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To be cited as: *ChemMedChem* 10.1002/cmdc.201800295

Link to VoR: http://dx.doi.org/10.1002/cmdc.201800295

A Journal of

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FULL PAPER

A minimalist approach to the design of complexity-enriched bioactive small molecules: discovery of phenanthrenoid mimics as antiproliferative agents.

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Supporting information for this article is given via a link at the end of the document.

Abstract: Over the last decades, much effort has been devoted to the design of the "ideal" library for screening, the most promising strategies being those which draw inspiration from biogenic compounds, as they seek to add biological relevance to such libraries. On the other hand, there is a growing understanding of the role that molecular complexity plays in the discovery of new bioactive small molecules. Nevertheless, the introduction of molecular complexity must be balanced with synthetic accessibility. In this work, we show that both concepts can be efficiently merged -in a minimalist way- by using very simple guidelines during the design process along with the application of multicomponent reactions as key steps in the synthetic process. Natural phenanthrenoids, a class of plant aromatic metabolites, served as inspiration for the synthesis of a library where complexity-enhancing features were introduced in few steps using multicomponent reactions. These resulting chemical entities were not only more complex than the parent natural products, but also interrogated an alternative region of the chemical space, which led to an outstanding hit rate in an antiproliferative assay: four out of twenty-six compounds showed *in vitro* activity, one of them being more potent than the clinically useful drug 5-fluorouracil.

Introduction

Despite the advances on the synthesis of large compound libraries and on high-throughput screening technologies, the discovery of new biological active molecules that might help in fighting against deadly diseases remains a slow and somehow unpromising task, mainly because the structural factors necessary to create compound collections with a potent and specific biochemical activity are not fully understood.[1] Moreover, there is an increasing awareness that most drugs not only act on a single, but on multiple targets,[2] and perhaps the extensive use during the past decades of target-based approaches for finding active chemotypes may account for the decrease in the pace of discovery of new drugs.[3–5]

In recent years, a trend has emerged towards addressing this issue, based on the design of libraries that take inspiration from natural products (NPs). Some of these approaches employ a strategy that incorporates scaffold usually present on NPs into the design process, the widely known Biology Oriented Synthesis (BIOS), introduced by Waldmann, being one of the most promising.[6,7] The rationale behind BIOS is that natural product fragments have a higher probability of binding to protein domains, and as proteins are built up modularly from a limited number of domain types, similar small molecules should be expected to bind to evolutionarily rather than functionally related proteins. Thus, focused libraries are designed around core scaffolds delineated from NPs in order to generate diversity from biologically pre-validated starting points.[8] The most crucial step in this process is identifying the relevant fragments that might confer biological relevance to the compounds to be synthesised. Only recently chemoinformatic tools have been developed for mining the information contained in the rapidly growing chemical databases, which have shed some light on this problem. Using such tools, Waldmann proposes a classification of the most biologically relevant scaffolds found in NPs, which might serve as a guideline for the selection of fragments on which to base the synthesis of rather small chemical libraries of less than 500 members, where a hit rate in biological screenings as high as 1.5% can be achieved.[7,9] As a consequence, the chemical modification of isolated NPs, which are inherently complex molecules, using synthetic procedures suitable to further increase the complexity, is a logical approach for generating small synthetic molecules that, although would be not direct analogs of a particular NP, could be considered as mimics enriched in the structural features of NPs, thus rising the chance of discovering new biologically relevant chemotypes.

Another important conclusion derived from chemoinformatic analysis of vast collections of compounds is that the complexity of a molecule has a paramount role in drug discovery.[10,11] Although there is no universally accepted definition, the concept of molecular complexity seems quite intuitive, and some simple and easily interpretable measures to assess it have been proposed during the last decade. For example, Lovering *et al.* [12] introduced the Fsp^3 metric (number of sp^3 hybridised carbons/total carbon count). In turn, Clemons *et al*. defined stereochemical complexity as the proportion of stereogenic carbon atoms in a molecule, and demonstrated that these descriptors highly correlate with the frequency and binding selectivity of a given compound to biological targets,[13] probably because the presence of non-flat molecular frameworks capable of orienting peripheral functional groups in all three dimensions might add the complementarity needed for binding to protein surfaces.[14] Only recently these appealing ideas have been implemented for the synthesis of Fsp^3-rich libraries.[15–17]

In the same vein, an extensive statistical analysis reported by Feher and Schmidt[18] suggests that some simple principles should be considered when designing new chemotypes for screening. In other words, increasing the number of chiral centres, fused rings, nitrogen and oxygen atoms while keeping the ratio between aromatic and saturated rings low may render a more biologically relevant compound. Taken together, these studies suggest that the aforementioned principles might serve as an intuitive guideline when designing such new chemical entities. But, as stated by Feher and Schmidt, introducing the required complexity-enhancing features into a molecule may imply substantial synthetic costs, such as the need for multiple cyclisation reactions and the formation of a number of carbon-carbon bonds.[18] This is the case of the work performed by Paciaroni *et al*., who described the synthesis of a 70-member library of compounds inspired on the alkaloidal scaffold of yohimbine, from which some bioactive compounds were discovered.[19] The ring distortion strategy reported by these researchers is a good example of the sophisticated but rather complex chemistry needed to achieve this goal. As an alternative, multicomponent reactions (MCRs) are powerful, highly convergent synthetic procedures that might help to overcome the synthetic costs associated to the efficient introduction of complexity-enhancing features. Most of these reactions are operationally simple and are especially useful for generating, in few steps, scaffolds with a structure closely resembling that of NPs. The Ugi four-component reaction (U-4CR), which is based on the exceptional reactivity of the isocyanide functional group, is probably the most powerful MCR and occurs when a carbonyl compound, an amine, a carboxylic acid and an isocyanide react together to give an α-aminoacylamide.[20] As a consequence, at least two oxygen and two nitrogen atoms, along with two sp^3 centres, can be introduced in one step, with the concomitant formation of three new bonds, all this without considering the variety of scaffolds that can be introduced through the appropriate choice of the components participating in the reaction.

