMGA1): Structure, Biological Function, and Therapeutic Potential. *Int. J. Biol. Sci.* **2022**, *18*, 4414−4431.
(14) Huso, T. H.; Resar, L. M. S. The high mobility group A1 molecular switch: turning on cancer - can we turn it off? *Expert Opin. Ther. Targets* **2014**, *18*, 541−553.
(15) Pegoraro, S.; et al. Targeting the intrinsically disordered architectural High Mobility Group A (HMGA) oncoproteins in breast cancer: learning from the past to design future strategies. *Expert Opin. Ther. Targets* **2020**, *24*, 953−969.
(16) Shah, S. N.; et al. HMGA1: a master regulator of tumor progression in triple-negative breast cancer cells. *PLoS One* **2013**, *8*, No. e63419.
(17) Pegoraro, S.; et al. HMGA1 promotes metastatic processes in basal-like breast cancer regulating EMT and stemness. *Oncotarget* **2013**, *4*, 1293−1308.
(18) Wegner, M.; Grummt, F. Netropsin, distamycin and berenil interact differentially with a high-affinity binding site for the high mobility group protein HMG-I. *Biochem. Biophys. Res. Commun.* **1990**, *166*, 1110−1117.
(19) Baron, R. M.; et al. Distamycin A inhibits HMGA1-binding to the P-selectin promoter and attenuates lung and liver inflammation during murine endotoxemia. *PLoS One* **2010**, *5*, No. e10656.
(20) Grant, M. A.; et al. Netropsin improves survival from endotoxaemia by disrupting HMGA1 binding to the NOS2 promoter. *Biochem. J.* **2009**, *418*, 103−112.
(21) D'Incalci, M.; Badri, N.; Galmarini, C. M.; Allavena, P. Trabectedin, a drug acting on both cancer cells and the tumour microenvironment. *Br. J. Cancer* **2014**, *111*, 646−650.
(22) D'Angelo, D.; et al. The impairment of the High Mobility Group A (HMGA) protein function contributes to the anticancer activity of trabectedin. *Eur. J. Cancer Oxf. Engl.* **2013**, *49*, 1142−1151.
(23) Resar, L.; Chia, L.; Xian, L. Lessons from the Crypt: HMGA1-Amping up Wnt for Stem Cells and Tumor Progression. *Cancer Res.* **2018**, *78*, 1890−1897.
(24) Ghirga, F.; et al. A unique high-diversity natural product collection as a reservoir of new therapeutic leads. *Org. Chem. Front.* **2021**, *8*, 996−1025.
(25) Platella, C.; et al. Identification of Effective Anticancer G-Quadruplex-Targeting Chemotypes through the Exploration of a High Diversity Library of Natural Compounds. *Pharmaceutics* **2021**, *13*, 1611.
(26) Infante, P.; et al. Gli1/DNA interaction is a druggable target for Hedgehog-dependent tumors. *EMBO J.* **2015**, *34*, 200−217.

(27) Quaglio, D.; et al. Chalcones and Chalcone-mimetic Derivatives as Notch Inhibitors in a Model of T-cell Acute Lymphoblastic Leukemia. *ACS Med. Chem. Lett.* **2019**, *10*, 639−643.
(28) Quaglio, D.; et al. ent-Beyerane Diterpenes as a Key Platform for the Development of ArnT-Mediated Colistin Resistance Inhibitors. *J. Org. Chem.* **2020**, *85*, 10891−10901.
(29) Fonfría-Subirós, E.; et al. Crystal structure of a complex of DNA with one AT-hook of HMGA1. *PLoS One* **2012**, *7*, No. e37120.
(30) Morris, G. M.; et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30*, 2785−2791.
(31) Stahl, M.; Mauser, H. Database clustering with a combination of fingerprint and maximum common substructure methods. *J. Chem. Inf. Model.* **2005**, *45*, 542−548.
(32) Mori, M.; et al. Identification of a novel chalcone derivative that inhibits Notch signaling in T-cell acute lymphoblastic leukemia. *Sci. Rep.* **2017**, *7*, 2213.
(33) Brunetti, A.; Manfioletti, G.; Chiefari, E.; Goldfine, I. D.; Foti, D. Transcriptional regulation of human insulin receptor gene by the high-mobility group protein HMGI(Y). *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2001**, *15*, 492−500.
(34) Calixto, J. B.; et al. Pharmacological analysis of the methanolic extract and sorocein A, a new Diels-Alder compound isolated from the roots of Sorocea bonplandii Bailon in the isolated rat uterus and guinea pig ileum. *Gen. Pharmacol.* **1993**, *24*, 983−989.
(35) Messana, I.; et al. Three New Diels-Alder Type Adducts from the Roots of Sorocea bonplandii Baillon. *Heterocycles* **1991**, *32*, 1287.
