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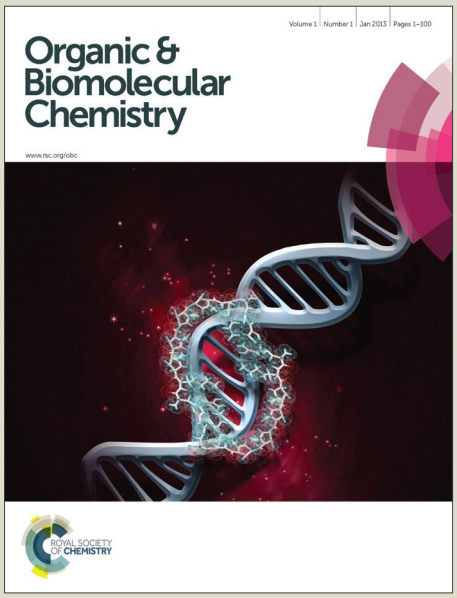

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ARTICLE

An Approach to "Escape from Flatland": Chemo-enzymatic Synthesis and Biological Profiling of a Library of Bridged Bicyclic Compounds

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A major reason for the low success rate in current drug development through chemical synthesis has been ascribed to the large fraction of quasi planar candidate molecules. Therefore, an "escape from flatland" strategy has been recommended for the generation of bioactive chemical entities. In a first attempt to test this recommendation, we synthesized a small collection of bridged bicyclic compounds possessing a rigid spherical core structure by combining a group of cyclic dienes with a collection of dienophiles. We started from planar biphenyl analogues and, by enzymatic dioxygenation, transformed them into hydroxylated diene structures. Using a small library of newly synthesized dienophiles, the dienes were converted into bridged bicycles via the Diels-Alder reaction. The resulting collection of 78 structures was first tested for bioactivity in a generic assay based on interference with the proliferation of mammalian cells. A more mechanism-targeted bioactivity profiling method, exploiting cellular impedance monitoring, was subsequently used to obtain suggestions for the mode of action exerted by those compounds that were the most active in the proliferation assay. Proteasome inhibition could be confirmed for 8 of a series of 9 respective candidates. Whilst 7 of these molecules showed relatively weak interference with proteasome activity, one candidate exerted a moderate but distinct inhibition. This result appears remarkable in view of the small size of the compound library, which was synthesized following a few basic considerations. It encourages the application of diverse synthetic approaches to further investigate the role of spherical shape for the success of compound libraries.

INTRODUCTION

Within the last 50 years, the rate of drug discovery is almost unchanged and clearly below the required level.[1] Synthetic compound libraries as source for new drug candidates typically suffer from low success in clinical development.[2,3-5] An obvious reason for this failure may be the chemistry-driven synthetic strategies by which compounds are produced in view of easy accessibility rather than structural complexity. Strategies for the chemical synthesis of compound libraries should increasingly take into account structural features that distinguish drugs and bioactive compounds from compounds in general.

A comparison between marketed drugs, compounds originating from combinatorial synthesis and natural products indicated that the structural diversity of combinatorial compound collections is substantially lower than that of either drugs or natural products and that the former are restricted to only a confined region of the chemical space.[6] A recent study by Reymond and co-workers[7], considering molecular shape as one of the important characteristics of bioactive molecules, revealed that the vast majority of synthesized molecules were distributed in space regions characteristic for flat molecules (rod or disc shapes), while very few compounds were found in the space harboring three-dimensional structures. Proteins as the main drug targets, however, typically use three-dimensional sub-structures to bind small molecules. Thus, the synthesis of small molecules with intrinsic three-dimensionality appears of considerable interest in order to populate unexplored regions of the chemical space and to find candidates complementary to protein cavities. The presence of a rigid scaffold[6] and few rotatable bonds is frequently observed in bioactive molecules.[6] A respective survey indeed showed that molecules with a significant three-dimensional portion showed higher success rates in drug development.[8] The rationale for this success may be their rigid spherical structure, as the rigid nature of a small molecule can play a crucial role in binding to macromolecules, due to lower entropic losses in comparison to a flexible ligand with the same interaction pattern.[9] Moreover, the rigidity of a ligand typically contributes to increase the selectivity.[10] This may be achieved directly or, in cases of a flexible target and a rigid decoy, by inducing a fit only in the desired target.[10] For these reasons, an "escape from flatland" has been suggested as a

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Electronic Supplementary Information (ESI) available: ^{1}H NMR, ^{13}C NMR spectra of all the compounds and the table of numerical values obtained in the impedance measurement. See DOI: 10.1039/x0xx00000x

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strategy to increase the success rate in the synthesis of bioactive compounds and potential drug candidates.[7,8]

The above-mentioned features of spherical structures encouraged us to throw focus on the synthesis of rigid sp^3-rich compounds. Out of the many strategies to obtain such molecules, we decided on a brief two-step synthetic pathway that permits the combination of building blocks containing sub-structures that are related to "privileged" scaffolds. As one of the starting components we used aromatic compounds, more precisely, hetero-aromatic analogues of biphenyl, which is regarded as a privileged structure. We decided for *N*-substituted analogues in order to introduce additional hydrogen bond donors and acceptors into the final products. Aromatic molecules are chemically highly stable. However, they may be enzymatically functionalized in a mild and selective way, for example, by introducing simultaneously two hydroxy groups and a conjugated diene structure (**Fig. 1**). This reaction is catalyzed by bacterial aryl-hydroxylating dioxygenases (ARHDOs). The resulting versatile dienediol (DD) synthons may, for example, be used in [4+2] cycloaddition reactions that introduce a bridged bicyclic structure (**Fig. 1**). If required, protection and deprotection steps for the two hydroxy groups may additionally be carried out. A wide range of dienophiles (DPs) was used as partners in the cycloaddition reactions, among them molecules that contain privileged sub-structures.

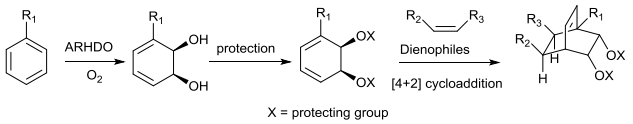


Fig. 1 Devised chemo-enzymatic route to synthesize bridged bicycles as a compound type that possesses a rigid three-dimensional structure. ARHDO: aryl-hydroxylating dioxygenase. Only one of the possible stereoisomers is shown.

As our approach was a structure-oriented, not a target-oriented synthetic strategy, we first used a generic poly-target biological screen for a broad assessment of bioactivity in the obtained compound collection. In order to elucidate the mode of action of the most active candidates, we applied a profiling method that exploits cellular impedance monitoring. Finally, we attempted to verify positive profiling results by appropriate biochemical assays.

Results & discussion

Transformation of aromatic compounds into cyclic *cis*-dienediols

In order to obtain the diene components for the synthesis of bridged bicycles (BBs), five partly hetero-aromatic biphenyl analogues, 2-, 3-, and 4-phenylpyridine, 4-phenylpyrimidine and 1-phenyl-1H-imidazole, were converted into *cis*-dienediols through dioxygenation by recombinant bacterial cells that efficiently synthesize an ARHDO (H. Overwin *et al.*, in preparation). The resulting DD structures are shown in **Fig. 2**. In addition to these compounds, a commercially available DD, the dioxygenation product derived from bromobenzene was also used in the synthesis of BBs.

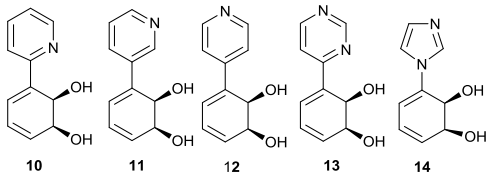
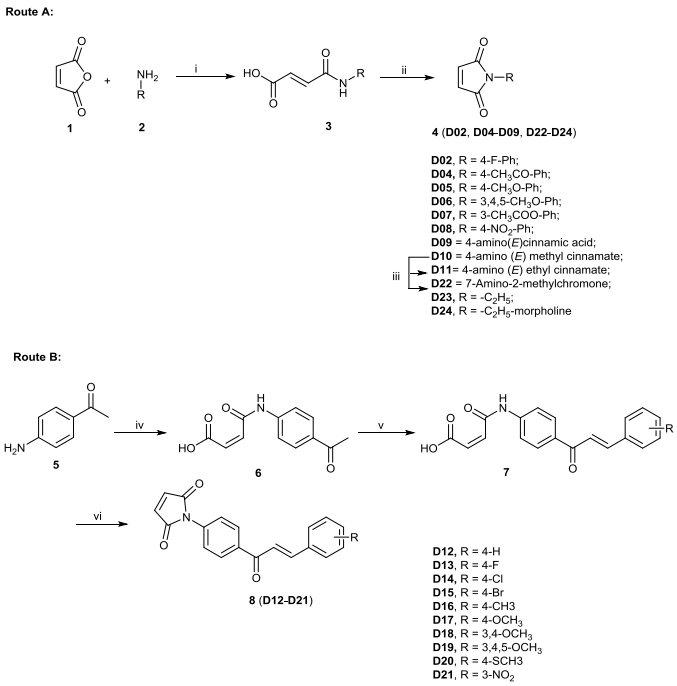


Fig. 2 Structures of hetero-aryl dienediols prepared for the synthesis of bridged bicycles.

Synthesis of dienophiles (DPs)

An overview on the DPs used in this project is given in **Fig. 3**. Five of the molecules (**D01**, **D03**, **D23**, **D25** and **D26**) were obtained from commercial sources. The others were synthesized, following two routes (**Scheme 1**). Chemically, the DPs may be subdivided into groups i - vii, as indicated in the legend of Fig. 3. Route A was used for the synthesis of DPs belonging to groups ii, iii, v and vi. Route B was applied to the synthesis of DPs belonging to group iv. All of the dienophiles synthesized were *N*-substituted maleimides, as the electrophilic maleimide moiety is expected to show good reactivity towards dienes in Diels-Alder reactions.[11]



Scheme 1 *Reagents and conditions*: (i) EtOAC, RT, 4 h; (ii) NaOAc, Ac_2O, RT- 80 °C, 2 h; (iii) H^+, MeOH/EtOH, reflux, overnight; (iv) maleicanhydride, EtOAc, RT, 4 h; (v) ArCHO, aq NaOH, 0 °C-RT, overnight; (vi) NaOAc, Ac_2O, RT- 80 °C, 2 h.

The maleimides of route A (**Scheme 1**) were readily obtained by reacting the appropriate substituted amine (**2**) with maleic anhydride (**1**) in ethyl acetate, leading to the corresponding *N*-aryl or -alkylmaleamic acid (**3**), which precipitates in ethyl acetate. Without any further purification, this open intermediate was subsequently cyclized in acetic anhydride in the presence of sodium acetate to the desired

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maleimide (**4**). Privileged structures like cinnamic acid and chromone were included as precursors for the synthesis of *N*-substituted maleimides. The 4′-cinnamate-based maleimides (**D09**-**D11**) were obtained by reacting the (*E*)-4-aminocinnamic acid with maleic anhydride and a subsequent cyclization, in two instances followed by esterification. For the preparation of **D22**, 7-amino-2-methylchromone was used as building block.

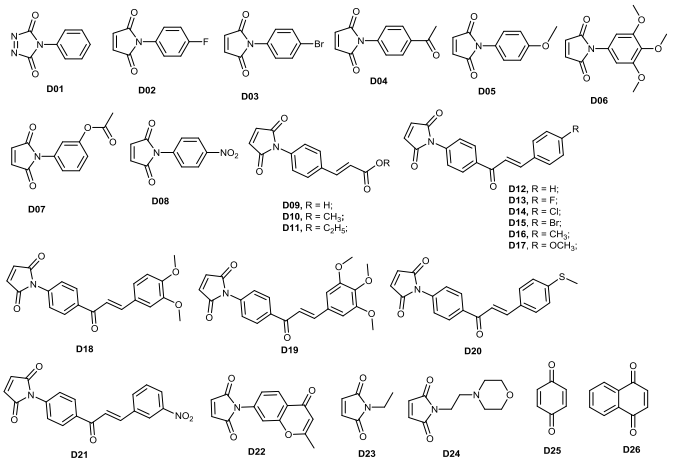
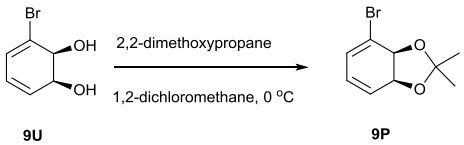


Fig. 3 Overview of structures of dienophiles used for the synthesis of bridged bicycles. They may chemically be subdivided into the following groups: (i) triazoline derivatives (**D01**), (ii) aryl maleimides (**D02**-**D08**), (iii) cinnamate maleimides (**D09**-**D11**), (iv) chalcone maleimides (**D12**-**D21**), (v) chromone maleimides (**D22**), (vi) alkyl maleimides (**D23**-**D24**) and (vii) quinones (**D25**-**D26**).

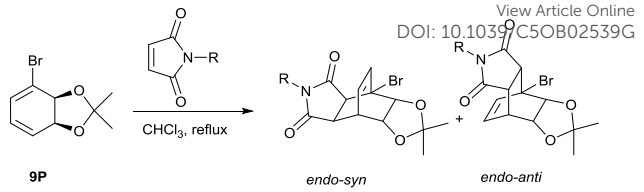
The maleimides of route B (**D12**-**D21**) were obtained by Claisen-Schmidt condensation.[12] Therefore, 4-aminoacetophenone (**5**) was reacted with maleic anhydride to yield the *N*-substituted maleamic acid (**6**). The intermediate was further reacted with appropriate arylaldehydes under basic conditions to afford the intermediate, maleamic acid with an *α*,β-unsaturated carbonyl skeleton (**7**), which was cyclized to yield the desired maleimide-chalcone conjugates (**8**). Of these compounds, the chalcone-based maleimides **D13**, **D20**, **D21** and the chromone-based maleimide **D22** were not previously described in the literature.

Synthesis of bridged bicycles

A first round of BB syntheses was carried out with the commercial DD, (*1S*-*cis*)-3-bromo-3,5-cyclohexadiene-1,2-diol (**9U**, **Scheme 2**). As synthetic applications involving *cis*-diol metabolites often require protection of the hydroxy groups,[13] they were derivatized to an acetonide (**9P**, **Scheme 2**), as previously reported.[14]



Scheme 2 Protection of the vicinal dienediol hydroxy groups with 2,2-dimethoxy propane.



Scheme 3 Isomers formed in cycloadditions between dienediol **9P** and various dienophiles. *Syn* and *anti* are assigned with respect to the isopropylidene ring.

Table 1 Bridged bicyclic compounds obtained from cycloaddition reactions between dienediols **11U** and/or **11P** and collection of dienophiles.[a]

Dieno phile	Bridged bicycle	Dieno phile	Bridged bicycle	Dieno phile	Bridged bicycle
D01	BrDUD01S	D07	BrDPD07S	D18	BrDUD18S
D01	BrDPD01A	D07	BrDPD07A	D18	BrDPD18A
D02	BrDUD02S	D08	BrDUD08S	D19	BrDUD19S
D02	BrDPD02A	D08	BrDUD08A	D20	BrDUD20S
D02	BrDPD02S	D09	BrDPD09S	D20	BrDPD20S
D03	BrDUD03S	D10	BrDUD10S	D20	BrDPD20A
D03	BrDPD03S	D10	BrDUD10A	D21	BrDUD21S
D03	BrDPD03A	D10	BrDPD10S	D21	BrDPD21A
D04	BrDUD04S	D10	BrDPD10A	D22	BrDUD22S
D04	BrDPD04S	D11	BrDPD11A	D23	BrDUD23S
D04	BrDPD04A	D12	BrDUD12S	D23	BrDPD23S
D05	BrDUD05S	D13	BrDUD13S	D23	BrDPD23A
D05	BrDUD05A	D13	BrDPD13S	D24	BrDUD24S
D05	BrDPD05S	D13	BrDPD13A	D24	BrDPD24S
D05	BrDPD05A	D14	BrDUD14S	D24	BrDPD24A
D06	BrDUD06S	D15	BrDPD15S	D25	BrDUD25S
D06	BrDUD06A	D15	BrDPD15A	D26	BrDUD26S
D06	BrDPD06S	D16	BrDUD16S		
D06	BrDPD06A	D17	BrDUD17S		
D07	BrDUD07S	D17	BrDPD17S		
D07	BrDUD07A	D17	BrDPD17A		

[a]Compounds are ordered by the dienophile number (for structures see **Fig. 3**). The compound codes shown contain the following information. BrD designates that the dienediol derived from bromobenzene was used. 'P' or 'U' stands for protected or unprotected, respectively; this is followed by the dienophile number; the final letter indicates the stereochemistry of the structure, either *endo–syn* (S) or *endo–anti* (A).