In this context, the aim of this work is to explore whether a library whose design relies on the combination of the BIOS philosophy to choose a relevant scaffold and further complexity enhancement using the principles suggested by recent chemoinformatic studies, while still maintaining the synthetic chemistry involved as easy as possible, will indeed lead to a set of relevant chemotypes with a higher hit rate of bioactive compounds.

Results and Discussion

A library of phenanthrenoid mimics was outlined using simple design principles

The first step in the design of the library is the proper choice of a scaffold as a core for generating more complex chemotypes, bearing some constraints in mind: it should be one of the fragments proposed by the BIOS approach and it should have, at the same time, a low structural complexity as measured by the Fsp^3 metrics. Therefore, we conducted a thorough search in the literature, from which the relatively unexplored phenanthrene ring system emerged as a suitable scaffold. Natural phenanthrenes, collectively known as phenanthrenoids, are a class of aromatic metabolites belonging to the broader family of stilbenoids.[21] Phenanthrenoids act as phytoalexins during the plant response to pathogens, and have been demonstrated to possess various types of biological activity, such as cytotoxic, anti-inflammatory and antimicrobial, that have been reviewed recently.[22] For example, denbinobin, isolated from *Dendrobium moniliforme*, prevents the binding of the transcription factor NF-κB to DNA, inducing apoptosis in human leukemic cells,[23] whereas the 9,10-dihydro-2,5-dimethoxyphenanthrene RSCL-0520, isolated from *Eulophia ochreata*, inhibits the inflammatory signalling mediated by toll-like receptor TLR4 in RAW264.7 macrophages [24] (Figure 1).

To better characterise the complexity of this family of natural compounds, we mined the literature[22,25] and selected 72 natural phenanthrenoids –whose structures are collected in the Supporting Information file– as a representative sample. We calculated their Fsp^3 scores and found a mean value of 0.22 (median=0.20). This value is significantly lower than that typically reported for NPs, which range from 0.41 to 0.59, depending on the set of compounds analysed.[1,26] Interestingly, all natural phenanthrenoids contain oxygen atoms, but none has nitrogen or other heteroatoms, which are known to contribute to the biological relevance of a given chemotype, in their structures.

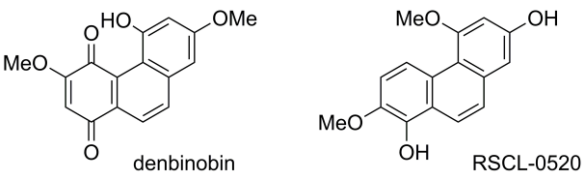
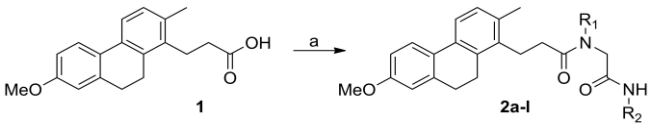


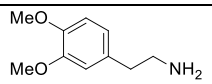
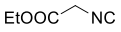
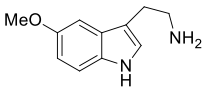
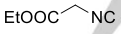
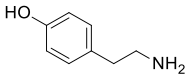
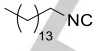
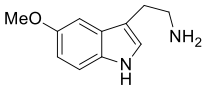
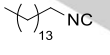
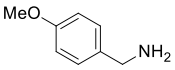
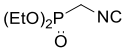
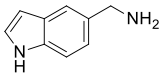
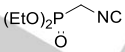
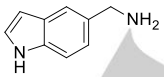
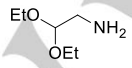
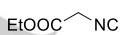
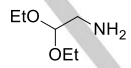
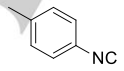
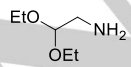
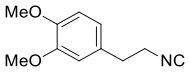
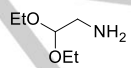
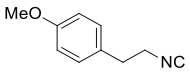
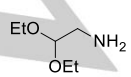
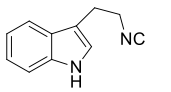
Figure 1. Examples of biologically active natural phenanthrenoids.



Scheme 1. U-4CR on phenanthrene **1**.
Reagents and conditions: a. R_1-NH_2 / R_2-NC / HCHO / methanol or ethanol; r.t; 48h