(36) Messana, I.; Ferrari, F.; Delle Monache, F.; Yunes, R. A.; Gacs-Baitz, E. Three New Flavanone Derivatives from the Root Bark of Sorocea bonplandii Baillon. *Heterocycles* **1994**, *38*, 1287−1297.
(37) Mascarello, A.; et al. Naturally occurring Diels-Alder-type adducts from Morus nigra as potent inhibitors of Mycobacterium tuberculosis protein tyrosine phosphatase B. *Eur. J. Med. Chem.* **2018**, *144*, 277−288.
(38) Iovine, V.; et al. Total Synthesis of (±)-Kuwanol E. *J. Nat. Prod.* **2016**, *79*, 2495−2503.
(39) Lau, K.-M.; Chan, Q. K. Y.; Pang, J. C. S.; Ma, F. M. T.; Li, K. K. W.; Yeung, W. W.; Cheng, A. S. L.; Feng, H.; Chung, N. Y. F.; Li, H. M.; Zhou, L.; Wang, Y.; Mao, Y.; Ng, H. K. Overexpression of HMGA1 deregulates tumor growth via cdc25A and alters migration/invasion through a cdc25A-independent pathway in medulloblastoma. *Acta Neuropathol. (Berl.)* **2012**, *123*, 553−571.
(40) Schneider, K.; et al. Proximicins A, B, and C-antitumor furan analogues of netropsin from the marine actinomycete Verrucosispora induce upregulation of p53 and the cyclin kinase inhibitor p21. *Angew. Chem. Int. Ed Engl.* **2008**, *47*, 3258−3261.
(41) Ouchi, K.; et al. Oncogenic role of HMGA2 in fusion-negative rhabdomyosarcoma cells. *Cancer Cell Int.* **2020**, *20*, 192.
(42) Jaroszeski, M. J.; Coppola, D.; Pottinger, C.; Gilbert, R. A.; Heller, R. Electrochemotherapy for the treatment of human sarcoma in athymic rats. *Technol. Cancer Res. Treat.* **2002**, *1*, 393−399.
(43) Orlowski, S.; Belehradek, J.; Paoletti, C.; Mir, L. M. Transient electropermeabilization of cells in culture. *Increase of the cytotoxicity of anticancer drugs. Biochem. Pharmacol.* **1988**, *37*, 4727−4733.
(44) Peter, S.; Yu, H.; Ivanyi-Nagy, R.; Dröge, P. Cell-based high-throughput compound screening reveals functional interaction between oncofetal HMGA2 and topoisomerase I. *Nucleic Acids Res.* **2016**, *44*, No. e162.
(45) Wu, Z.; et al. HMGA2 as a potential molecular target in KMT2A-AFF1-positive infant acute lymphoblastic leukaemia. *Br. J. Haematol.* **2015**, *171*, 818−829.
(46) Zhu, L.; Jones, C. The high mobility group AT-hook 1 protein stimulates bovine herpesvirus 1 productive infection. *Virus Res.* **2017**, *238*, 236−242.
(47) Miao, Y.; Cui, T.; Leng, F.; Wilson, W. D. Inhibition of high-mobility-group A2 protein binding to DNA by netropsin: a biosensor-surface plasmon resonance assay. *Anal. Biochem.* **2008**, *374*, 7−15.
(48) De Martino, M.; Fusco, A.; Esposito, F. HMGA and Cancer: A Review on Patent Literatures. *Recent Patents Anticancer Drug Discov.* **2019**, *14*, 258−267.
(49) D'Angelo, D.; Mussnich, P.; Arra, C.; Battista, S.; Fusco, A. Critical role of HMGA proteins in cancer cell chemoresistance. *J. Mol. Med. Berl. Ger.* **2017**, *95*, 353−360.
(50) Chinta Rao, T. S.; et al. Microwave assisted Westphal condensation and its application to synthesis of sempervirine and related compounds. *Tetrahedron Lett.* **2013**, *54*, 487−490.
(51) Papastephanou, C. Tubocurarine Chloride. In *Analytical Profiles of Drug Substances* (ed. Florey, K.) vol. 7 477−500 (Academic Press, 1978).
(52) Delle-Monache, F.; Goncalves de Lima, O.; Mello, J. F.; Delle-Monache, G. I. U. L. I. A. N. O.; Marini-Bettolo, G. B. Chroman- and cromene-chalcones from cordoin and isocordoin. *Gaz Chim Ital* **1974**.
(53) Noro, B.; et al. Molecular dissection of the architectural transcription factor HMGA2. *Biochemistry* **2003**, *42*, 4569−4577.
(54) Alley, M. C.; et al. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589−601.

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