Table 2 Bridged bicyclic compounds obtained from cycloaddition reactions between dienediols of heterocyclic biphenyl analogues and nine selected dienophiles.[a]

Dienop hile	Bridged bicycle	Dienop hile	Bridged bicycle	Dienop hile	Bridged bicycle
D01	Py2DUD01S	D10	PmDUD10S	D19	Py3DUD19S
D01	Py3DUD01S	D10	ImDUD10S	D19	PmDUD19S
D01	Py4DUD01S	D13	Py3DUD13S	D20	Py3DUD20S
D01	PmDUD01S	D13	PmDUD13S	D20	PmDUD20S
D06	Py3DUD06S	D17	Py3DUD17S	D21	Py3DUD21S
D06	PmDUD06S	D17	PmDUD17S	D21	PmDUD21S
D10	Py3DUD10S				

[a]Compounds are ordered according to the dienophile number (for structures see **Fig. 3**). In the compound codes, **Py2D**, **Py3D**, **Py4D**, **PmD** and **ImD** designate dienediols **12** to **16** (**Fig. 2**). Further details of compound codes are given in the legend of **Table 1**.

The acetonide was reacted with all dienophiles shown in **Fig. 3**, except for **D08**, **D12**, **D14**, **D16**, **D25** and **D26**, which were

synthesized later, to afford bridged bicyclic structures in a simple one-step reaction. The rates of these reactions, when performed at RT, were sluggish, thus requiring long reaction times (4-7 days). Refluxing the solution of the protected DD (**9P**) and a dienophile in chloroform resulted in good yields. Except **D01**, all of the dienophiles yielded a mixture of two products. NMR characterization showed that they were the *endo* isomers of the expected product. These structures were identified by two-dimensional NMR spectroscopy with NOE data. A through-space interaction between one of the methyl groups of the isopropylidene ring and the methine protons of the maleimide moiety identified the *endo-syn* isomer. *syn* refers to the isopropylidenedioxy ring face of the diene (**Scheme 3**). For the *endo-anti* isomer, a similar proximity was observed between the methine protons of the maleimide ring and the protons on the carbons bonded to the two oxygens of the isopropylidene ring. With four exceptions, the *syn* product was the major isomer. With **D10**, **D11** and **D23**, the *anti* isomer had been formed in moderate excess (60-65%). In case of **D01**, the *anti* isomer was exclusively obtained, as previously reported.[15] The typically observed preference for the *syn* isomer is in accordance with previous findings,[16] which had shown that, while most DPs yield *anti* isomers in high excess, maleimides have a significant tendency to increase formation of the *syn* isomer. A strong *syn* selection is generally noticed when the diol groups of the dienes are unprotected (see results below).[17,18] This is ascribed to the formation of hydrogen bonds between the diol and the dienophile, which direct the latter to the *syn* face.[17] For maleimides, we propose a similar effect also for protected diols. The two oxygens are still available as hydrogen bond acceptors. Hydrogens at double- or triple-bonded carbons possess a low acidity. In the case of maleimides, this is enhanced not only by the vicinal oxygens, but additionally by the vicinal nitrogen. Therefore, the formations of weak bonds between these hydrogens and the acetonide oxygens may exert a weak directing effect towards the *syn* face. The only striking exception was **D01**, which exclusively yielded the *anti* isomer, in agreement with literature data.[15,17,19] This has been ascribed to electrostatic repulsion between the lone electron pairs of the acetonide oxygens and of the triazolidinone nitrogens upon approach of the dienophile at the *syn* face.[16,17] We note that the absence of any hydrogens at the reacting bond of this DP may also disfavour *syn* formation. In case of unprotected DDs, when hydrogen bond formation between the reaction partners is possible, a significant increase of the *syn* isomer was observed.[17]

To deprotect cycloadducts, compounds were refluxed under acidic conditions, as previously reported.[20] This resulted in poor yields (less than 30%). Therefore, Diels-Alder reactions were attempted without prior protection of the hydroxy groups. Cycloadditions were performed with the DD **9U** and the entire collection of dienophiles (**Fig. 3**). Cycloaddition products from all dienophiles were obtained successfully in moderate to good yields in the range of 40-70%, except with quinone-based dienophiles (**D25** and **D26**), where yields were lower (25-30%). In cycloaddition reactions reported previously with protected and unprotected DDs, higher reactivity was observed with protected DDs under otherwise identical conditions.[16] However, with DDs **9U** and **9P** the reactivity observed towards several dienophiles was the same. All DPs yielded about 90% of the *endo-syn* isomer, probably due to the directing effect of bond formation with the diol hydrogens.[17] A list of all cycloaddition products obtained from DDs **9U** and **9P** is shown in **Table 1**. The compound codes given there, together with the structures shown in **Fig. 2**, **Fig. 3** and **Scheme 3**, allow to deduce the sterical structures of all listed compounds. Additionally, all structures are shown in the Supplementary Information.

Nine dienophiles were selected for the synthesis of cycloadducts with hetero-aryl DDs. This was based on reactivity and yields with DDs **9U** and **9P** and on the initial results of the biological activity screening (see below) of the first collection of bridged bicycles derived from them. Six of the selected dienophiles (**D13**, **D17**, **D18**, **D19**, **D20** and **D21**, see **Fig. 3**), were chalcone-based maleimides. The others were N-(3,4,5-trimethoxy phenyl)maleimide (**D06**) and 4-phenyl-1,2,4-triazoline-3,5-dione (**D01**). Because of the low yields with the acetonide deprotection of the bridged bicyclic products from the DD **9P**, all cycloadditions were carried out with unprotected hetero-aryl DDs. The first cycloaddition reactions with the selected dienophiles were performed with DD **14** (**Fig. 2**) and dienophiles **D06**, **D10** and **D17** under the same conditions used for DDs **9U** and **9P**. However, they resulted in only about 10% yields of the desired products, accompanied by several by-products. Therefore, different reaction conditions were explored. As a result, preparative reactions were repeated in a sealed tube with chloroform as solvent under elevated temperature and pressure for 3 days. This increased yields with DD **14** to about 16%. However, reactions performed with DDs **11** and **13** (**Fig. 2**) under these conditions afforded products in significantly increased yields of 30-45% and 40-60%, respectively, with all of the selected dienophiles. DDs **10** and **12** were reacted with dienophile **D01**. They showed similar reactivity to that of DD **11**. Altogether, 20 bridged bicyclic compounds were synthesized with the five hetero-aryl DDs (**Table 2** and Supplementary Information). The predominant products formed were the *endo-syn* isomers with an approximately 9-fold excess over the *endo-anti* isomers. The lower reactivity of the hetero-aryl-substituted DDs in the cycloaddition reactions, compared to the bromo-substituted DD **9U**, may be due to increased electron-withdrawal from the DD ring, caused by the aryl nitrogens, in conjunction with the extended conjugated system of the molecules. Reduced electron densities of the diene carbons are expected to make them more stable towards attack by the DP.[21] All bridged bicyclic compounds synthesized were not previously reported.

Assessment of bioactivity

As there is no assay for a general assessment of any kind of bioactivity, we determined the influence of our compounds on the proliferation of a mammalian cell line [22], which appeared to us to be the best available option. The half maximal inhibitory concentration

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values for proliferation (IC_{50}) of the bridged bicycles derived from bromobenzene and the hetero-aromatic biphenyl analogues are shown in **Table 3**. As can be seen, BBs synthesized with cinnamate- and chalcone-based dienophiles typically showed higher bioactivities than the other compounds.

Table 3 Bioactivity values of bridged bicyclic compounds as determined in a cell viability assay.[a]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
Group (i)		**Group (iv)**	
BrDUD01S	41.2±2.8	BrDUD12S	38.5±1.4
BrDPD01A	59.2±2.8	BrDUD13S	47±2.8
Py2DUD01S	82.4±3.5	BrDPD13S	21.8±1.06
Py3DUD01S	68.7±4.2	BrDPD13A	9.1±0.35
Py4DUD01S	82.4±1.4	Py3DUD13S	39.2±0.7
PmDUD01S	96±4.9	PmDUD13S	29.3±2.1
Group (ii)		BrDUD14S	34.1±0.7
BrDUD02S	NC*	BrDPD15S	19.6±0.7
BrDPD02A	NC	BrDPD15A	19.6±1.4
BrDPD02S	NC	BrDUD16S	6.9±0.14
BrDUD03S	NC	BrDUD17S	13.4±0.35
BrDPD03S	NC	BrDPD17S	35.5±2.1
BrDPD03A	NC	BrDPD17A	8.8±0.21
BrDUD04S	NC	Py3DUD17S	38.3±1.4
BrDPD04S	NC	PmDUD17S	67±4.9
BrDPD04A	NC	BrDUD18S	18.1±2.8
BrDUD05S	NC	BrDPD18A	16.9±1.06
BrDUD05A	NC	BrDUD19S	4.3±0.14
BrDPD05S	NC	Py3DUD19S	12±0.7
BrDPD05A	NC	PmDUD19S	30.9±0.35
BrDUD06S	44.1±1.4	BrDUD20S	22.3±2.1
BrDUD06A	NC	BrDPD20S	29.4±1.06
BrDPD06S	NC	BrDPD20A	24.2±1.06
BrDPD06A	NC	Py3DUD20S	37.2±2.8
Py3DUD06S	NC	PmDUD20S	37.1±0.35
PmDUD06S	64±2.83	BrDUD21S	31.6±0.7
BrDUD07S	NC	BrDPD21A	32.9±1.4
BrDUD07A	NC	Py3DUD21S	33.5±5.3
BrDPD07S	NC	PmDUD21S	40.1±1.4
BrDPD07A	NC	**Group (v)**	
BrDUD08S	39.2±1.06	BrDUD22S	NC
BrDUD08A	NC	**Group (vi)**	
Group (iii)		BrDUD23S	NC
BrDPD09S	25.4±2.1	BrDPD23S	NC
BrDUD10S	NC	BrDPD23A	NC
BrDUD10A	NC	BrDUD24S	NC
BrDPD10S	37±0.7	BrDPD24S	NC
BrDPD10A	12.3±1.06	BrDPD24A	NC
Py3DUD10S	49.3±3.53	**Group (vii)**	
PmDUD10S	80.5±1.43	BrDUD25S	23.5±1.4
ImDUD10S	64.4±5.6	BrDUD26S	40.2±0.7
BrDPD11A	NC		

[a]Standard deviations are indicated. Compound groups are defined in the legend of **Fig. 3**. Compound codes are explained in **Tables 1** and **2**. *NC-Not considered (IC_{50} > 100 μM).

More specifically, such differences were also observed in the comparisons of the IC_{50} values of pairs of compounds that are identical except for the absence or presence, respectively, of the α,β-unsaturated keto moiety. For example, compound **BrDUD06S**, carrying a 3,4,5-trimethoxyphenyl moiety, showed an IC_{50} value of 44.1 μM, but its analogue, carrying a 3,4,5-trimethoxychalcone moiety, compound **BrDUD19S**, exerted the same effect at an almost 10-fold lower concentration (4.3 μM). Protection of the diol hydroxy groups resulted in no clear tendency. Both, increases and decreases in activity were observed. When hydroxy groups were protected, the *anti*-isomer always showed higher bioactivity than the *syn*-isomer. This may be seen, for example, in the case of compounds **BrDPD17A** and **BrDPD17S**. Amongst the cycloadducts with a chalcone moiety, different substitutions of the terminal phenyl ring result in different IC_{50} values. The 3,4,5-trimethoxy and 4-methyl substitutions (**BrDUD19S** and **BrDUD16S**) yielded particularly low IC_{50} values of 4.3 or 6.9 μM, respectively. When comparing the cycloaddition products derived from hetero-aromatic biphenyl analogues with their counterparts derived from the bromobenzene (**Table 3**), the former in most cases showed higher IC_{50} values than the latter. It is also noteworthy that, among the former compounds, again a compound which possesses the 3,4,5-trimethoxy substitution at the aromatic ring (**Py3DUD19S**) exhibited the lowest IC_{50} value (12 μM).

Bioactivity profiling

In order to obtain suggestions about the mode of action with which the most active of these molecules interfere, a label-free bioactivity profiling method, measurement of cellular impedance, was applied.[23] The 16 most active of the bromobenzene-derived molecules and the 14 most active of the biphenyl-analog-derived molecules (**Table 3**) were selected for this analysis.

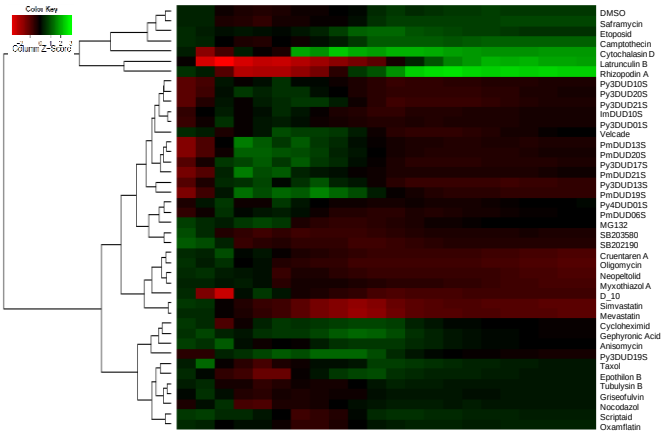

Fig. 4 Heatmap deduced from the cellular impedance measurement of 14 BBs derived from biphenyl analogues, clustered with 26 reference compounds. On the x-axis, basis spline coeficients derived from the cubic smoothing splines fitting of the TCRPs (corresponding to time-segments of the TCRPs, for details see Materials and Methods) are shown for the measurement time after compound addition of 66.5 h. The values of the basis spline coeficients were the Z-transpormed, indicates how many standard deviations the descriptors deviate from the mean value 0 of the descriptors. The profiles were clustered leading to a dendrogram representation of TCRP similarity on the y-axis. Compound names or codes (**Tables 1** and **2**) are indicated at the right margin.

It had been observed that cellular impedance is sensitive to perturbations, as brought about, for example, by the interaction of cellular components with small molecules.[24] Moreover, it has been shown that the change of this parameter over time can be characteristic for the type of perturbation.[23] Thus, the similarity of a recorded impedance curve, a so-called time-dependent cellular response profile (TCRP) with the curves generated by compounds with known biological activities, yields suggestions about the cellular process the molecule in question may interfere with. Dimensionality reduction of the TCRPs can be achieved by a spline-fitting method.[25] The basis spline coefficients can be used as descriptors to calculate distance values (Euclidean distance) between test and reference compounds. The descriptor profiles of the TRCPs can be visualized in the form of heat maps, where an associated dendrogram constructed by hierarchical clustering of the descriptor profiles of the TCRPs indicates curve similarities.