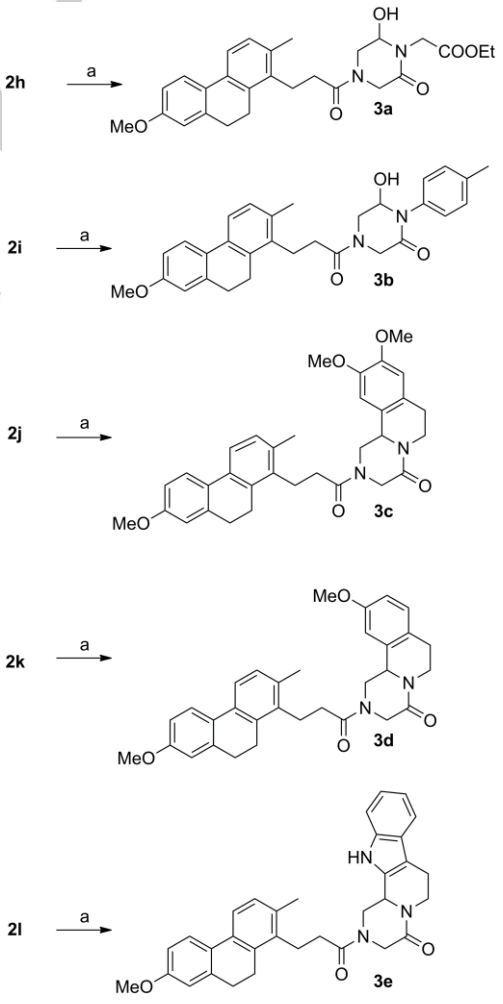
The next step comprised the selection of an appropriate synthetic strategy to generate the desired library. With this purpose, we chose the 9,10-dihydrophenanthrene **1**, which can be easily obtained from the readily available steroid estrone [27] as the carboxylic component for an U-4CR, and selected some amines and isocyanides containing both aliphatic and aromatic moieties and N, O or P-based functional groups (depicted in Table 1), pursuing, at the same time, the introduction of additional scaffolds suggested by the BIOS strategy into the final compounds. From these components, we straightforwardly obtained the first members of the library, compounds **2a – l** (Scheme 1).

Table 1. Amines and isocyanides used in the U-4CR depicted in Scheme 1.

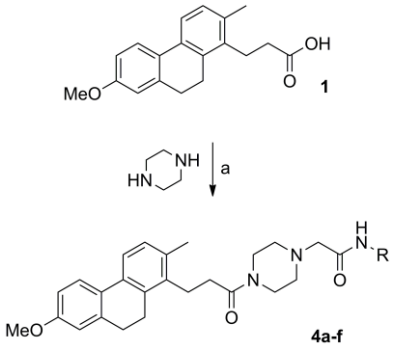
Compound	R_1-NH_2	R_2-NC
2a		
2b		
2c		
2d		
2e		
2f		
2g		
2h		
2i		
2j		
2k		
2l		

One of the most remarkable features of MCRs is that they offer additional opportunities for post-condensation modifications of the reaction products,[28] which in this context might help to expand the complexity of the final chemotypes. Thus, we designed a series of compounds were, by using 2,2-diethoxyethanamine and suitable isocyanides in the U-4CR, intermediates **2h – l** were obtained, which were then subjected to subsequent treatment under acidic conditions, to give the corresponding cyclisation products **3a** and **3b**. In addition, intermediates derived from electron-rich aromatic isocyanides suffered an additional Pictet-Spengler type reaction [29] to give the complex polycyclic compounds **3c - e** (Scheme 2).

A third subset of compounds was designed taking into account that the dihydrophenanthrene **1** could also be a component of a variant of the U-4CR, known as the N-split-Ugi reaction, where the primary amine is replaced by a secondary diamine. [30] In this case, the use of piperazine as the amine component along with different isocyanides (Table 2) led to the corresponding adducts of general structure **4** (Scheme 3), with an alternative backbone that introduces a protonable tertiary amine and four sp^3 carbons, enriching the complexity of the final compound in a single step.



Scheme 2. Acidic cyclisation of the U-4CR products **2h-l** to give the polycyclic compounds **3a-e**.
Reagents and conditions: a. HCOOH; -10°C; 1h

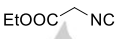
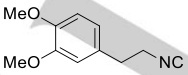
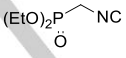
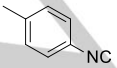
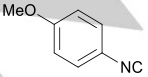
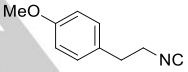


Scheme 3. N-split Ugi reaction between the 9,10-dihydrophenanthrene **1** and piperazine to give compounds **4a-f**.
Reagents and conditions: a. R-NC / HCHO / methanol or ethanol; r.t; 48h

The library designed was synthesised via MCRs and post-condensation reactions.

The detailed experimental procedures for the synthesis of the library is described in the Supporting Information file. The new twenty-six compounds were obtained from **1** with yields ranging from 38 to 78% after purification, and their structures were corroborated by 1D and 2D NMR. In most cases, the NMR spectra showed the compounds as a mixture of conformers which are expected to originate from cis/transrotation around the N-substituted amide bonds.[31] As the interchange between the isomers is generally slower than the NMR time scale, the spectrum is the composite of the NMR spectra of the corresponding conformational isomers. For simplicity purposes, only the chemical shifts and coupling constants for the most populated conformer were described. Moreover, a direct analysis via high resolution electrospray ionisation mass spectrometry was performed in order to obtain an unambiguous characterisation of the compounds.

Table 2. Isocyanides used in the N-split Ugi reaction of Scheme 3.

Compound	R-NC
4a	
4b	
4c	
4d	
4e	
4f	

The phenanthrenoids designed are more complex than natural ones and interrogate a different region of the chemical space.

Once the library was designed a question arose: how does the complexity of its members compare to that of natural phenanthrenoids? The resulting mean value of the Fsp^3 metrics for the designed compounds was 0.40 (median = 0.39), which is significantly higher (99% confidence level in a Mann-Whitney test) than that found for the natural phenanthrenoids taken as reference (Figure 2a) and proves that this simple synthetic strategy is a powerful complexity-enhancing tool.