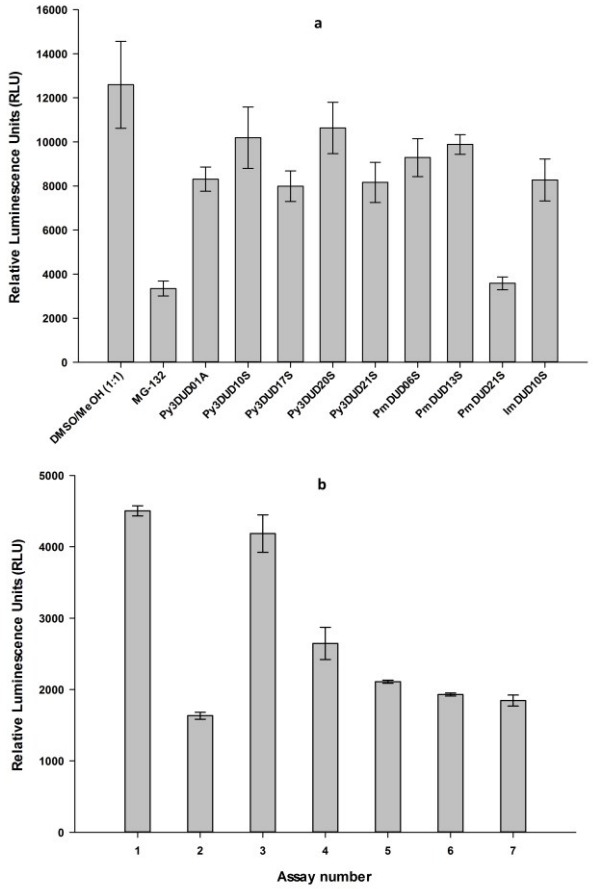

Fig. 5. Bioluminescent proteasome inhibiton assay (a) with the compounds selected from the cellular impedance measurement profiling and (b) with the most active compound at different concentrations. Proteolysis is proportional to the relative luminescence units determined. In part (a), each compound was used at a concentration of 50 µg/mL (92 µM). The leftmost columns show the solvent control (DMSO and methanol in 1:1 ratio (v/v)) and the positive control (4 uM MG-132). In part (b), column 1 shows the solvent control, DMSO and MeOH in a 1:1 ratio (v/v); column 2 shows MG-132 as positive control (4 µM); columns 3-7 show the effects of compound PmDUD21S at 20, 25, 30, 35 and 40 µg/mL, corresponding to 37, 46, 56, 65 and 74 µM. Standard deviations are indicated.

While the impedance measurements with the 16 bromobenzene-derived compounds yielded no clear suggestions, the data from the 14 selected bridged bicyclic compounds based on the biphenyl analogues indicated curve similarities with the known proteasome inhibitors MG-132 and velcade (**Fig. 4**).

Verification of profiling suggestions

The proteasome, a multi-catalytic complex, is responsible for most of the intracellular protein degradation, including proteins that regulate cell cycle and apoptosis. Malignant cells have a defective cell cycle with high proliferation rate. Therefore, these cells largely depend on the proteasome degradation for further cell cycle progression. Proteasome inhibition thus leads to preferential death of proliferating malignant cells.[26]

Nine compounds were selected for a specific assay monitoring proteasome inhibition. The assay performed measures the proteolytic activity associated with the proteasome via bioluminescence. A proteasome substrate peptide that was conjugated with a luminogenic reporter group, aminoluciferin, was added to KB 3–1 cells. The cellular proteasome cleaves the peptide to release the aminoluciferin. The luminescence signal is proportional to proteasome activity. A known proteasome inhibitor, MG-132, was used as positive control. Eight out of the nine compounds showed weak inhibition, whereas one compound, **PmDUD21S**, showed stronger inhibition. (**Fig. 5a**) The assay was repeated with different amounts of this compound to determine the concentration-dependency of the effect (**Fig. 5b**). This clearly confirmed the inhibitory effect at concentrations of 25 µg/mL or above. The compound showed similar proteasome inhibition activity to the known proteasome inhibitor MG-132 at an approximately eightfold higher concentration.

Conclusions

The described results show that in a small collection of 78 compounds, which was synthesized based just on a few general considerations, it was possible to identify a molecule with a moderate, but distinctive biological activity. The principal consideration was to synthesize molecules that possess a rigid spherical structure. Another consideration was to include compounds with privileged (sub-) structures into the building blocks used.

One type of such molecules are biphenyl derivatives. Such aromatic compounds are chemically rather inert. However, enzyme-catalyzed reactions may be used for their functionalization. For the current work, we successfully used dioxygenation that transforms aromatic rings into synthetically versatile dienediols, which may readily be converted into rigid spherical structures. One should be cautious to draw too far-reaching conclusions from the limited survey of the current work. This initial study, however, encouraged us to extend our research on spherical molecules. This is particularly true, as many of the physico-chemical parameters regarded important

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for bioactivity have not yet been optimized in this initial approach. Thus, there is considerable space for improvement along these lines. One of the next steps envisaged is the diversification of compound libraries in terms of sizes and structure types of the three-dimensional scaffolds. With a simultaneous improvement of (and also the adoption of additional) bio-profiling methods, it appears promising to further explore the influence of spherical shape on the success rate of compound collections.

Experimental section

Materials and Methods

NMR data (^{1}H and ^{13}C) was recorded with instruments Bruker DPX-Bruker AV II-300 (300.1 MHz for 1H NMR, ^{13}C NMR with 75.5 MHz, T = 296 K), AV III-400 (400.1 MHz for 1H NMR, ^{13}C NMR with 100.6 MHz, T = 296 K), Bruker DRX-400 (400.1 MHz for 1H NMR, ^{13}C NMR with 100.6 MHz, T = 300 K), and Bruker AV II-600 (600.1 MHz for 1H NMR; ^{13}C NMR with 150.3 MHz). The chemical shifts δ are given in ppm and referenced to the internal solvent standard. Multiplicities of NMR signals are indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets) and br. s (broad singlet). TLC was performed on pre-coated aluminum sheets, silica gel 60 F_{254} with concentrating zone 20 x 2.5 cm (Merck). Silica gel (J. T. Baker) with pore size of 60 Å and HPLC grade solvents were used for the column chromatography purification. High-resolution mass spectra were recorded with nano-spray ionization (HRMS-NSI) in the positive mode, using a Thermo Fischer Scientific LTQ Orbitrap Velos Pro instrument. All non-aqueous reactions were conducted in dried glassware under nitrogen atmosphere. All yields refer to isolated compounds after the final purification process, unless otherwise stated.

General procedure for the synthesis of Dienophiles

Route A:[27] Appropriate aryl or alkyl amine (**2**, 5.8 mmol) was added to the solution of maleic anhydride (**1**, 5.1 mmol) in ethyl acetate (15 mL) and stirred at RT for 1 h. The resultant solid precipitate was filtered and dried under vacuum to get the intermediate, *N*-substituted aminobutenoic acid (**3**). A mixture of NaOAc (2.55 mmol) and acetic anhydride (25.5 mmol) was heated to 80 °C in an oil bath until all NaOAc had dissolved. The intermediate (**3**) was added to this solution and stirred at 80 °C. After 1 h, the reaction was cooled to RT and diluted with ice-cold water (15 mL). The aqueous layer was extracted with ethyl acetate (2 x 15 mL). The organic layers were combined and dried over Na_2SO_4. The organic layer was evaporated at 30 °C under reduced pressure. The obtained crude compounds were purified by column chromatography using petroleum ether and ethyl acetate as eluents to obtain compounds in 40-65% yield.

Route B:[12] A solution of the appropriate aromatic aldehyde (**8,** 1.5 mmol) in ethanol (1 mL) was cooled in an ice bath to 0 °C, after which a precooled solution of sodium hydroxide in water (2.3 mmol in 2 mL) was added dropwise with stirring. A precooled mixture of oxoaminobutenoic acid (**7**, 1.3 mmol), and sodium hydroxide (2.6 mmol) in water (2 mL) was added dropwise to the stirring solution of the aryl aldehyde over a period of 15 min with stirring. The reaction mixture was stirred at RT for 14 h, acidified with 2 N hydrochloric acid and stirred for another 1h. The resultant precipitate was filtered, washed with water and vacuum-dried. The completely dried solid intermediate (**9**) was added to the mixture of NaOAc (0.7 mmol) and acetic anhydride (7.7 mmol), which was preheated to 90 °C for 20 min. The resultant reaction mixture was stirred at 90 °C. After 1 h, the reaction was allowed to cool to RT and quenched with ice-cold water (5 mL). The aqueous layer was extracted with ethyl acetate (2 x 15 mL), and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by column chromatography using dichloromethane as eluent to afford the desired compounds in 40-60% yields.

(E)-1-(4-(3-(4-Fluorophenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (D13)

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.90 (s, 2H), 7.09-7.14 (m, 2H), 7.43 (d, *J* = 15.72 Hz, 1H), 7.54-7.58 (m, 2H), 7.61-7.66 (m, 2H), 7.78 (d, *J* = 15.56 Hz, 1H), 8.11 (d, *J* = 8.85 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 116.3, 121.5, 125.5, 129.4, 130.5, 131.1, 134.5, 135.3, 137.0, 144.0, 163.2, 165.2, 169.0, 189.3; MS (NSI): *m/z* 321 $[M+H]^+$.

(E)-1-(4-(3-(4-(Methylthio)phenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (D20)

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.51 (s, 3H), 6.89 (s, 2H), 7.22-7.28 (m, 2H), 7.46 (d, *J* = 15.72 Hz, 1H), 7.56 (d, *J* = 8.54 Hz, 4H), 7.78 (d, *J* = 15.56 Hz, 1H), 8.07-8.12 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.2, 120.7, 125.5, 126.0, 128.9, 129.5, 131.3, 134.5, 135.1, 137.2, 142.7, 144.9, 169.0, 189.4; MS (NSI): *m/z* 350 $[M+H]^+$.

(E)-1-(4-(3-(3-Nitrophenyl)acryloyl)phenyl)-1H-pyrrole-2,5-dione (D21)

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.24 (s, 2H), 7.60 (d, *J* = 8.70 Hz, 2H), 7.76 (t, *J* = 8.01 Hz, 1H), 7.89 (d, *J* = 15.72 Hz, 1H), 8.20 (d, *J* = 15.72 Hz, 1H), 8.28 (ddd, *J* = 8.24, 2.29, 0.92 Hz, 1H), 8.32 (d, *J* = 8.70 Hz, 2H), 8.34-8.37 (m, 1H), 8.80 (t, *J* = 1.91 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 123.1, 124.8, 126.3, 129.4, 130.4, 134.9, 135.2, 135.9, 136.0, 136.6, 141.7, 148.5, 169.5, 188.3; MS (NSI): *m/z* 349 $[M+H]^+$.

1-(2-Methyl-4-oxo-4H-chromen-7-yl)-1H-pyrrole-2,5-dione (D22)

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.39 (d, J=0.57 Hz, 4H), 6.18 (d, J = 0.75 Hz, 1H), 6.92 (s, 2H), 7.49 (dd, J = 8.67, 1.88 Hz, 1H), 7.57 (d, J = 1.70 Hz, 1H), 8.25 (d, J = 8.67 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.61, 110.9, 114.3, 121.7, 122.4, 126.6, 134.5, 135.8, 156.5, 166.6, 168.7, 177.4; MS (NSI): m/z 256 $[M+H]^+$.

Procedure for the protection of (1S-cis)-3-bromo-3,5-cyclohexadiene-1,2-diol (9U)[14]

2,2-Dimethoxypropane (1.1 mmol) and a catalytic amount of p-toluenesulfonic acid were added to a pre-cooled solution of the DD (**9U**, 0.52 mmol) in dichloromethane (4 mL). The reaction mixture was allowed to warm to RT and stirred until the reaction was complete (monitored by TLC). The reaction mixture was neutralized with triethylamine (0.2 mmol), and dichloromethane was evaporated under reduced pressure at 25 °C. The crude compound was purified by flash column chromatography using ethylacetate and petroleum ether as eluents to obtain the pure compound in 90% yield.

General procedure for [4+2] cycloadditions with protected (*1S-cis*)-3-bromo-3,5-cyclohexadiene-1,2-diol (9P)

A mixture of the protected DD (**9P**, 0.07 mmol) and a dienophile (0.08 mmol) in chloroform (4 mL) was refluxed under a drying tube for 2-4 days.[28] Reaction progress was monitored by TLC. After the reaction was completed, the solvent was removed under reduced pressure at 25 °C and the crude compound was purified by column chromatography using ethyl acetate and petroleum ether as eluents to afford cycloadducts in 40-70% yield.

General procedure for [4+2] cycloadditions with unprotected (1S-cis)-3-bromo-3,5-cyclohexadiene-1,2-diol (9P)[28]

A mixture of DD (**9U**, 0.07 mmol) and a dienophile (0.08 mmol) in toluene (5 mL) was refluxed with a drying tube for 2-4 days. Reaction progress was monitored by TLC. After the reaction was completed, the solvent was removed under reduced pressure at 25 °C and the crude compound was purified by column chromatography using ethyl acetate and petroleum ether as eluents to afford cycloadducts in 40-70% yield.

5-Bromo-10,11-dihydroxy-2-phenyl-5,8-dihydro-1H-5,8-ethano-[1,2,4]triazolo[1,2-a]pyridazine-1,3(2H)-dione

BrDUD01S (*syn*): ^{1}H NMR (500 MHz, Acetone-d_6): δ 4.03-4.14 (m, 2H,) 4.92-4.96 (m, 1H), 5.07 (d, J= 2.75 Hz, 1H), 5.42 (br. s., 1H), 6.61-6.65 (m, 1H), 6.67-6.72 (m, 1H), 7.38-7.51 (m, 5H); ^{13}C NMR (125 MHz, Acetone-d_6): δ 56.1, 63.9, 70.0, 71.2, 126.3, 128.1, 128.8, 130.3, 132.2, 136.8, 153.9, 154.3; HRMS (NSI): Calcd for For $C_{14}H_{13}BrN_3O_4$ $[M+H]^+$ 366.0089, found 366.0084.

4-Bromo-2,2-dimethyl-7-phenyl-3a,4,10,10a-tetrahydro-6H-4,10-etheno[1,3]dio-xolo[4,5-d][1,2,4]triazolo[1,2-a]pyridazine-6,8(7H)-dione

BrDPD01A (*anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (d, J = 5.65 Hz, 6H), 4.62-4.82 (m, 2H), 5.20 (ddd, J = 5.56, 4.05, 1.32 Hz, 1H), 6.30 (ddd, J = 8.43, 5.79, 0.85 Hz, 1H), 6.59 (dt, J = 8.48, 1.32 Hz, 1H), 7.32-7.52 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.5, 25.6, 51.9, 68.0, 74.9, 81.3, 112.5, 125.8, 128.3, 128.7, 129.2, 130.9, 134.8, 154.3, 154.8; HRMS (NSI): Calcd for $C_{17}H_{17}BrN_3O_4$ $[M+H]^+$ 406.0402, found 406.0397.

4-Bromo-2-(4-fluorophenyl)-8,9-dihydroxy-3a,4,7,7a-tetrahydro-1H-4,7-ethano-isoindole-1,3(2H)-dione

BrDUD02S (*endo-syn*): ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.07-3.14 (m, 1H), 3.49-3.55 (m, 1H), 3.61-3.67 (m, 1H), 3.68-3.78 (m, 2H), 5.46-5.63 (m, 2H), 6.23 (dd, J = 8.67, 6.40 Hz, 1H), 6.33-6.40 (m, 1H), 7.19-7.29 (m, 2H), 7.31-7.42 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 44.4, 64.2, 70.5, 115.7, 116.0, 128.4, 129.0, 131.2, 135.9, 159.8, 163.0, 175.0, 177.1; HRMS (NSI): Calcd for $C_{16}H_{14}BrFNO_4$ $[M+H]^+$ 382.009, found 382.0085.