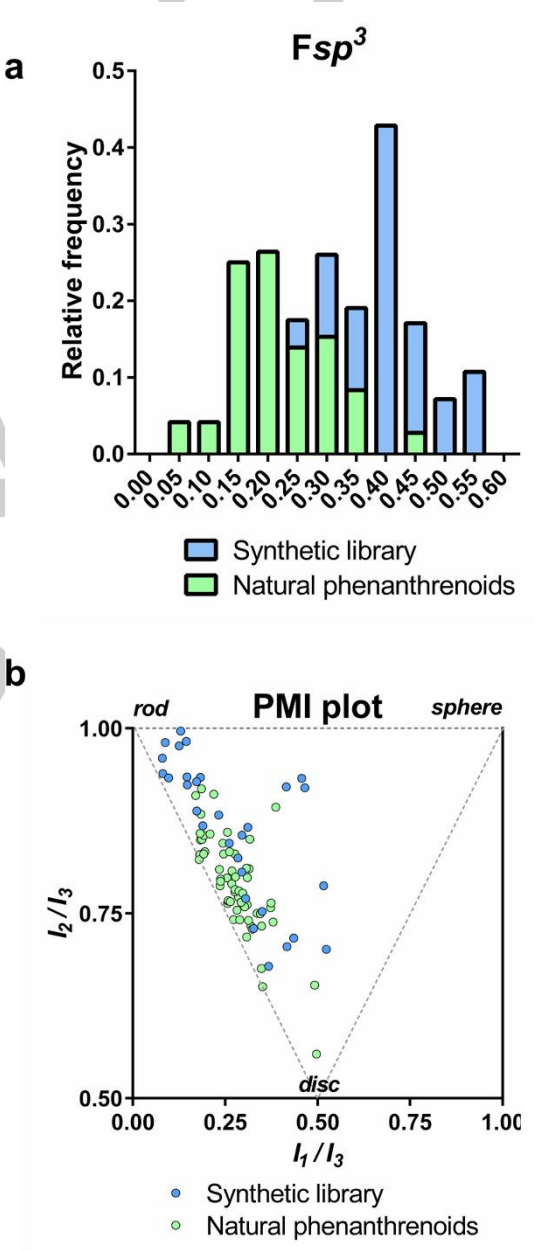


Figure 2. a) Comparison of the frequency distribution of the Fsp^3 score for the synthetic library and a set of natural phenanthrenoids. b) PMI plot showing the three-dimensional shape of the lowest-energy conformer of each compound in both sets. Calculations are described in the text.

As the Fsp^3 score is a topological metric based on the 2D structure of a molecule, a principal moment of inertia (PMI) analysis[32] was also performed in order to compare the three-dimensional shapes of the lowest-energy conformations of our synthetic library members with the phenanthrenoid reference set. Figure 2b shows that the synthetic compounds span across a wider region of the shape space, and that several of them lie near the rod-like vertex, a molecular shape that is usually found in bologically relevant compounds.[33,34]

However, as previously discussed, there are several other factors that might contribute to the overall biological relevance of a library. To gain further insight into the similarities and differences between the sets of synthetic and natural phenanthrenoids, we performed an additional chemoinformatic analysis to calculate 16 suitable molecular descriptors which are meant to grasp the topological, structural and physicochemical properties of a set of compounds. These descriptors, previously validated by several researchers, [35,36] were taken as a base for a principal component analysis (PCA): the 16-dimension chemical space defined by these descriptors was projected onto 15 orthogonal axes (principal components), of which the first three (F1, F2 and F3) allow to represent 72% of the initial variability of the data. The plot depicted in Figure 3 shows that the two sets of compounds span across rather disjoint clustered regions of the reduced chemical space defined by these principal components. Moreover, the larger contributions to these axes can be seen to come from several original variables related to molecular complexity, not only the Fsp^3 index, but also the number of heteroatoms, number of rings and rotable bonds. These analyses, when taken together, suggest not only that the designed library is enriched from the shape complexity point of view when compared to natural phenanthrenoids, but also that it comprises additional features that are often regarded to be crucial for a chemical entity to have a higher probability of exerting biological activity.

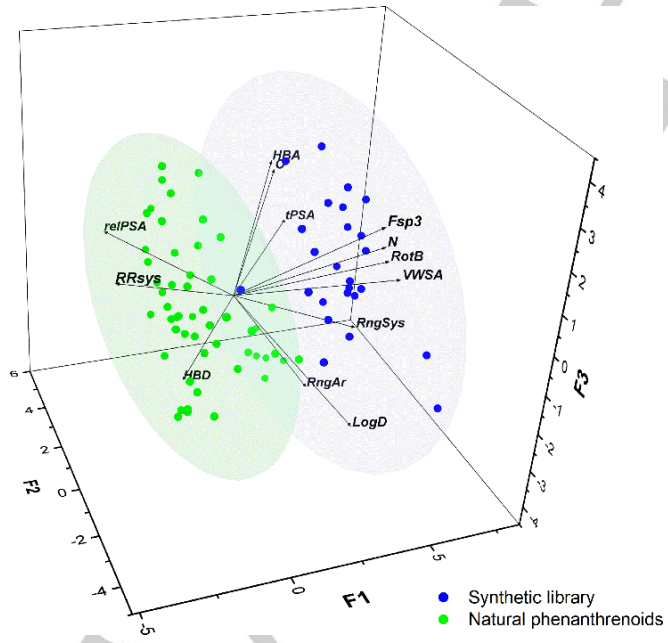


Figure 3. Three-dimension biplot showing the different regions that the synthetic and natural phenanthrenoids occupy in a chemical space defined by the principal components F1, F2 and F3. The contribution of the most relevant descriptors (listed in the SI file) to these components is also depicted.