4-Bromo-6-(4-fluorophenyl)-2,2-dimethyl-3a,4,4a,7a,8,8a-hexa-hydro-5H-4,8-eth-eno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD02S (*endo-syn*): ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.58 (s, 3H), 3.52-3.56 (m, 1H), 3.64 (dd, J = 8.44, 2.93 Hz, 1H), 3.74 (d, J = 8.44 Hz, 1H), 4.31 (dd, J = 8.25, 3.85 Hz, 1H), 4.40 (d, J = 8.44 Hz, 1H), 6.18 (dd, J = 8.44, 6.24 Hz, 1H), 6.40 (d, J = 8.44 Hz, 1H), 7.10-7.15 (m, 2H), 7.18-7.22 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.4, 26.3, 36.6, 40.2, 44.2, 57.5, 75.1, 82.0, 113.4, 116.0, 116.3, 127.5, 128.2, 128.3, 131.0, 137.1, 161.1, 163.5, 174.4, 176.5; HRMS (NSI): Calcd for $C_{19}H_{18}BrFNO_4$ $[M+H]^+$ 422.0403, found 422.0398. **BrDPD02A** (*endo-anti*): ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.35 (s, 3H), 1.40 (s, 3H), 3.05 (dd, J = 8.44, 2.93 Hz, 1H), 3.15 (d, J = 8.44 Hz, 1H), 3.56-3.59 (m, 1H), 4.41-4.47 (m, 2H), 6.07 (dd, J = 8.25, 6.42 Hz, 1H), 6.29 (d, J = 8.80 Hz, 1H), 7.10-7.15 (m, 2H), 7.16-7.21 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.1, 25.4, 35.9, 42.2, 46.7, 57.6, 78.0, 83.7, 110.7, 116.1, 116.4, 127.3, 128.2, 128.3, 129.7, 134.5, 160.7, 164.0, 172.4, 174.6; HRMS (NSI): Calcd for $C_{19}H_{18}BrFNO_4$ $[M+H]^+$ 422.0403, found 422.0398.

4-Bromo-2-(4-bromophenyl)-8,9-dihydroxy-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD03S (*endo-syn*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 3.25 (dtd, J = 6.31, 3.06, 3.06, 1.32 Hz, 1H), 3.60-3.66 (m, 1H), 3.72-3.77 (m, 1H), 3.84-3.93 (m, 2H), 4.83 (d, J = 3.39 Hz, 1H), 5.17 (d, J = 3.58 Hz, 1H), 6.24 (dd, J = 8.67, 6.40 Hz, 1H), 6.35-6.40 (m, 1H), 7.20 (d, J = 8.85 Hz, 2H), 7.65 (d, J = 8.67 Hz, 2H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 40.2, 40.9, 45.3, 63.9, 65.5, 71.8, 122.2, 129.6, 132.1, 132.7, 137.4, 175.3, 177.3; HRMS (NSI): Calcd for $C_{16}H_{14}Br_2NO_4$ $[M+H]^+$ 441.9289, found 441.9284.

4-Bromo-6-(4-bromophenyl)-2,2-dimethyl-3a,4,4a,7a,8,8a-hexa-hydro-5H-4,8-eth-eno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD03S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.58 (s, 3H), 3.51-3.57 (m, 1H), 3.60-3.66 (m, 1H), 3.71-3.76 (m, 1H), 4.27-4.34 (m, 1H), 4.37-4.42 (m, 1H), 6.17 (dd, J = 8.48, 6.59 Hz, 1H), 6.40 (d, J = 8.48 Hz, 1H), 7.09-7.15 (m, 2H), 7.53-7.60 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.4, 26.3, 36.6, 40.2, 44.3, 47.1, 57.4, 66.3, 75.0, 82.0, 113.4, 122.6, 124.8, 127.5, 127.9, 131.0, 131.8, 132.3, 137.1, 174.1, 176.3; HRMS (NSI): Calcd for $C_{19}H_{18}Br_2NO_4$ $[M+H]^+$ 481.9602, found 481.9597. **BrDPD03A** (*endo-anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (s, 3H), 1.41 (s, 3H), 3.03-3.08 (m, 1H), 3.13-3.18 (m, 1H), 3.55-3.63 (m, 1H), 4.41-4.50 (m, 2H), 6.07 (dd, J = 8.57, 6.31 Hz, 1H), 6.26-6.32 (m, 1H), 7.07-7.15 (m, 2H), 7.54-7.62 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.1, 25.4, 35.9, 42.3, 46.7, 57.6, 78.0, 83.7, 110.7, 122.8, 127.9, 129.7, 130.4, 132.4, 134.5, 172.1,

174.4; HRMS (NSI): Calcd for $C_{19}H_{18}Br_2NO_4$ $[M+H]^+$ 481.9602, found 481.9597.

2-(4-Acetylphenyl)-4-bromo-8,9-dihydroxy-3a,4,7,7a-tetrahydro-1H-4,7-ethano-isoindole-1,3(2H)-dione

BrDUD04S (*endo-syn*): ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.64 (s, 3H), 3.08-3.15 (m, 1H), 3.55 (dd, J = 8.39, 2.80 Hz, 1H), 3.67 (d, J = 8.14 Hz, 1H), 3.69-3.75 (m, 2H), 5.50 (d, J = 4.07 Hz, 1H), 5.60 (d, J = 4.58 Hz, 1H), 6.24 (dd, J = 8.65, 6.61 Hz, 1H), 6.38 (d, J = 8.65 Hz, 1H), 7.37 (d, J = 8.65 Hz, 2H), 8.09 (d, J = 8.65 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.8, 44.5, 64.2, 70.5, 127.0, 128.8, 131.3, 136.0, 136.4, 174.8, 176.8, 197.2; HRMS (NSI): Calcd for $C_{18}H_{17}BrNO_5$ $[M+H]^+$ 406.029, found406.024 .

6-(4-Acetylphenyl)-4-bromo-2,2-dimethyl-3a,4,4a,7a,8,8a-hexahydro-5H-4,8-eth-eno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD04S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 3H), 1.58 (s, 3H), 2.61 (s, 3H), 3.52 - 3.59 (m, 1H), 3.63-3.69 (m, 1H), 3.73-3.79 (m, 1H), 4.28-4.35 (m, 1H), 4.38-4.43 (m, 1H), 6.19 (dd, J = 8.57, 6.50 Hz, 1H), 6.42 (d, J = 8.48 Hz, 1H), 7.33-7.41 (m, 2H), 7.99-8.06 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.4, 26.3, 26.7, 36.6, 40.3, 44.3, 57.4, 75.0, 82.0, 113.4, 126.4, 129.1, 131.0, 135.7, 136.9, 137.1, 174.0, 176.2, 196.9; HRMS (NSI): Calcd for $C_{21}H_{21}BrNO_5$ $[M+H]^+$ 446.0603, found 446.0598. **BrDPD04A** (*endo-anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (s, 3H), 1.42 (s, 3H), 2.61 (s, 3H), 3.06-3.12 (m, 1H), 3.16-3.22 (m, 1H), 3.60 (dt, J = 5.89, 2.80 Hz, 1H), 4.41-4.51 (m, 2H), 6.09 (dd, J = 8.57, 6.31 Hz, 1H), 6.31 (d, J = 8.67 Hz, 1H), 7.33-7.40 (m, 2H), 8.00-8.07 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.1, 25.4, 26.7, 35.9, 42.3, 46.8, 57.6, 78.0, 83.7, 110.7, 126.4, 129.1, 129.8, 134.5, 135.4, 137.0, 172.0, 174.3, 196.9; HRMS (NSI): Calcd for $C_{21}H_{21}BrNO_5$ $[M+H]^+$ 446.0603, found 446.0598.

4-Bromo-8,9-dihydroxy-2-(4-methoxyphenyl)-3a,4,7,7a-tetrahydro-1H-4,7-etha-noisoindole-1,3(2H)-dione

BrDUD05S (*endo-syn*): ^{1}H NMR (500 MHz, Acetone-d_6): δ 3.28 (br. s., 1H), 3.54-3.57 (m, 1H), 3.63-3.67 (m, 1H), 3.73-3.76 (m, 3H), 3.85 (d, J = 3.20 Hz, 2H), 6.12 (s, 1H), 6.29-6.33 (m, 1H), 6.84-6.89 (m, 2H), 7.02-7.06 (m, 2H); ^{13}C NMR (125 MHz, Acetone-d_6): δ 39.2, 39.9, 44.1, 55.3, 62.7, 64.5, 70.7, 114.2, 124.8, 127.9, 131.0, 136.7, 159.5, 162.4, 175.2, 177.2; HRMS (NSI): Calcd for $C_{17}H_{17}BrNO_5$ $[M+H]^+$ 394.029, found 394.0285. **BrDUD05A** (*endo-anti*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 3.30 (s, 2H), 3.36-3.43 (m, 1H), 3.82 (s, 3H), 4.08 (d, J = 7.35 Hz, 1H), 4.22-4.31 (m, 1H), 4.59 (d, J = 4.33 Hz, 1H), 4.77 (br. s., 1H), 6.14 (dd, J = 8.67, 6.03 Hz, 1H), 6.22-6.31 (m, 1H), 6.93-7.02 (m, 2H), 7.05-7.15 (m, 2H); ^{13}C NMR (125 MHz, Acetone-d_6): δ 39.6, 43.4, 48.6, 55.8, 64.8, 70.1, 75.2, 114.8, 126.2, 128.9, 131.3, 135.5, 160.4, 174.1, 176.3; HRMS (NSI): Calcd for $C_{17}H_{17}BrNO_5$ $[M+H]^+$ 394.029, found 394.0285.

4-Bromo-2,2-dimethyl-6-(3,4,5-trimethoxyphenyl)-3a,4,4a,7a,8,8a-hexahydro-5H-4,8-etheno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD06S (*endo-syn*): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.58 (s, 3H), 3.52-3.57 (m, 1H), 3.60-3.65 (m, 1H), 3.74 (d, J = 8.14 Hz, 1H), 3.79-3.86 (m, 9H), 4.32 (dd, J = 8.14, 4.07 Hz, 1H), 4.38-4.43 (m, 1H), 6.20 (dd, J = 8.39, 6.36 Hz, 1H), 6.39-6.45 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.4, 26.3, 36.6, 40.1, 44.2, 56.3, 57.5, 60.9, 75.1, 82.1, 104.1, 113.4, 127.1, 131.0, 137.1, 138.4, 153.5, 174.5, 176.7; HRMS (NSI): Calcd for $C_{22}H_{25}BrNO_7$ $[M+H]^+$ 494.0814, found 494.0809. **BrDPD06A** (*endo-anti*):^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33-1.39 (m, 3H), 1.42 (s, 3H), 3.01-3.09 (m, 1H), 3.12-3.19 (m, 1H), 3.59 (dt, J = 5.75, 2.78 Hz, 1H), 3.80-3.88 (m, 9H), 4.39-4.51 (m, 2H), 6.10 (dd, J = 8.67, 6.22 Hz, 1H), 6.32 (d, J = 8.67 Hz, 1H), 6.40 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.1, 25.4, 35.9, 42.2, 46.6, 56.3, 57.6, 60.9, 78.1, 83.7, 104.1, 110.7, 126.9, 129.7, 134.6, 138.4, 153.5, 172.5, 174.7; HRMS (NSI): Calcd for $C_{22}H_{25}BrNO_7$ $[M+H]^+$ 494.0814, found 494.0809.

4-Bromo-6-(4-methoxyphenyl)-2,2-dimethyl-3a,4,4a,7a,8,8a-hexahydro-5H-4,8-etheno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD05S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.58 (s, 3H), 3.51-3.57 (m, 1H), 3.59-3.65 (m, 1H), 3.70-3.75 (m, 1H), 3.81 (s, 3H), 4.28-4.34 (m, 1H), 4.37-4.43 (m, 1H), 6.17 (dd, J = 8.48, 6.40 Hz, 1H), 6.40 (d, J = 8.48 Hz, 1H), 6.94 (d, J = 9.04 Hz, 2H), 7.07-7.16 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.4, 26.3, 36.6, 40.1, 44.2, 55.5, 57.6, 75.1, 82.1, 113.3, 114.4, 124.3, 127.6, 131.0, 137.0, 159.6, 174.7, 176.9; HRMS (NSI): Calcd for $C_{20}H_{21}BrNO_5$ $[M+H]^+$ 434.0603, found 434.0598. **BrDPD05A** (*endo-anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (s, 3H), 1.41 (s, 3H), 3.01-3.07 (m, 1H), 3.11-3.17 (m, 1H), 3.55-3.62 (m, 1H), 3.82 (s, 3H), 4.41-4.49 (m, 2H), 6.07 (dd, J = 8.48, 6.22 Hz, 1H), 6.29 (d, J = 8.48 Hz, 1H), 6.92-6.98 (m, 2H), 7.08-7.15 (m, 2H); ^{13}C NMR (75 MHz $CDCl_3$): δ 25.1, 25.4, 35.9, 42.2, 46.6, 55.5, 57.8, 78.1, 83.7, 110.6, 114.5, 124.1, 127.6, 129.7, 134.5, 159.7, 172.7, 175.0; HRMS (NSI): Calcd for $C_{20}H_{21}BrNO_5$ $[M+H]^+$ 434.0603, found 434.0598.

4-Bromo-8,9-dihydroxy-2-(3,4,5-trimethoxyphenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD06S (*endo-syn*): ^{1}H NMR (400 MHz, Acetone-d_6): δ 3.21-3.26 (m, 1H), 3.59 (dd, J = 8.39, 2.80 Hz, 1H), 3.71 (d, J = 8.14 Hz, 1H), 3.74 (s, 3H), 3.79 (s, 6H), 3.84-3.93 (m, 2H), 4.82 (d, J = 4.07 Hz, 1H), 5.16 (d, J = 4.07 Hz, 1H), 6.24 (dd, J = 8.90, 6.36 Hz, 1H), 6.37 (d, J = 8.65 Hz, 1H), 6.49 (s, 2H); ^{13}C NMR (100 MHz, Acetone-d_6): δ 40.2, 40.7, 45.2, 56.6, 60.6, 64.0, 65.5, 71.8, 105.9, 129.2, 132.0, 137.4, 139.3, 154.3, 175.4, 177.5; HRMS (NSI): Calcd for $C_{19}H_{21}BrNO_7$ $[M+H]^+$ 454.0501, found 454.0496. **BrDUD06A** (*endo-anti*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 3.29-3.36 (m, 2H), 3.36-3.43 (m, 1H), 3.72-3.80 (m, 9H), 4.04-4.14 (m, 1H), 4.22-4.31 (m, 1H), 4.59 (d, J = 4.52 Hz, 1H) 4.78 (d, J = 6.22 Hz, 1H), 6.11-6.20 (m, 1H), 6.24-6.30 (m, 1H), 6.48 (s, 2H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 39.6, 43.4, 48.6, 56.6, 60.5, 64.8, 70.1, 75.2, 105.9, 127.0, 129.1, 131.3, 135.5, 154.3, 173.8, 176.1; HRMS (NSI): Calcd for $C_{19}H_{21}BrNO_7$ $[M+H]^+$ 454.0501, found 454.0496.

3-(4-Bromo-8,9-dihydroxy-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-ethanoiso-indol-2-yl)phenyl acetate

BrDUD07S (*endo-syn*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 2.26 (s, 3H), 3.25 (dtd, J = 6.29, 3.03, 3.03, 1.41 Hz, 1H), 3.61-3.66 (m, 1H), 3.72-3.78 (m, 1H), 3.83-3.96 (m, 2H), 4.85 (d, J = 3.96 Hz, 1H), 5.17 (d, J = 4.52 Hz, 1H), 6.24 (dd, J = 8.67, 6.40 Hz, 1H), 6.38 (d, J = 8.67 Hz, 1H), 7.03 (t, J = 2.17 Hz, 1H), 7.15 (dddd, J = 12.24, 8.10, 2.07, 0.94 Hz, 2H), 7.44-7.53 (m, 1H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 20.9, 40.2, 40.9, 45.3, 47.4, 65.5, 71.8, 121.2, 122.6, 124.8, 130.1, 132.1, 134.4, 137.4, 151.9, 169.4, 175.2, 177.3; HRMS (NSI): Calcd for $C_{18}H_{17}BrNO_6$ $[M+H]^+$ 422.0239, found 422.0234. **BrDUD07A** (*endo-anti*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 2.26 (s, 4H), 3.28-3.36 (m, 3H), 3.40-3.45 (m, 1H), 4.05-4.13 (m, 1H), 4.27 (d, J = 4.52 Hz, 1H), 4.60 (d, J = 4.90 Hz, 1H), 4.75-4.81 (m, 1H), 6.11-6.20 (m, 1H), 6.25-6.31 (m, 1H), 7.02 (t, J = 2.07 Hz, 1H), 7.09-7.21 (m, 2H), 7.44-7.54 (m, 1H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 20.9, 39.6, 43.5, 48.7, 70.1, 75.2, 121.2, 122.7, 124.8, 130.1, 131.4, 134.3, 135.5, 151.9, 169.4, 173.7, 175.9; HRMS (NSI): Calcd for $C_{18}H_{17}BrNO_6$ $[M+H]^+$ 422.0239, found 422.0234.

3-(4-Bromo-2,2-dimethyl-5,7-dioxo-3a,4,4a,5,7,7a,8,8a-octahydro-6H-4,8-etheno-[1,3]dioxolo[4,5-f]isoindol-6-yl)phenyl acetate

BrDP07S (*endo-syn*): ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.58 (s, 3H), 2.28 (s, 3H), 3.52-3.55 (m, 1H), 3.63 (dd, J = 8.44, 2.93 Hz, 1H), 3.73 (d, J = 8.44 Hz, 1H), 4.30 (dd, J = 8.25, 3.85 Hz, 1H), 4.39 (d, J = 8.07 Hz, 1H), 6.17 (dd, J = 8.44, 6.60 Hz, 1H), 6.39 (d, J = 8.80 Hz, 1H), 7.07 (t, J = 2.02 Hz, 1H), 7.14 (ddd, J = 7.89, 5.50, 2.02 Hz, 2H), 7.43 (t, J = 8.07 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 21.1, 24.4, 26.3, 36.6, 40.2, 44.2, 57.5, 75.0, 77.2, 82.0, 113.3, 119.6, 121.9, 123.4, 129.6, 131.0, 137.1, 150.8, 168.9, 174.1, 176.2; HRMS (NSI): Calcd for $C_{21}H_{21}BrNO_6$ $[M+H]^+$ 462.0548, found 462.0547. **BrDP07A** (*endo-anti*): ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.36 (s, 3H), 1.41 (s, 3H), 2.29 (s, 3H), 3.04 (dd, J = 8.44, 2.93 Hz, 1H), 3.14 (d, J = 8.80 Hz, 1H), 3.58 (dt, J = 5.96, 2.71 Hz, 1H), 4.41-4.47 (m, 2H), 6.04-6.09 (m, 1H), 6.29 (d, J = 8.44 Hz, 1H), 7.06 (t, J = 2.02 Hz, 1H), 7.12-7.16 (m, 2H), 7.44 (t, J = 8.25 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 21.1, 25.1, 25.4, 35.9, 42.2, 46.6, 57.7, 77.2, 78.0, 83.7, 110.7, 119.7, 122.1, 123.4, 129.7, 132.2, 134.5, 150.8, 168.9, 172.1, 174.3; HRMS (NSI): Calcd for $C_{21}H_{21}BrNO_6$ $[M+H]^+$ 462.0548, found 462.0547.

4-Bromo-8,9-dihydroxy-2-(4-nitrophenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoiso-indole-1,3(2H)-dione

BrDUD08S (*endo-syn*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 3.25-3.34 (m, 1H), 3.65-3.73 (m, 1H), 3.77-3.84 (m, 1H), 3.85-3.97 (m, 2H), 4.85 (d, J = 3.96 Hz, 1H), 5.16-5.22 (m, 1H), 6.27 (dd, J = 8.67, 6.40 Hz, 1H), 6.36-6.44 (m, 1H), 7.55-7.63 (m, 2H), 8.30-8.40 (m, 2H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 40.2, 41.1, 45.5, 63.8, 65.4, 71.7, 124.8, 128.5, 132.2, 137.4, 139.1, 147.9, 175.1, 177.2; HRMS (NSI): Calcd for $C_{16}H_{14}BrN_2O_6$ $[M+H]^+$ 409.0035, found 409.0029. **BrDUD08A** (*endo-anti*): ^{1}H NMR (300 MHz, Acetone-d_6); δ 3.33-3.44 (m, 2H), 3.47-3.52 (m, 1H), 4.09-4.16 (m, 1H), 4.26-4.33 (m, 1H), 4.61 (d, J = 4.90 Hz, 1H), 4.78-4.84 (m, 1H), 6.14-6.21 (m, 1H), 6.25-6.33 (m, 1H), 7.58 (d, J = 9.23 Hz, 2) 8.36 (d, J = 9.23 Hz, 2H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 39.1, 40.6, 43.2, 48.4, 69.5, 74.7, 124.4, 128.0, 131.0, 131.7, 135.1, 147.5, 173.0, 175.3; HRMS (NSI): Calcd for $C_{16}H_{14}BrN_2O_6$ $[M+H]^+$ 409.0035, found 409.0029.

(E)-3-(4-(4-Bromo-2,2-dimethyl-5,7-dioxo-3a,4,4a,5,7,7a,8,8a-octahydro-6H-4,8-etheno[1,3]dioxolo[4,5-f]isoindol-6-yl)phenyl)-acrylic acid

BrDPD09S (*endo-syn*): ^{1}H NMR (400 MHz, Acetone-d_6): δ 1.41 (s, 3H), 1.55 (s, 3H), 3.41-3.47 (m, 1H), 3.70 (dd, J = 8.65, 3.05 Hz, 1H), 3.76-3.83 (m, 1H), 4.39-4.48 (m, 2H), 6.31 (dd, J = 8.39, 6.36 Hz, 1H), 6.42 (d, J = 8.65 Hz, 1H), 6.57 (d, J = 15.77 Hz, 1H), 7.27-7.33 (m, 2H), 7.70 (d, J = 15.77 Hz, 1H), 7.78 (d, J = 8.65 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6): δ 24.6, 26.3, 37.6, 41.4, 45.6, 59.7, 76.0, 83.0, 113.6, 120.4, 128.0, 129.3, 132.4, 135.0, 135.4, 137.4, 144.2, 167.5, 175.0, 177.2; HRMS (NSI): Calcd for $C_{22}H_{21}BrNO_6$ [M+H 474.0552, found 474.0547.

Methyl-(E)-3-(4-(4-bromo-2,2-dimethyl-5,7-dioxo-3a,4,4a,5,7,7a,-8,8a-octahydro-6H-4,8-etheno[1,3]dioxolo[4,5-f]isoindol-6-yl)phenyl)acrylate

BrDUD10S (*endo-syn*): ^{1}H NMR (400 MHz, Acetone-d_6): δ 3.26 (dtd, J = 6.36, 2.92, 2.92, 1.53 Hz, 1H), 3.64 (dd, J = 8.14, 3.05 Hz, 1H), 3.72-3.79 (m, 4H), 3.85-3.96 (m, 2H), 4.83 (d, J = 4.07 Hz, 1H), 5.18 (d, J = 4.07 Hz, 1H), 6.25 (dd, J = 8.65, 6.61 Hz, 1H), 6.39 (d, J = 8.65 Hz, 1H), 6.59 (d, J = 16.28 Hz, 1H), 7.31 (d, J = 8.65 Hz, 2H), 7.69 (d, J = 16.28 Hz, 1H), 7.78 (d, J = 8.65 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6): δ 40.4, 41.1, 45.5, 47.6, 51.9, 64.1, 65.6, 71.9, 119.9, 128.2, 129.4, 135.3, 137.5, 144.3, 167.4, 175.5, 177.5; HRMS (NSI): Calcd for $C_{20}H_{19}BrNO_6$ $[M+H]^+$ 448.0395, found 448.0390. **BrDUD10A** (*endo-anti*): ^{1}H NMR (400 MHz, Acetone-d_6): δ 3.32-3.37 (m, 2H), 3.40-3.47 (m, 1H), 3.76 (s, 3H), 4.11 (t, J = 6.10 Hz, 1H), 4.28 (d, J = 4.58 Hz, 1H), 4.60 (d, J = 5.09 Hz, 1H), 4.79 (d, J = 6.10 Hz, 1H), 6.13-6.20 (m, 1H), 6.28 (d, J = 8.14 Hz, 1H), 6.59 (d, J = 16.28 Hz, 1H), 7.30 (d, J = 8.65 Hz, 2H), 7.69 (d, J = 16.28 Hz, 1H), 7.78 (d, J = 8.65 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6): δ 39.6, 43.6, 48.7, 51.8, 64.7, 70.1, 75.2, 119.8, 128.1, 129.3, 131.4, 135.0, 135.3, 135.5, 144.1, 167.3, 173.7, 176.0; HRMS (NSI): Calcd for $C_{20}H_{19}BrNO_6$ $[M+H]^+$ 448.0395, found 448.0390.

Methyl-(E)-3-(4-(4-bromo-2,2-dimethyl-5,7-dioxo-3a,4,4a,5,7,-7a,8,8a-octahydro-6H-4,8-etheno[1,3]dioxolo[4,5-f]isoindol-6-yl)phenyl)acrylate

BrDPD10S (*endo-syn*): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.58 (s, 3H), 3.55 (dt, J = 6.36, 3.43 Hz, 1H), 3.63-3.67 (m, 1H), 3.73-3.77 (m, 1H), 3.81 (s, 3H), 4.31 (dd, J = 8.14, 4.07 Hz, 1H), 4.38-4.42 (m, 1H), 6.18 (dd, J = 8.39, 6.36 Hz, 1H), 6.38-6.47 (m, 2H), 7.28 (d, J = 8.14 Hz, 2H), 7.59 (d, J = 8.65 Hz, 2H), 7.68 (d, J = 15.77 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.4, 26.3, 36.6, 40.3, 44.3, 51.8, 57.5, 75.0, 82.0, 113.4, 119.2, 126.7, 128.6, 131.0, 133.1, 134.8, 137.1, 143.5, 167.1, 174.2, 176.4; HRMS (NSI): Calcd for $C_{23}H_{23}BrNO_6$ $[M+H]^+$ 488.0709, found 488.0703. **BrDPD10A** (*endo-anti*): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 3H), 1.41 (s, 3H), 3.07 (dd, J = 8.39, 2.80 Hz, 1H), 3.15-3.20 (m, 1H), 3.57-3.62 (m, 1H), 3.81 (s, 3H), 4.42-4.49 (m, 2H), 6.08 (dd, J = 8.65, 6.10 Hz, 1H), 6.30 (d, J = 8.65 Hz, 1H), 6.45 (d, J = 16.28 Hz, 1H), 7.28 (s, 2H), 7.57-7.62 (m, 2H), 7.68 (d, J =

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15.77 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.1, 25.4, 35.9, 42.3, 46.7, 51.9, 57.6, 78.0, 83.7, 110.7, 119.3, 126.7, 128.7, 129.7, 132.8, 134.5, 134.9, 143.4, 167.1, 172.2, 174.5; HRMS (NSI): Calcd for $C_{23}H_{23}BrNO_6$ $[M+H]^+$ 488.0709, found 488.0703.

Ethyl-(E)-3-(4-(4-bromo-2,2-dimethyl-5,7-dioxo-3a,4,4a,5,7,7a,8,8a-octahydro-6H-4,8-etheno[1,3]dioxolo[4,5-f]isoindol-6-yl)phenyl)acrylate

BrDPD11A (*endo-anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28-1.47 (m, 9H), 3.04-3.12 (m, 1H), 3.14-3.21 (m, 1H), 3.56-3.63 (m, 1H), 4.27 (q, *J* = 7.03 Hz, 2H), 4.41-4.51 (m, 2H), 6.08 (dd, *J* = 8.57, 6.31 Hz, 1H), 6.30 (d, *J* = 8.67 Hz, 1H), 6.44 (d, *J* = 16.01 Hz, 1H), 7.23-7.31 (m, 2H), 7.59 (d, *J* = 8.48 Hz, 2H), 7.63-7.72 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 25.1, 25.4, 35.9, 42.3, 46.7, 57.5, 60.7, 78.0, 83.7, 110.7, 119.8, 126.7, 128.6, 129.7, 132.7, 134.5, 135.0, 143.1, 166.7, 172.2, 174.5; HRMS (NSI): Calcd for $C_{24}H_{22}NO_6Br$ $[M+H]^+$ 502.0865, found 502.0854.

4-Bromo-2-(4-cinnamoylphenyl)-8,9-dihydroxy-3a,4,7,7a-tetra-hydro-1H-4,7-eth-anoisoindole-1,3(2H)-dione

BrDUD12S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.48 (dddd, *J* = 5.46, 4.39, 2.29, 1.14 Hz, 1H), 3.68-3.73 (m, 2H), 3.91-4.01 (m, 2H), 6.21 (dd, *J* = 8.62, 6.49 Hz, 1H), 6.42 (dd, *J* = 8.70, 1.22 Hz, 1H), 7.39-7.45 (m, 5H), 7.48 (d, *J* = 15.72 Hz, 1H), 7.61-7.67 (m, 2H), 7.81 (d, *J* = 15.72 Hz, 1H), 8.05-8.11 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.7, 40.0, 44.0, 62.7, 64.8, 71.1, 121.8, 126.4, 128.6, 128.8, 129.1, 129.3, 130.8, 131.2, 134.7, 135.4, 136.3, 138.1, 145.6, 174.3, 176.3, 189.6; HRMS (NSI): Calcd for $C_{25}H_{21}NO_5Br$ $[M+H]^+$ 494.0603, found 494.0598.

(E)-4-Bromo-2-(4-(3-(4-fluorophenyl)acryloyl)phenyl)-8,9-dihydr-oxy-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD13S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.48 (ddd, *J* = 6.41, 3.28, 1.60 Hz, 1H), 3.71 (d, *J* = 1.53 Hz, 2H) 3.92-3.96 (m, 1H), 3.97-4.01 (m, 1H), 6.21 (dd, *J* = 8.70, 6.41 Hz, 1H), 6.42 (dd, *J* = 8.62, 1.14 Hz, 1H), 7.12 (t, *J* = 8.62 Hz, 2H), 7.38-7.43 (m, 3H), 7.64 (dd, *J* = 8.54, 5.34 Hz, 2H), 7.78 (d, *J* = 15.72 Hz, 1H), 8.07 (d, *J* = 8.70 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.7, 40.0, 43.9, 62.6, 64.7, 71.0, 116.1, 116.3, 121.5, 126.4, 128.6, 129.2, 130.5, 131.2, 135.4, 136.3, 137.9, 144.2, 163.2, 165.2, 174.2, 176.3, 189.3; HRMS (NSI): Calcd for $C_{25}H_{20}BrFNO_5$ $[M+H]^+$ 512.0509, found 512.0503.