Four hits with antiproliferative activity were identified in an *in vitro* screening.

At this point the remaining question was whether the complexity-generating features introduced on the dihydrophenanthrene core did give the resulting chemotypes a higher chance of having biological relevance. Thus, we evaluated them in a cell-based biological assay, as this approach does not require prior knowledge of a particular molecular mechanism of action. [37,38] Taking into account the interesting antiproliferative and cytotoxic effects exerted by several natural phenantrenoids, our choice was a cell viability screening on human tumour cell lines. Needless to say, finding new compounds able to inhibit cell proliferation is a pressing need, not only for their potential clinical use but also because, in some instances, the effects seen for small molecules in cell-based screenings have revealed novel biological mechanisms underlying tumour progression.[39–41]

A preliminary screening was initially performed in the human colorectal adenocarcinoma HT-29 cell line using the well-established MTT assay [42] after cell treatment with each compound during 48 h. Most of the synthetic phenanthrenoids did not exert any effect below a 100 μM concentration, but compounds **2e**, **2f**, **2g** and **4f** elicited a significant inhibition of cell viability, with IC50 values (the concentration needed to inhibit 50% of cell viability compared to that of the control) of 17, 63, 31 and 36 μM, respectively. Compound **2e** proved even more potent than 5-fluorouracil (5-FU), which showed an IC50 of 32 μM in this experiment. Noteworthily, these compounds, depicted in Figure 4, share similar structural features and occupy a rather defined portion of the chemical space, suggesting the presence of a defined pharmacophore linked to a particular macromolecular target, which might pave the way for a further structure-activity relationship study based on the exploration of this particular region of the space.

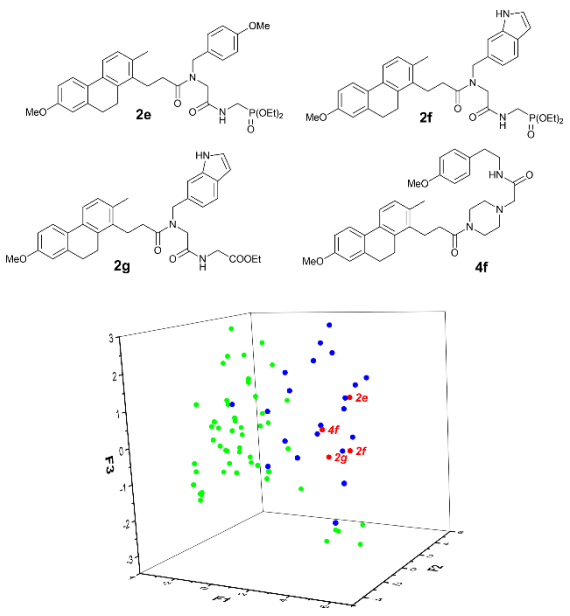


Figure 4. Three-dimension biplot showing that the phenanthrenoid mimics that exerted antiproliferative activity in vitro on HT-29 cells share similar coordinates in the chemical space (labelled red dots).

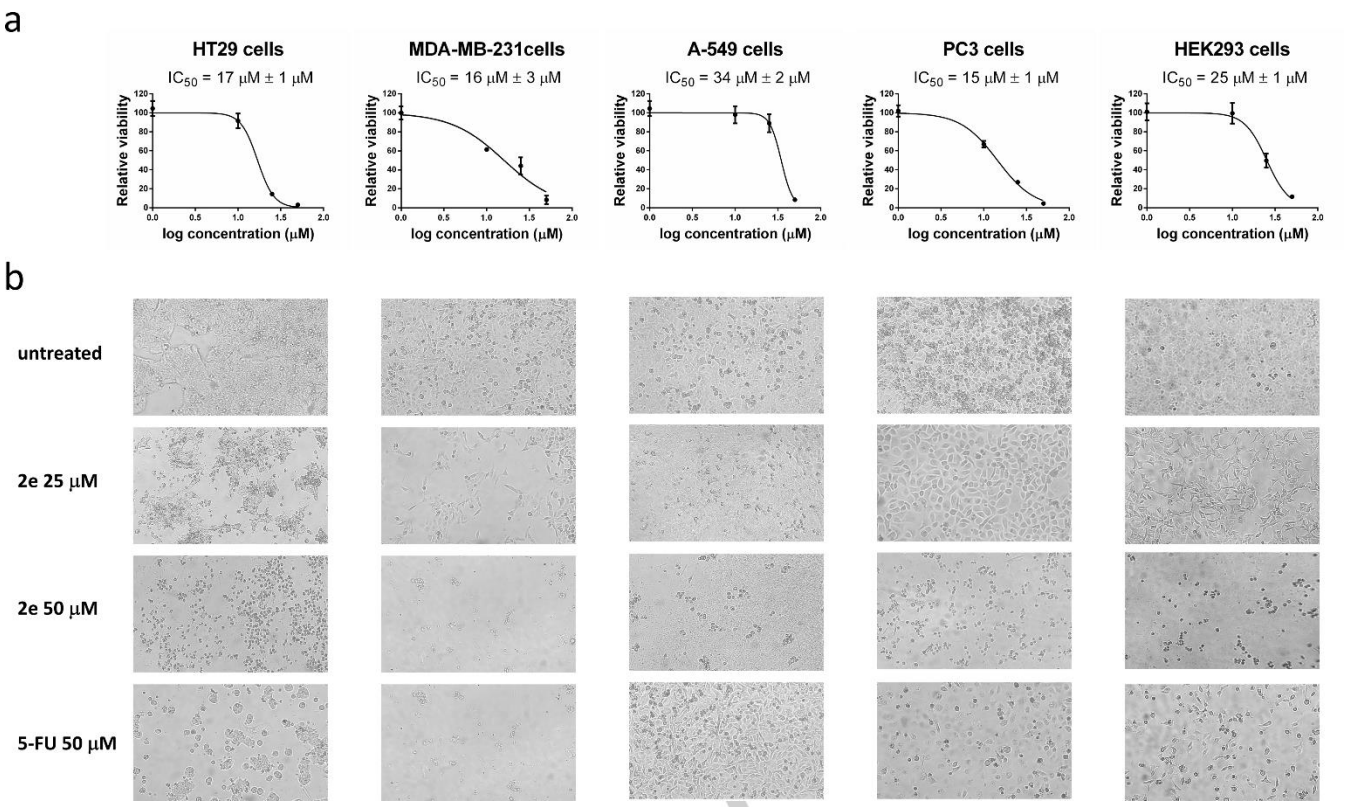


Figure 5. **a)** Dose-dependent effect of compound **2e** on HT29, MDA-MB-231, A-549, PC3 and HEK293 cell viability by MTT assay. Data are expressed as relative viability (percentage of untreated control cells) and the IC50 values were obtained from three independent experiments. **b)** HT29, MDA-MB-231, A-549, PC3 and HEK293 cells untreated or treated with compound 2e or 5-FU examined under a light microscope in randomly selected fields.

Taking these results into account, **2e** was selected as the most active compound to further study the selectivity of its effect on additional cell lines. Thus, similar viability assays were performed on malignant breast ductal carcinoma MDA-MB-231 cells, lung adenocarcinoma A-549 cells and prostate cancer PC3 cells, along with non-cancerous human embryonic kidney HEK293 cells. As shown in Figure 5, compound **2e** proved to decrease the viability of all these cell lines with IC50s ranging from 15 to 35 μM. Additional microscopy observations revealed significant changes in cell morphology.

These results, obtained using the short-term MTT assay, were complemented with a longer *in vitro* cell survival assay –the colony formation assay–, which might be regarded as an indirect estimation of the antineoplastic effect of a compound.[43] Therefore, the ability of HT29 cells to grow and form *foci* was measured in the presence of **2e**. Figure 6 shows that these cells, when treated with **2e**, formed fewer and smaller colonies in a concentration-dependent manner compared to the control, which further proves **2e** antiproliferative properties.

Compound 2e exerts its antiproliferative activity *via* an apoptotic mechanism

As apoptosis can be regarded as one of the most relevant molecular mechanisms in drug-induced cell death, and since some natural phenanthrenoids such as denbinobin were shown to induce cell death by this mechanism in different cell lines,[23,44,45] we decided to determine whether apoptosis could be involved in growth inhibition triggered by **2e**. Thus, we performed a morphological analysis of cell death using fluorescence microscopy after staining nuclear DNA of HT29 cells with 4′,6-diamidino phenylindole (DAPI), [46] and found that treated cells showed the typical morphological characteristics of apoptotic cells: nuclear condensation and fragmentation, cell shrinkage and fragmentation into apoptotic bodies.

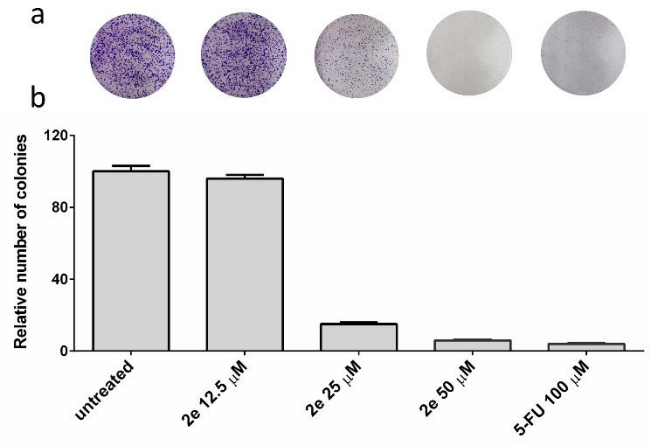


Figure 6. a) Clonogenic assay of HT29 cells untreated or treated with compound **2e** or 5-FU for 24 h. Photographs of petri-dishes in representative experiments. b) Percentage of colonies formed with respect to untreated control (relative number of colonies)

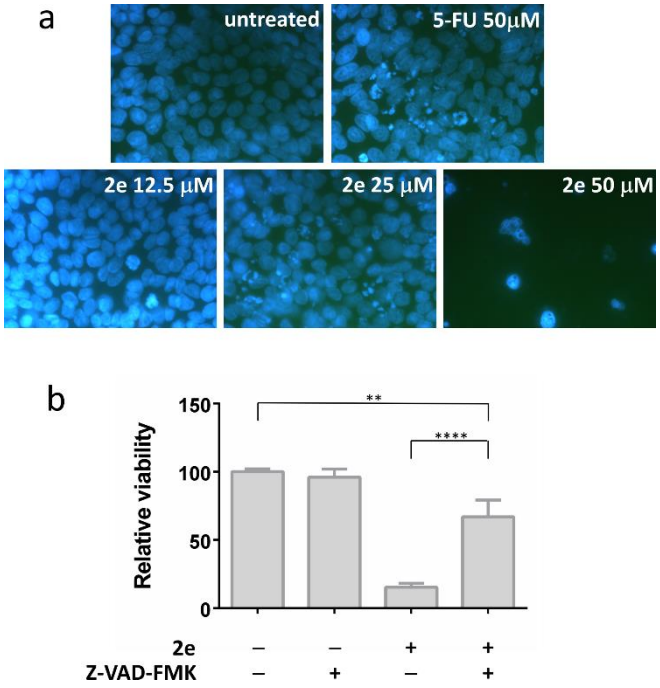


Figure 7. a) Representative fluorescence microscopy images after staining nuclear DNA with DAPI. b) Cell death induced by compound **2e** in the presence absence of a pan-caspase inhibitor. Activity on cell viability determined by MTT assay. Results are expressed as the mean ± standard deviation of three independent experiments performed in duplicate, ** $p < 0.01$, **** $p < 0.001$.)