(E)-4-Bromo-6-(4-(3-(4-fluorophenyl)acryloyl)phenyl)-2,2-dimethyl-3a,4,4a,7a,8,-8a-hexahydro-5H-4,8-etheno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD13S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 3H), 1.59 (s, 3H), 3.53-3.60 (m, 1H), 3.65-3.71 (m, 1H), 3.76-3.81 (m, 1H), 4.30-4.35 (m, 1H), 4.40-4.44 (m, 1H), 6.21 (dd, *J* = 8.48, 6.59 Hz, 1H), 6.43 (d, *J* = 8.48 Hz, 1H), 7.08-7.16 (m, 2H), 7.37-7.45 (m, 3H), 7.60-7.67 (m, 2H), 7.78 (d, *J* = 15.82 Hz, 1H), 8.07 (d, *J* = 8.67 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.4, 26.3, 36.7, 40.3, 44.4, 57.5, 75.0, 82.0, 113.4, 116.4, 121.5, 126.4, 129.3, 129.6, 130.6, 131.1, 131.8, 135.4, 137.1, 138.0, 139.5, 144.2, 162.6, 165.9, 174.1, 176.3, 189.3; HRMS (NSI): Calcd for $C_{28}H_{24}BrFNO_5$ $[M+H]^+$ 552.0822, found 552.0816. **BrDPD13A** (*endo-anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (s, 3H), 1.41 (s, 3H), 3.06-3.13 (m, 1H), 3.16-3.23 (m, 1H), 3.57-3.65 (m, 1H), 4.41-4.51 (m, 2H), 6.06-6.14 (m, 1H), 6.32 (d, *J* = 8.67 Hz, 1H), 7.11 (t, *J* = 8.57 Hz, 2H), 7.35-7.44 (m, 3H), 7.63 (dd, *J* = 8.76, 5.37 Hz, 2H), 7.77 (d, *J* = 15.82 Hz, 1H), 8.07 (d, *J* = 8.48 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.2, 25.5, 36.0, 42.5, 46.9, 57.7, 78.1, 83.8, 110.9, 116.3, 116.5, 121.6, 121.6, 126.5, 129.4, 129.9, 130.6, 130.6, 131.1, 134.7, 135.3, 138.3, 144.4, 163.1, 165.6, 172.2, 174.4, 189.4; HRMS (NSI): Calcd for $C_{28}H_{24}BrFNO_5$ $[M+H]^+$ 552.0822, found 552.0816.

(E)-4-Bromo-2-(4-(3-(4-chlorophenyl)acryloyl)phenyl)-8,9-dihydro-xy-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD14S (*endo-syn*): ^{1}H NMR (500 MHz, Acetone-d_6): δ 3.32 (dtd, *J* = 6.33, 3.09, 3.09, 1.22 Hz, 1H), 3.72 (dd, *J* = 8.54, 3.05 Hz, 1H), 3.83 (d, *J* = 8.39 Hz, 1H), 3.90-3.99 (m, 2H), 5.00 (d, *J* = 4.27 Hz, 1H), 5.31 (t, *J* = 5.11 Hz, 1H), 6.31 (dd, *J* = 8.70, 6.41 Hz, 1H), 6.45 (d, *J* = 8.70 Hz, 1H), 7.49 (d, *J* = 8.70 Hz, 2H), 7.55 (d, *J* = 8.54 Hz, 2H), 7.81-7.85 (m, 1H), 7.91-7.98 (m, 3H), 8.27 (d, *J* = 8.70 Hz, 2H); ^{13}C NMR (125 MHz, Acetone-d_6): δ 40.2, 41.0, 45.4, 63.9, 65.3, 71.7, 123.5, 127.8, 129.8, 129.9, 131.1, 132.1, 134.8, 136.6, 137.4, 137.5, 138.4, 143.6, 175.3, 177.4, 189.1; HRMS (NSI): Calcd for $C_{25}H_{20}BrClNO_5$ $[M+H]^+$ 528.0213, found 528.0208.

(E)-4-Bromo-6-(4-(3-(4-bromophenyl)acryloyl)phenyl)-2,2-dimeth-yl-3a,4,4a,7a,8,-8a-hexahydro-5H-4,8-etheno[1,3]-dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD15S (*endo-syn*): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 3H), 1.59 (s, 3H), 3.57 (dt, *J* = 6.48, 3.12 Hz, 1H), 3.68 (dd, *J* = 8.65, 3.05 Hz, 1H), 3.76-3.80 (m, 1H), 4.32 (dd, *J* = 8.14, 4.07 Hz, 1H), 4.41 (d, *J* = 8.14 Hz, 1H), 6.21 (dd, *J* = 8.39, 6.36 Hz, 1H), 6.43 (d, *J* = 8.65 Hz, 1H), 7.39-7.60 (m, 7H), 7.74 (d, *J* = 15.77 Hz, 1H), 8.07 (d, *J* = 8.65 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.3, 26.3, 36.6, 40.3, 44.3, 57.4, 75.0, 81.9, 113.4, 122.2, 125.1, 126.4, 129.2, 129.8, 131.0, 132.3, 133.6, 135.4, 137.1, 137.8, 144.1, 174.0, 176.2,189.2; HRMS (NSI): Calcd for $C_{28}H_{24}Br_2NO_5$ $[M+H]^+$ 612.0021, found 612.0016. **BrDPD15A** (*endo-anti*): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 3H), 1.42 (s, 3H), 3.10 (dd, *J* = 8.39, 2.80 Hz, 1H), 3.17-3.23 (m, 1H), 3.59-3.65 (m, 1H), 4.44-4.50 (m, 2H), 6.07-6.14 (m, 1H), 6.32 (d, *J* = 8.65 Hz, 1H), 7.39-7.60 (m, 7H), 7.74 (d, *J* = 15.77 Hz, 1H), 8.08 (d, *J* = 8.14 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.0, 25.3, 35.9, 42.3, 46.7, 57.6, 78.0, 83.6, 110.7, 122.2, 125.1, 126.4, 129.3, 129.9, 132.3, 133.6, 134.5, 135.2, 137.9, 144.1, 172.0, 174.3, 189.2; HRMS (NSI): Calcd for $C_{28}H_{24}Br_2NO_5$ $[M+H]^+$ 612.0021, found 612.0016.

(E)-4-Bromo-8,9-dihydroxy-2-(4-(3-(p-tolyl)acryloyl)phenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD16S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.40 (s, 3H), 3.46-3.50 (m, 1H), 3.68-3.73 (m, 2H), 3.92-4.00 (m, 2H), 6.19-6.24 (m, 1H), 6.42 (dd, *J* = 8.62, 1.30 Hz, 1H), 7.24 (d, *J* = 7.93 Hz, 2H), 7.39-7.46 (m, 3H), 7.54 (d, *J* = 8.24 Hz, 2H), 7.79 (d, *J* = 15.72 Hz, 1H), 8.05-8.09 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.6, 38.7, 40.0, 44.0, 62.6, 64.8, 71.1, 120.8, 126.4,

128.6, 129.3, 129.8, 131.2, 132.0, 135.2, 136.3, 138.2, 141.4, 145.7, 174.3, 176.3, 189.8; HRMS (NSI): Calcd for $C_{26}H_{23}NO_5Br$ $[M+H]^+$ 508.0759, found, 508.0754.

(E)-4-Bromo-8,9-dihydroxy-2-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD17S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27 (dd, J = 18.08, 3.20 Hz, 2H), 3.48 (ddd, J = 4.76, 3.16, 1.51 Hz, 1H), 3.71 (d, J = 1.51 Hz, 2H), 3.86 (s, 3H), 3.92-4.02 (m, 2H), 6.21 (dd, J = 8.67, 6.40 Hz, 1H), 6.42 (dd, J = 8.67, 1.13 Hz, 1H), 6.90-6.99 (m, 2H), 7.31-7.44 (m, 3H), 7.55-7.64 (m, 2H), 7.79 (d, J = 15.64 Hz, 1H), 8.04-8.10 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.7, 40.0, 44.0, 55.5, 62.7, 64.8, 71.1, 114.5, 119.6, 126.4, 127.5, 129.2, 129.9, 130.4, 131.2, 135.2, 136.4, 138.4, 145.5, 161.9, 174.3, 176.3, 189.7; HRMS (NSI): Calcd for $C_{26}H_{23}BrNO_6$ $[M+H]^+$ 524.0709, found 524.0703.

(E)-4-Bromo-6-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)-2,2-dimethyl-3a,4,4a,-7a,8,8a-hexahydro-5H-4,8-etheno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD17S (*endo-syn*): ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.43 (s, 3H), 1.59 (s, 3H), 3.55-3.59 (m, 1H), 3.67 (dd, J = 8.44, 2.93 Hz, 1H), 3.76-3.80 (m, 1H), 3.86 (s, 3H), 4.32 (dd, J = 8.07, 3.67 Hz, 1H), 4.41 (d, J = 8.07 Hz, 1H), 6.20 (dd, J = 8.44, 6.60 Hz, 1H), 6.43 (d, J = 8.44 Hz, 1H), 6.93-6.96 (m, 2H), 7.36 (d, J = 15.41 Hz, 1H), 7.39-7.42 (m, 2H), 7.58-7.62 (m, 2H), 7.78 (d, J = 15.77 Hz, 1H), 8.07 (d, J = 8.80 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.4, 26.3, 36.6, 40.3, 44.3, 55.5, 57.5, 75.0, 82.0, 113.4, 114.5, 119.6, 126.3, 127.5, 129.2, 130.4, 131.0, 135.1, 137.1, 138.4, 145.4, 161.9, 174.0, 176.3, 189.6; HRMS (NSI): Calcd for $C_{29}H_{27}BrNO_6$ $[M+H]^+$ 564.1022, found 564.1016. **BrDPD17A** (*endo-anti*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37 (s, 3H), 1.42 (s, 3H), 3.10 (dd, J = 8.54, 2.75 Hz, 1H), 3.20 (d, J = 8.39 Hz, 1H), 3.60-3.63 (m, 1H), 3.86 (s, 3H), 4.44-4.50 (m, 2H), 6.11 (dd, J = 8.62, 6.33 Hz, 1H), 6.32 (dd, J = 8.70, 0.76 Hz, 1H), 6.93-6.96 (m, 2H), 7.36 (d, J = 15.56 Hz, 1H), 7.38-7.41 (m, 2H), 7.58-7.62 (m, 2H), 7.79 (d, J = 15.56 Hz, 1H), 8.07 (d, J = 8.70 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.1, 25.4, 35.9, 42.3, 46.7, 55.5, 57.6, 78.0, 83.7, 110.7, 114.5, 119.5, 126.3, 127.4, 129.2, 129.8, 130.4, 134.5, 134.8, 138.6, 145.5, 161.9, 172.1, 174.4, 189.6; HRMS (NSI): Calcd for $C_{29}H_{27}BrNO_6$ $[M+H]^+$ 564.1022, found 564.1016.

(E)-4-Bromo-2-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)-8,9-dihydroxy-3a,4,-7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD18S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.34-3.52 (m, 3H), 3.60-3.73 (m, 3H), 3.75-3.88 (m, 1H), 3.95 (d, J = 4.71 Hz, 6H), 6.20 (dd, J = 8.67, 6.40 Hz, 1H), 6.42 (dd, J = 8.67, 1.13 Hz, 1H), 6.91 (d, J = 8.48 Hz, 1H), 7.15 (d, J = 1.88 Hz, 1H), 7.21-7.25 (m, 1H), 7.32 (d, J = 15.64 Hz, 1H), 7.38-7.43 (m, 2H), 7.75 (d, J = 15.64 Hz, 1H), 8.06 (d, J = 8.67 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.7, 40.0, 44.0, 56.0, 62.6, 64.8, 71.0, 110.2, 111.2, 120.0, 123.4, 126.4, 127.7, 129.2, 131.2, 135.2, 136.4, 138.4, 145.9, 149.3, 151.7, 174.3, 176.4, 189.9; HRMS (NSI): Calcd for $C_{27}H_{25}BrNO_7$ $[M+H]^+$ 554.0814, found 554.0809.

(E)-4-Bromo-6-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)-2,2-dimethyl-3a,4,-4a,7a,8,8a-hexahydro-5H-4,8-etheno-[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD18A (*endo-anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (s, 3H), 1.42 (s, 3H), 3.10 (dd, J = 8.48, 2.83 Hz, 1H), 3.17-3.23 (m, 1H), 3.58-3.65 (m, 1H), 3.95 (d, J = 4.33 Hz, 6H), 4.46 (s, 2H), 6.06-6.14 (m, 1H), 6.32 (d, J = 8.67 Hz, 1H), 6.91 (d, J = 8.29 Hz, 1H), 7.15 (d, J = 1.70 Hz, 1H), 7.20-7.25 (m, 1H), 7.28-7.36 (m, 1H), 7.40 (d, J = 8.48 Hz, 2H), 7.75 (d, J = 15.64 Hz, 1H), 8.07 (d, J = 8.48 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.1, 25.4, 35.9, 42.3, 46.8, 56.0, 57.6, 78.0, 83.7, 110.2, 110.8, 111.2, 119.9, 123.4, 126.4, 127.7, 129.3, 129.8, 134.6, 134.9, 138.6, 145.9, 149.4, 151.7, 172.1, 174.4, 189.9; HRMS (NSI): Calcd for $C_{30}H_{29}BrNO_7$ $[M+H]^+$ 594.1127, 594.1122.

(E)-4-Bromo-8,9-dihydroxy-2-(4-(3-(3,4,5-trimethoxyphenyl)-acryloyl)phenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD19S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.48 (td, J = 3.16, 1.22 Hz, 1H), 3.71 (d, J = 1.32 Hz, 3H), 3.88-3.99 (m, 11H), 6.20 (dd, J = 8.67, 6.59 Hz, 1H), 6.42 (dd, J = 8.67, 1.13 Hz, 1H), 6.86 (s, 2H), 7.33 (d, J = 15.64 Hz, 1H), 7.41 (d, J = 8.48 Hz, 2H), 7.70 (d, J = 15.64 Hz, 1H), 8.06 (d, J = 8.48 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.7, 40.0, 44.0, 56.3, 61.1, 62.6, 64.8, 71.0, 105.8, 121.5, 126.4, 129.3, 130.2, 131.2, 135.3, 136.4, 138.2, 140.7, 145.9, 153.6, 174.3, 176.4, 190.0; HRMS (NSI): Calcd for $C_{28}H_{27}NO_8Br$ $[M+H]^+$ 584.092 found, 584.0915.

(E)-4-Bromo-8,9-dihydroxy-2-(4-(3-(4-(methylthio)phenyl)acryloyl)phenyl)-3a,4,-7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD20S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.52 (s, 3H), 3.44-3.49 (m, 1H), 3.67-3.73 (m, 2H), 3.92-4.00 (m, 2H), 6.20 (dd, J = 8.70, 6.56 Hz, 1H), 6.42 (dd, J = 8.70, 1.07 Hz, 1H), 7.24-7.28 (m, 2H), 7.38-7.46 (m, 3H), 7.55 (d, J = 8.39 Hz, 2H), 7.76 (d, J = 15.72 Hz, 1H), 8.07 (d, J = 8.85 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.7, 40.0, 44.0, 62.6, 64.8, 71.0, 120.7, 126.0, 126.4, 128.9, 129.2, 131.2, 135.3, 136.4, 138.2, 142.8, 145.2, 174.3, 176.4, 189.6; HRMS (NSI): Calcd for $C_{26}H_{23}BrNO_5S$ $[M+H]^+$ 540.048, found 540.0475.