However, the precise mechanism of denbinobin apoptosis induction has not been fully delineated, but the cytotoxic activity seems to involve activation of caspase-3.[44] Therefore, our cytological observations were complemented with pre-incubation of HT29 cells with 10 μM of Z-VAD-FMK for 4 h, a pan-caspase inhibitor that blocks apoptosis,[47] and co-incubation of the cells treated with 25 μM **2e** for another 48 h. Figure 7 shows that cell death promoted by **2e** was partially reversed when cells were treated with the pan-caspase inhibitor, which suggests that caspases are at least in part involved in the mechanism of the cell death induced by this compound. Therefore, the results showed herein strongly suggest that the mimics described in this work share some pharmacological features with the NPs that served as inspiration for their design.

Conclusions

With the advent of high-throughput screening techniques, much effort has been devoted to the design of the "ideal" library for screening, both in terms of size and synthetic efficiency.[48] In this vein, the BIOS approach is probably one of the most appealing methods, which, as stated above, builds on the fact that macromolecules and natural products have co-evolved to render highly diverse and complex structures by combining a limited set of fragments.

On the other hand, it is becoming clearer nowadays that, for a library to contain biologically relevant chemical entities, it should incorporate some degree of structural complexity, a concept which, even if quite intuitive, has no sharp quantitative definition. Although the simple guidelines for introducing complexity proposed by Feher and Schmidt in their seminal work have been discussed by several researchers, they have been usually applied *ex post facto* in order to rationalise the discovery of new bioactive molecules among members of chemistry-driven designed libraries. In fewer cases, these guidelines served for designing new interesting chemotypes, but almost no further studies on their biological properties have been reported.[15,19,49]

The present work might then be considered a proof of concept showing that the BIOS approach can be complemented with a simple complexity-enhancing synthetic methodology in order to obtain smaller, but still biologically relevant libraries with active members when assayed in a phenotypic screening. In this sense, Nilar *et al*. have proposed that compounds having an increased degree of complexity improve the likelihood of activity in phenotypic screenings,[50] as these assays simultaneously measure interactions with multiple macromolecular targets, each one having in turn various degrees of complexity.

In this case, four antiproliferative compounds have emerged from a set of only twenty-six members, an outstanding hit rate. Moreover, one of them showed a more potent *in vitro* activity when compared to that of a clinically useful antitumour drug. It is important to point out that it this work constitutes the first report on the synthesis of mimics of this family of natural products, and that the simplicity of the synthetic approach might also pave the way for an efficient hit-to-lead optimisation. In addition, such simplicity offers a tool to face the important problem posed by the low abundance of NPs when searching for novel bioactive compounds. For example denbinobin, the most studied natural phenanthrenoid, can be isolated from its natural source with a 0.002% yield, which has hampered its more detailed pharmacological investigation until the development of total synthesis that, in turn, comprises several steps and the need of sophisticated synthetic procedures.[51]

Taken as a whole, our results suggest that the rather simple approach presented herein, which we have dubbed as a minimalist design approach, might be envisaged as an efficient way to address the challenging first steps of the drug discovery process.

Finally, as many of the natural phenanthrenoids that have inspired our synthetic library show biological activities other than antiproliferative, thus, it would be interesting to perform phenotypic screenings on alternative biological systems. Further studies are underway to address this issue.

Experimental Section

Synthesis of the library

For a full experimental description of the synthesis and characterisation of the new compounds, please see the SI file. The structures were confirmed by 1D and 2D NMR spectroscopy from spectra recorded on a Bruker AM-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C). Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. Coupling constant (J) values are in Hz. HRMS (ESI) analysis were performed on a Bruker micrOTOF-Q II. Furthermore, combustion analyses for the new compounds were performed on an Exeter CE 440 Elemental Analyzer and were within ± 0.4 % of the theoretical values. Melting points were determined on a Fisher Johns apparatus and are uncorrected.

Compound **1** was obtained by adapting the procedure described by Boots and Johnson.[27] The synthesis of two representative members of the library are described below:

Compound 2e: The acid 1 (50 mg, 0.16 mmol) was added to a solution of (4-methoxybenzyl)amine (22 µL, 0.16 mmol) and 15 µL of 37% aq formaldehyde (0.18 mmol) in 3 mL of ethanol. The mixture was stirred for 15 min at room temperature and then 0.18 mmol (35 µL) of diethyl (isocyanomethyl)phosphonate were added. The reaction was kept under the same conditions until total disappearance of the acid (usually 48 h). The solvent was evaporated under reduced pressure and the residue was taken in dichloromethane and washed with NaOH (5% aq.). The combined organic layers were dried over anhydrous sodium sulfate and evaporated. The crude products were purified by silica gel column chromatography (hexane/EtOAc gradient) to give compound 2e as a white powder (65 mg, 65% yield); m.p.: 103°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.26 (t, *J* = 7.1 , 3H), 1.34 (t, *J* = 7.0 , 3H), 2.30 (s, 3H), 2.58 (m, 2H), 2.73 (m, 2H), 2.75 (m, 2H), 3.08 (m, 2H), 3.71 (dd, *J* = 12.1 and 5.9, 2H), 3.78 (s, 3H), 3.83 (s, 3H), 4.03 (s, 2H), 4.12 (q, J = 7.0, 2H), 4.15 (q, J = 7.1, 2H), 4.55 (s, 2H), 6.74 (NH, t, *J* = 5.9, 1H), 6.76 (d, *J* = 2.7, 1H), 6.82 (dd, *J* = 8.8 and 2.8 , 1H), 6.85 (d, *J* = 8.8, 2H), 7.03 (d, *J* = 8.2, 2H), 7.07 (d, *J* = 8.0, 1H), 7.47 (d, *J* = 7.9, 1H), 7.60 (d, *J* = 8.6, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 16.6, 16.63, 20.2, 25.0, 25.2, 29.6, 32.7, 34.3 (d, $J_{C\text{-}P}$ = 157), 50.1, 51.9, 55.4, 55.5, 62.7 62.8, 112.5, 113.1, 114.6, 121.7, 125.1, 127.5, 127.8, 127.9, 128.9, 133.0, 134.9, 134.9, 136.4, 138.5, 158.9, 159.5, 169.0, 173.9; HRMS (ESI) m/z calcd for ($M+Na^+$) $C_{34}H_{43}N_2O_7PNa$: 645.2700, found: 645.2689.

Compound 4f: The same procedure described before was followed, but using 14 mg (0.16 mmol) of piperazine as the amine component and 1-(2-isocyanoethyl)-4-methoxybenzene (29 mg, 0.18 mmol) as the isocyanide component. After purification, 47 mg of compound 2f (53% yield) as a pale yellow powder was obtained; m.p.: 181°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.22 (t, *J* = 5.0, 2H), 2.34 (t, *J* = 5.0, 2H), 2.36 (s, 3H), 2.44 (m, 2H), 2.78 (t, *J* = 6.8, 2H), 2.81 (m, 2H), 2.84 (m, 2H), 2.92 (s, 2H), 3.07 (m, 2H), 3.19 (t, *J* = 4.9, 2H), 3.51 (t, *J* = 5.0, 2H), 3.54 (m, 2H), 3.76 (s, 3H), 3.84 (s, 3H), 6.78 (d, *J* = 2.7, 1H), 6.83 (d, *J* = 8.6, 2H), 6.84 (dd, *J* = 8.6 and 2.7, 1H), 7.00 (t, *J* = 5. , 1H), 7.09 (d, *J* = 8.6, 2H), 7.10 (d, *J* = 7.9, 1H), 7.49 (d, *J* = 7.9, 1H), 7.62 (d, *J* = 8.6, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 20.3, 25.1, 25.2, 29.6, 32.6, 34.7, 39.9, 41.7, 45.5, 53.2, 53.6, 55.4, 61.5, 112.5, 113.1, 114.2, 121.7, 125.2, 128.0, 128.9, 129.7, 130.8, 133.1, 134.9, 135.0, 136.6, 138.5, 158.5, 159.0, 169.5, 170.9; HRMS (ESI) m/z calcd for ($M+Na^+$) $C_{34}H_{41}N_3NaO_4$: 578.2989, found: 578.2991.

Chemoinformatic analysis

The structures of the natural phenanthrenoids were obtained from the literature and are listed in the SI file. JChem for Excel was used for calculations of the F*sp*^{3} metrics, other structure-based properties and generation of the lower -energy conformations of the compounds (release 16.10.1700.1231, 2016, ChemAxon; http://www.chemaxon.com). Conformations were re-optimized to the AM1 semiempirical level and the principal moments of inertia for the resulting structures were calculated using Hyperchem (release 7.5 for Windows, HyperCube Inc; http://www.hyper.com). Principal component and additional statistical analysis were performed using OriginPro 2017 (release b9.4.0.220; http://www.OriginLab.com).

Biological assays

Cell viability in all experiments was determined by the colourimetric MTT assay. Detailed experimental procedures can be found in the SI file. The statistical analysis of the experiments was performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA, USA; www.graphpad.com).

Acknowledgements

This work was supported by grants from Universidad de Buenos Aires (UBACyT 20020130100826BA and 20020130100584BA) and ANPCyT (PICT 2331/2013 and 2281/2013). We are also grateful to Ms María Marta Rancez for her assistance with English language editing.

Keywords: natural products mimics • molecular complexity • multicomponent reactions • antiproliferative • phenanthrenes

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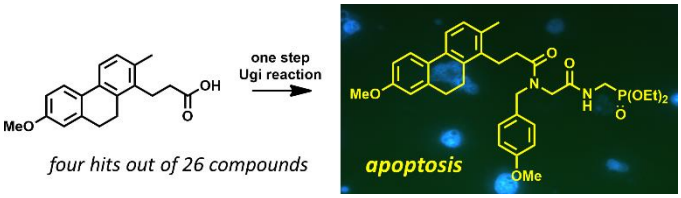

We describe the design of a small library of natural product mimics where structural complexity was introduced following simple guidelines, while maintaining a low synthetic cost, by using multicomponent reactions. The resulting compounds, inspired on the phenanthrenoid scaffold, yielded a new antiproliferative chemical entity which resulted more potent than 5-fluorouracil in cell-based assays.