(E)-4-Bromo-2,2-dimethyl-6-(4-(3-(4-(methylthio)phenyl)-acryloyl)phenyl)-3a,4,4a,7a,8,8a-hexahydro-5H-4,8-etheno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD20S (*endo-syn*): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 3H), 1.59 (s, 3H), 2.52 (s, 3H), 3.57 (dt, J = 6.61, 3.31 Hz, 1H), 3.65-3.70 (m, 1H), 3.76-3.80 (m, 1H), 4.32 (dd, J = 8.14, 3.56 Hz, 1H), 4.40-4.43 (m, 1H), 6.21 (dd, J = 8.39, 6.36 Hz, 1H), 6.43 (d, J = 8.14 Hz, 1H), 7.24-7.29 (m, 2H), 7.39-7.46 (m, 3H), 7.55 (d, J = 8.65 Hz, 2H), 7.77 (d, J = 15.77 Hz, 1H), 8.07 (d, J = 8.65 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.1, 24.4, 26.3, 36.7, 40.3, 44.3, 57.5, 75.0, 77.2, 82.0, 113.4, 120.8, 126.0, 126.4, 128.9, 129.2, 131.0, 131.2, 135.3, 137.1, 138.2, 142.8, 145.0, 174.1, 176.3,

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189.5; HRMS (NSI): Calcd for $C_{29}H_{27}BrNO_5S$ $[M+H]^+$ 580.0793, found 580.0788. **BrDPD20A** (*endo-anti*): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (s, 3H), 1.41 (s, 3H), 2.51 (s, 3H), 3.09 (dd, *J* = 8.39, 2.80 Hz, 1H), 3.16-3.21 (m, 1H), 3.58-3.63 (m, 1H), 4.43-4.49 (m, 2H), 6.07-6.12 (m, 1H), 6.31 (d, *J* = 8.65 Hz, 1H), 7.24-7.28 (m, 2H), 7.37-7.45 (m, 3H), 7.54 (d, *J* = 8.14 Hz, 2H), 7.76 (d, *J* = 15.77 Hz, 1H), 8.06 (d, *J* = 8.65 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.2, 25.1, 25.4, 35.9, 42.3, 46.8, 57.6, 78.0, 83.7, 110.8, 120.7, 126.0, 126.4, 128.9, 129.3, 129.8, 131.2, 134.6, 135.0, 138.4, 142.8, 145.1, 172.1, 174.3, 189.5; HRMS (NSI): Calcd for $C_{29}H_{27}BrNO_5S$ $[M+H]^+$ 580.0793 found, 580.0788.

(E)-4-Bromo-8,9-dihydroxy-2-(4-(3-(3-nitrophenyl)acryloyl)phenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD21S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.49 (ddq, *J* = 6.30, 3.29, 1.53, 1.53, 1.53 Hz, 1H), 3.72 (d, *J* = 1.68 Hz, 2H), 3.93-3.99 (m, 2H), 6.22 (dd, *J* = 8.62, 6.49 Hz, 1H), 6.43 (dd, *J* = 8.62, 1.30 Hz, 1H), 7.45 (d, *J* = 8.70 Hz, 2H), 7.58-7.66 (m, 2H), 7.84 (d, *J* = 15.72 Hz, 1H), 7.93 (d, *J* = 7.78 Hz, 1H), 8.11 (d, *J* = 8.85 Hz, 2H), 8.27 (ddd, *J* = 8.20, 2.25, 0.99 Hz, 1H), 8.51 (t, *J* = 1.91 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.7, 40.0, 44.0, 62.6, 64.8, 71.1, 122.5, 124.3, 124.9, 126.6, 129.4, 130.2, 131.2, 134.4, 135.8, 136.4, 136.5, 137.4, 142.4, 148.8, 174.2, 176.3, 188.7; HRMS (NSI): Calcd for $C_{25}H_{20}N_2O_7Br$ $[M+H]^+$ 539.0454, found 539.0448.

(E)-4-Bromo-2,2-dimethyl-6-(4-(3-(3-nitrophenyl)acryloyl)phenyl)-3a,4,4a,7a,8,-8a-hexahydro-5H-4,8-etheno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD21A (*endo-anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (s, 3H), 1.42 (s, 3H), 3.07-3.24 (m, 2H), 3.59-3.65 (m, 1H), 4.41-4.51 (m, 2H), 6.11 (dd, *J* = 8.67, 6.22 Hz, 1H), 6.30-6.36 (m, 1H), 7.41-7.48 (m, 2H), 7.56-7.67 (m, 2H), 7.80-7.88 (m, 1H), 7.92 (d, *J* = 7.91 Hz, 1H), 8.09-8.15 (m, 2H), 8.28 (dd, *J* = 9.32, 1.22 Hz, 1H), 8.51 (t, *J* = 1.88 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.1, 25.4, 35.9, 42.4, 46.8, 57.6, 77.2, 78.0, 83.7, 110.8, 122.5, 123.7, 124.3, 124.9, 126.6, 129.4, 129.8, 130.1, 134.4, 134.6, 135.6, 136.5, 137.5, 138.2, 142.4, 148.8, 172.1, 174.3, 188.7; HRMS (NSI): Calcd for $C_{28}H_{24}BrN_2O_7$ $[M+H]^+$ 579.0767, found 579.0761.

4-Bromo-8,9-dihydroxy-2-(2-methyl-4-oxo-4H-chromen-7-yl)-3a,4,7,7a-tetra-hydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD22S (*endo-syn*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 2.45 (d, *J* = 0.57 Hz, 3H), 3.32 (dtd, *J* = 6.22, 3.01, 3.01, 1.32 Hz, 1H), 3.68-3.75 (m, 1H), 3.80-3.86 (m, 1H), 3.88-4.01 (m, 2H), 4.89 (d, *J* = 4.14 Hz, 1H), 5.19-5.27 (m, 1H), 6.21 (d, *J* = 0.75 Hz, 1H), 6.32 (dd, *J* = 8.67, 6.40 Hz, 1H), 6.41-6.49 (m, 1H), 7.37 (dd, *J* = 8.48, 1.88 Hz, 1H), 7.47 (d, *J* = 1.70 Hz, 1H), 8.16 (d, *J* = 8.48 Hz, 1H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 20.3, 40.3, 40.9, 45.5, 63.9, 65.5, 71.8, 111.1, 117.0, 123.8, 124.1, 126.4, 132.2, 137.4, 137.8, 157.0, 167.7, 175.1, 176.9, 177.2; HRMS (NSI): Calcd for $C_{20}H_{17}BrNO_6$ $[M+H]^+$ 446.0239, found 446.0235.

4-Bromo-2-ethyl-8,9-dihydroxy-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD23S (*endo-syn*): ^{1}H NMR (400 MHz, Acetone-d_6): δ 1.02 (t, *J* = 7.12 Hz, 3H), 3.10-3.21 (m, 1H), 3.34-3.48 (m, 3H), 3.51-3.59 (m, 1H), 3.76-3.89 (m, 2H), 4.75 (br. s., 1H), 5.09 (br. s., 1H), 6.08 (dd, *J* = 8.65, 6.10 Hz, 1H), 6.23 (d, *J* = 8.65 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6): δ 13.1, 33.9, 39.9, 40.5, 44.9, 64.0, 65.4, 71.7, 131.8, 137.1, 176.0, 178.0; HRMS (NSI): Calcd for $C_{15}H_{19}BrNO_4$ $[M+H]^+$ 356.0497, found 356.0491.

4-Bromo-6-ethyl-2,2-dimethyl-3a,4,4a,7a,8,8a-hexahydro-5H-4,8-etheno[1,3]-dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD23S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10 (t, *J* = 7.25 Hz, 3H), 1.40 (s, 3H), 1.54 (s, 3H), 3.40-3.58 (m, 5H), 4.23-4.28 (m, 1H), 4.33-4.37 (m, 1H), 6.01-6.08 (m, 1H), 6.28 (d, *J* = 9.04 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.0, 24.4, 26.2, 34.0, 36.3, 40.1, 44.1, 57.7, 75.1, 82.1, 113.2, 130.7, 136.8, 175.3, 177.3; HRMS (NSI): Calcd for $C_{15}H_{19}BrNO_4$ $[M+H]^+$ 356.0497, found 356.0492. **BrDPD23A** (*endo-anti*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (t, *J* = 7.16 Hz, 3H), 1.34 (s, 3H), 1.39 (s, 3H), 2.86 (dd, *J* = 8.29, 2.64 Hz, 1H), 2.95-3.01 (m, 1H), 3.46-3.56 (m, 3H), 4.35-4.43 (m, 2H), 5.95 (dd, *J* = 8.57, 6.31 Hz, 1H), 6.18 (dd, *J* = 8.67, 0.94 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.9, 25.0, 25.4, 34.2, 35.6, 42.2, 46.6, 57.8, 78.1, 83.7, 110.6, 129.4, 134.2, 173.3, 175.4; HRMS (NSI): Calcd for $C_{15}H_{19}BrNO_4$ $[M+H]^+$ 356.0497, found 356.0492.

4-Bromo-8,9-dihydroxy-2-(2-morpholinoethyl)-3a,4,7,7a-tetra-hydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

BrDUD24S (*endo-syn*): ^{1}H NMR (700 MHz, DMSO-d_6): δ 2.30 (dt, *J* = 12.53, 6.10 Hz, 6H), 2.99 (dtd, *J* = 6.35, 3.07, 3.07, 1.29 Hz, 1H), 3.29-3.33 (m, 1H), 3.39-3.45 (m, 3H), 3.51 (t, *J* = 4.41 Hz, 4H), 3.60-3.68 (m, 2H), 5.38-5.41 (m, 1H), 5.45-5.48 (m, 1H), 6.03 (dd, *J* = 8.82, 6.45 Hz, 1H), 6.18 (d, *J* = 8.61 Hz, 1H); ^{13}C NMR (175 MHz, DMSO-d_6): δ 35.2, 38.7, 43.9, 52.9, 54.7, 64.2, 66.2, 70.5, 130.9, 135.7, 175.6, 177.7; HRMS (NSI): Calcd for $C_{16}H_{22}BrN_2O_5$ $[M+H]^+$ 401.0711, found 401.0707.

4-Bromo-2,2-dimethyl-6-(2-morpholinoethyl)-3a,4,4a,7a,8,8a-hexahydro-5H-4,8-etheno[1,3]dioxolo[4,5-f]isoindole-5,7(6H)-dione

BrDPD24S (*endo-syn*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (s, 3H), 1.55 (s, 3H), 2.37-2.54 (m, 6H), 3.38-3.51 (m, 2H), 3.53-3.69 (m, 7H), 4.23-4.31 (m, 1H), 4.32-4.39 (m, 1H), 6.04 (dd, *J* = 8.29, 6.78 Hz, 1H), 6.27 (d, *J* = 8.48 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.5, 26.4, 35.9, 36.4, 40.3, 44.2, 55.4, 57.8, 67.2, 75.2, 82.2, 113.4, 130.8, 136.9, 175.5, 177.6; HRMS (NSI): Calcd for $C_{19}H_{26}BrN_2O_5$ $[M+H]^+$ 441.1025, found 441.1020. **BrDPD24A** (*endo-anti*): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 3H), 1.39 (s, 3H), 2.38-2.51 (m, 6H), 2.89 (dd, *J* = 8.14, 2.54 Hz, 1H), 3.00 (d, *J* = 8.14 Hz, 1H), 3.48 (dt, *J* = 5.85, 2.67 Hz, 1H), 3.54-3.67 (m, 6H), 4.35-4.43 (m, 2H), 5.95 (dd, *J* = 8.65, 6.10 Hz, 1H), 6.17 (d, *J* = 8.65 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.1, 25.4, 35.6, 36.0, 42.2, 46.6, 53.4, 55.2, 57.8, 67.0, 78.1, 83.8, 110.6, 129.4, 134.2, 173.4, 175.6; HRMS (NSI): Calcd for $C_{19}H_{26}BrN_2O_5$ $[M+H]^+$ 441.1025, found 441.1020.

1-Bromo-9,10-dihydroxy-1,4,4a,8a-tetrahydro-1,4-ethano-naphthalene-5,8-dione

BrDUD25S (*endo-syn*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 3.22 (dtd, J = 6.38, 3.07, 3.07, 1.41 Hz, 1H), 3.59-3.68 (m, 2H) 3.79-3.85 (m, 2H), 4.75 (br. s., 1H), 5.13 (br. s., 1H), 6.15 (dd, J = 8.67, 6.59 Hz, 1H), 6.33 (dd, J = 8.57, 1.22 Hz, 1H), 6.64-6.69 (m, 1H) 6.74-6.79 (m, 1H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 43.3, 45.7, 49.0, 65.0, 70.8, 72.4, 132.7, 138.6, 142.6, 143.7, 196.1, 198.9; HRMS (NSI): Calcd for $C_{12}H_{12}BrO_4$ $[M+H]^+$ 298.9919, found 298.9913.

1-Bromo-11,12-dihydroxy-1,4,4a,9a-tetrahydro-1,4-ethano-anthracene-9,10-dione

BrDUD26S (*endo-syn*): ^{1}H NMR (300 MHz, Acetone-d_6): δ 3.30-3.37 (m, 1H), 3.75-3.90 (m, 4H), 4.81 (d, J = 3.77 Hz, 1H), 5.18 (d, J = 5.27 Hz, 1H), 5.95-6.10 (m, 2H), 7.72-7.89 (m, 4H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 43.8, 46.8, 49.8, 65.0, 66.6, 72.3, 126.4, 127.1, 132.7, 134.4, 134.9, 138.6, 195.5, 197.6; HRMS (NSI): Calcd for $C_{16}H_{14}BrO_4$ $[M+H]^+$ 349.0048, found 349.0070.

General procedure for [4+2] cycloadditions with hetero-aryl DDs

The appropriate dienophile (0.04 mmol) was added to a solution of a DD (0.03 mmol) in methanol (0.2 mL) and chloroform (3 mL). This mixture was heated to 100-110 °C in a sealed vessel and stirred for 3-4 days until the DDs had reacted completely (monitored by TLC). Subsequently, the solvent was evaporated under reduced pressure at 25 °C and the crude compound was purified by flash column column chromatography using methanol and dichloromethane as eluents to obtain pure compounds in 22-45% yields.

10,11-Dihydroxy-2-phenyl-5-(pyridin-2-yl)-5,8-dihydro-1H-5,8-ethano[1,2,4]-triazolo[1,2-a]pyridazine-1,3(2H)-dione

Py2DUD01S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.10-4.15 (m, 1H), 4.45 (d, J = 8.39 Hz, 1H), 5.13 (ddd, J = 6.22, 2.71, 1.45 Hz, 1H), 6.66-6.69 (m, 1H), 6.73-6.77 (m, 1H), 7.27-7.31 (m, 1H), 7.34-7.44 (m, 5H), 7.74 (dt, J = 7.97, 0.97 Hz, 1H), 7.89 (td, J = 7.82, 1.75 Hz, 1H),8.58-8.61 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 55.8, 64.0, 65.6, 66.2, 124.8, 125.4, 125.6, 125.8, 128.2, 129.0, 129.1, 130.9, 131.3, 133.6, 139.6, 146.3, 154.5; HRMS (NSI): Calcd for $C_{19}H_{17}N_4O_4$ $[M+H]^+$ 365.125, found 365.1244.

10,11-Dihydroxy-2-phenyl-5-(pyridin-3-yl)-5,8-dihydro-1H-5,8-ethano-[1,2,4]triazo-lo-[1,2-a]pyridazine-1,3(2H)-dione

Py3DUD01S (*endo-syn*):^{1}H NMR (500 MHz, CD_3OD): δ 4.03-4.10 (m, 2H), 5.01-5.04 (m, 1H), 6.73-6.75 (m, 2H), 7.34-7.41 (m, 3H), 7.43-7.51 (m, 3H), 8.14-8.22 (m, 1H), 8.48-8.56 (m, 1H), 8.91 (d, J = 1.83 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ 58.6, 64.3, 68.2, 68.6, 124.6, 127.6, 129.8, 130.3, 131.3, 132.9, 134.5, 136.0, 138.1, 149.4, 150.0, 156.4, 156.9; HRMS (NSI): Calcd for $C_{19}H_{17}N_4O_4$ $[M+H]^+$ 365.125, found 365.1244.

8,9-Dihydroxy-4-(pyridin-3-yl)-2-(3,4,5-trimethoxyphenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

Py3DUD06S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.28 (dd, J = 8.39, 2.59 Hz, 1H), 3.81 (d, J = 5.95 Hz, 11H), 3.97 (d, J = 6.41 Hz, 2H), 5.06-5.16 (m, 2H), 6.32 (s, 2H), 6.61 (dd, J = 8.70, 6.26 Hz, 1H), 6.75 (d, J = 8.70 Hz, 1H), 7.52-7.57 (m, 1H), 8.04 (d, J = 7.93 Hz, 1H), 8.64 (dd, J = 5.04, 1.37 Hz, 1H), 9.16 (br. s., 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.6, 32.1, 36.5, 44.4, 47.6, 53.5, 56.5, 61.0, 104.2, 122.4, 126.4, 126.9, 129.9, 131.1, 132.5, 138.7, 139.5, 145.9, 153.3, 153.7, 160.7, 174.5, 179.4; HRMS (NSI): Calcd for $C_{24}H_{25}N_2O_7$ $[M+H]^+$ 453.1662, found 453.1656.

Methyl-(E)-3-(4-(8,9-dihydroxy-1,3-dioxo-4-(pyridin-3-yl)-1,3,3a,4,-7,7a-hexahyd-ro-2H-4,7-ethanoisoindol-2-yl)phenyl)acrylate

Py3DUD10S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.37 (dt, J = 3.24, 1.66 Hz, 1H), 3.51 (d, J = 8.39 Hz, 1H), 3.73 (dd, J = 8.32, 2.98 Hz, 1H), 3.77 (s, 3H), 3.86 (dd, J = 8.54, 3.51 Hz, 1H), 4.12 (d, J = 8.39 Hz, 1H), 6.39 (d, J = 16.02 Hz, 1H), 6.42-6.51 (m, 2H), 7.15 (d, J = 8.54 Hz, 2H), 7.52 (d, J = 8.39 Hz, 2H), 7.61 (d, J = 16.02 Hz, 1H) 7.76 (dd, J = 8.24, 5.34 Hz, 1H), 8.43 (d, J = 8.24 Hz, 1H), 8.59 (d, J = 5.19 Hz, 1H), 8.96 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.7, 39.3, 40.3, 50.3, 51.8, 63.6, 70.4, 119.1, 125.1, 126.6, 128.6, 129.0, 129.1, 132.5, 132.9, 133.4, 134.7, 140.7, 141.2, 142.9, 143.5, 143.5, 167.2, 176.8, 177.6; HRMS (NSI): Calcd for $C_{25}H_{23}N_2O_6$ $[M+H]^+$ 447.1556, found 447.1551.

(E)-2-(4-(3-(4-Fluorophenyl)acryloyl)phenyl)-8,9-dihydroxy-4-(pyridin-3-yl)-3a,4,-7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

Py3DUD13S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.03 (d, J = 7.32 Hz, 1H), 3.42 (d, J = 8.39 Hz, 2H), 3.80 (dd, J = 8.47, 3.43 Hz, 1H), 4.11 (d, J = 8.39 Hz, 1H), 6.41-6.44 (m, 2H), 7.02 (t, J = 8.54 Hz, 2H), 7.23 (d, J = 8.70 Hz, 2H), 7.31 (d, J = 15.72 Hz, 1H), 7.54 (dd, J = 8.70, 5.34 Hz, 2H), 7.65 (d, J = 15.87 Hz, 1H), 7.70 (d, J = 5.49 Hz, 1H), 7.94 (d, J = 8.70 Hz, 2H), 8.34-8.40 (m, 1H), 8.53 (br. s., 1H), 8.90 (br. s., 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.6, 39.3, 40.3, 46.0, 63.5, 70.4, 116.0, 121.2, 125.0, 126.3, 128.7, 129.1, 130.5, 133.0, 135.3, 137.7, 139.6, 141.8, 142.4, 143.9, 144.5, 163.1, 165.1, 176.7, 177.6, 189.8; HRMS (NSI): Calcd for $C_{30}H_{24}N_2O_5F$ $[M+H]^+$ 511.1669, found 511.1664.

(E)-8,9-Dihydroxy-2-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)-4-(pyridin-3-yl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

Py3DUD17S (*endo-syn*): ^{1}H NMR (700 MHz, $CDCl_3$): δ 3.39 (d, J = 8.39 Hz, 2H), 3.63-3.66 (m, 1H), 3.73 (d, J = 1.29 Hz, 3H), 3.78 (dd, J = 8.39, 3.23 Hz, 1H), 4.08 (d, J = 8.17 Hz, 1H), 6.38-6.43 (m, 2H), 6.82 (d, J = 7.53 Hz, 2H), 7.18-7.21 (m, 2H), 7.23 (d, J = 15.70 Hz, 1H), 7.48 (d, J = 7.53 Hz, 2H), 7.62 (d, J = 15.49 Hz, 1H), 7.64-7.68 (m, 1H), 7.88-7.92 (m, 2H), 8.31 (d, J = 8.17 Hz, 1H), 8.50 (d, J = 4.95 Hz, 1H), 8.86 (br. s., 1H); ^{13}C NMR (175 MHz, $CDCl_3$): δ 38.5, 39.2, 40.2, 46.0, 55.1, 63.4, 70.3, 114.2, 119.0, 124.8, 126.2, 127.0, 128.9, 130.2, 132.7, 132.8, 134.9, 138.0, 139.9, 142.1, 144.2, 145.8, 161.8, 176.6, 177.6, 190.1; HRMS (NSI): Calcd for $C_{31}H_{27}N_2O_6$ $[M+H]^+$ 523.1869, found 523.1864.

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(E)-8,9-Dihydroxy-4-(pyridin-3-yl)-2-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phen-yl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

Py3DUD19S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.58-3.66 (m, 2H), 3.88-3.92 (m, 9H), 3.98 (dd, *J* = 8.32, 3.28 Hz, 1H), 4.24 (d, *J* = 8.39 Hz, 1H), 6.45-6.58 (m, 2H), 6.84 (s, 2H), 7.27-7.35 (m, 3H), 7.66 (d, *J* = 15.56 Hz, 1H), 7.74-7.82 (m, 1H), 8.00 (d, *J* = 8.54 Hz, 2H), 8.41 (d, *J* = 8.24 Hz, 1H), 8.52 (d, *J* = 4.58 Hz, 1H), 9.14 (br. s., 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.9, 39.2, 40.2, 50.4, 56.3, 61.0, 63.9, 71.0, 105.8, 105.8, 121.3, 125.5, 126.4, 129.2, 130.1, 132.5, 133.7, 135.1, 138.3, 140.7, 142.6, 144.0, 144.4, 145.8, 153.5, 174.4, 176.5, 177.0, 189.8; HRMS (NSI): Calcd for $C_{33}H_{31}N_2O_8$ $[M+H]^+$ 583.208, found 583.2075.

(E)-8,9-Dihydroxy-2-(4-(3-(4-(methylthio)phenyl)acryloyl)phenyl)-4-(pyridin-3-yl)-3a,4,7,7a-tetrahydro-1H-4,7-ethano-isoindole-1,3(2H)-dione

Py3DUD20S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.48 (s, 3H), 3.50-3.55 (m, 2H), 3.76-3.79 (m, 1H), 3.86-3.90 (m, 1H), 4.14 (d, *J* = 8.39 Hz, 1H), 6.49-6.55 (m, 2H), 7.22 (d, *J* = 8.54 Hz, 2H), 7.27 (d, *J* = 8.85 Hz, 2H), 7.37 (d, *J* = 15.56 Hz, 1H), 7.50 (d, *J* = 8.39 Hz, 2H), 7.70 (d, *J* = 15.72 Hz, 1H), 7.94 (dd, *J* = 8.24, 5.95 Hz, 2H), 7.99 (d, *J* = 8.70 Hz, 2H), 8.65 (s, 1H), 9.06 (d, *J* = 1.98 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.0, 38.7, 39.4, 40.3, 50.4, 63.5, 70.3, 120.5, 123.5, 124.2, 125.9, 126.3, 128.9, 129.2, 131.0, 131.7, 133.9, 138.2, 138.6, 141.3, 142.2, 142.9, 143.7, 145.3, 145.6, 176.7, 177.3, 189.8; HRMS (NSI): Calcd for $C_{31}H_{27}N_2O_5S$ $[M+H]^+$ 539.1640, found 539.1635.

(E)-8,9-Dihydroxy-2-(4-(3-(3-nitrophenyl)acryloyl)phenyl)-4-(pyridin-3-yl)-3a,4,-7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

Py3DUD21S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.58-3.61 (m, 1H), 3.63 (d, *J* = 8.54 Hz, 1H), 3.80 (dd, *J* = 8.39, 2.90 Hz, 1H), 3.97 (dd, *J* = 8.39, 3.36 Hz, 1H), 4.26 (d, *J* = 8.39 Hz, 1H), 6.49-6.52 (m, 2H), 7.38 (d, *J* = 8.70 Hz, 2H), 7.57 (d, *J* = 15.72 Hz, 1H), 7.62 (t, *J* = 7.93 Hz, 1H), 7.81 (d, *J* = 15.72 Hz, 1H) 7.90 (d, *J* = 7.63 Hz, 1H), 7.94-8.00 (m, 2H), 8.05 (d, *J* = 8.85 Hz, 2H), 8.13 (dd, *J* = 4.96, 1.30 Hz, 1H), 8.24-8.28 (m, 1H), 8.49 (t, *J* = 1.91 Hz, 1H), 8.76 (d, *J* = 2.14 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 38.8, 39.4, 40.3, 50.7, 63.8, 70.6, 122.5, 124.2, 124.9, 126.5, 129.6, 130.1, 132.4, 133.6, 134.4, 135.0, 135.6, 138.2, 142.4, 143.5, 148.7, 176.7, 177.3, 188.8, 192.0; HRMS (NSI): Calcd for $C_{30}H_{24}N_3O_7$$[M+H]^+$ 538.1614, found 538.1609.

10,11-Dihydroxy-2-phenyl-5-(pyridin-4-yl)-5,8-dihydro-1H-5,8-ethano[1,2,4]-triazolo-[1,2-a]pyridazine-1,3(2H)-dione

Py4DUD01A (*endo-syn*): ^{1}H NMR (500 MHz, CD_3OD): δ 3.77 (dd, *J* = 2.82, 1.91 Hz, 1H), 4.04 (s, 1H), 4.97 (ddd, *J* = 6.10, 2.82, 0.99 Hz, 1H), 6.77 (dd, *J* = 8.39, 6.10 Hz, 1H), 6.98 (dt, *J* = 8.39, 1.14 Hz, 1H), 7.29-7.34 (m, 2H), 7.41-7.50 (m, 4H), 7.77-7.81 (m, 2H), 8.59 (d, *J* = 5.95 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD): δ 59.8, 68.2, 76.1, 78.9, 124.9, 127.3, 127.5, 127.8, 129.3, 129.7, 130.0, 130.2, 130.3, 132.1, 132.6, 147.4, 149.8, 156.4, 158.1; HRMS (NSI): Calcd for $C_{19}H_{17}N_4O_4$ $[M+H]^+$ 365.125, found 365.1244.

8,9-Dihydroxy-4-(pyrimidin-4-yl)-2-(3,4,5-trimethoxyphenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

PmDUD06S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.54-3.58 (m, 1H), 3.70 (dd, *J* = 8.54, 2.90 Hz, 1H), 3.79-3.83 (m, 9H), 3.88-3.92 (m, 3H), 6.32 (s, 2H), 6.52 (dd, *J* = 8.62, 6.48 Hz, 1H), 6.61 (d, *J* = 8.54 Hz, 1H), 7.61 (dd, *J* = 5.42, 1.30 Hz, 1H), 8.80 (d, *J* = 5.34 Hz, 1H), 9.24 (d, *J* = 1.22 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.2, 40.6, 43.1, 49.7, 56.4, 61.0, 65.1, 67.8, 104.2, 121.0, 127.2, 130.5, 133.1, 138.5, 153.7, 157.2, 157.6, 169.3, 176.8, 177.7; HRMS (NSI): Calcd for $C_{23}H_{24}N_3O_7$ $[M+H]^+$ 454.1614, found 454.1609.

Methyl-(E)-3-(4-(8,9-dihydroxy-1,3-dioxo-4-(pyrimidin-4-yl)-1,3,3a,4,7,7a-hexa-hydro-2H-4,7-ethanoisoindol-2-yl)phenyl)-acrylate

PmDUD10S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.53-3.58 (m, 1H) 3.72 (dd, *J* = 8.47, 2.82 Hz, 1H) 3.80 (s, 3H) 3.88-3.93 (m, 3H) 6.42 (d, *J* = 16.02 Hz, 1H) 6.49 (dd, *J* = 8.62, 6.49 Hz, 1H) 6.59 (d, *J* = 8.54 Hz, 1H) 7.20 (d, *J* = 8.54 Hz, 2H) 7.55 (d, *J* = 8.54 Hz, 2H) 7.57-7.60 (m, 1H) 7.65 (d, *J* = 16.02 Hz, 1H) 8.78 (d, *J* = 5.34 Hz, 1H) 9.22 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.2, 40.5, 42.9, 49.7, 51.9, 64.9, 67.7, 119.2, 120.9, 126.6, 128.6, 130.4, 133.0, 134.7, 143.4, 157.3, 157.6, 167.1, 168.8, 176.3, 177.3; HRMS (NSI): Calcd for $C_{24}H_{22}N_3O_6$ $[M+H]^+$ 448.1508, found 448.1503.

(E)-2-(4-(3-(4-Fluorophenyl)acryloyl)phenyl)-8,9-dihydroxy-4-(pyrimidin-4-yl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

PmDUD13S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.56-3.60 (m, 1H), 3.75 (dd, *J* = 8.54, 2.90 Hz, 1H), 3.89-3.95 (m, 3 H), 6.50-6.54 (m, 1H), 6.61 (d, *J* = 8.54 Hz, 1H), 7.12 (t, *J* = 8.62 Hz, 2H), 7.33-7.36 (m, 2H), 7.38 (d, *J* = 15.87 Hz, 1H), 7.58-7.65 (m, 3H), 7.76 (d, *J* = 15.72 Hz, 1H), 8.03-8.06 (m, 2H), 8.82 (d, *J* = 5.34 Hz, 1H), 9.25 (d, *J* = 1.22 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.3, 40.6, 43.2, 49.6, 65.0, 67.6, 116.2, 116.3, 120.8, 121.5, 126.4, 129.3, 130.3, 130.5, 131.0, 133.1, 135.3, 138.0, 144.3, 157.5, 157.7, 163.2, 165.2, 168.7, 176.2, 177.1, 189.3; HRMS (NSI): Calcd for $C_{29}H_{23}N_3O_5F$ $[M+H]^+$ 512.1621, found 512.1616.

(E)-8,9-Dihydroxy-2-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)-4-(pyrimidin-4-yl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

PmDUD17S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.54-3.59 (m, 1H), 3.73 (dd, *J* = 8.54, 2.90 Hz, 1H), 3.85 (s, 3H), 3.89-3.94 (m, 3H), 6.51 (dd, *J* = 8.62, 6.49 Hz, 1H), 6.60 (d, *J* = 8.24 Hz, 1H), 6.93 (d, *J* = 8.85 Hz, 2H), 7.32 (dd, *J* = 12.13, 3.43 Hz, 3H), 7.58 (d, *J* = 8.85 Hz, 3H), 7.76 (d, *J* = 15.56 Hz, 1H), 8.03 (d, *J* = 8.70 Hz, 2H), 8.80 (d, *J* = 5.34 Hz, 1H), 9.23 (d, *J* = 1.22 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.3, 40.6, 43.1, 49.6, 55.5, 65.0, 67.7, 114.5, 119.5, 120.8, 126.3, 127.4, 129.2, 130.4, 133.1, 135.1, 138.4, 145.5, 157.4, 157.7, 161.9, 168.8, 176.2, 177.1, 189.6; HRMS (NSI): Calcd for $C_{30}H_{26}N_3O_6$ $[M+H]^+$ 524.1821, found 524.1816.

(E)-8,9-Dihydroxy-4-(pyrimidin-4-yl)-2-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)-phenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

PmDUD19S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.57 (dd, *J* = 3.81, 2.59 Hz, 1H), 3.75 (s, 2H), 3.89 - 3.92 (m, 11H), 6.52 (dd, *J* = 8.62, 6.48 Hz, 1H), 6.62 (d, *J* = 8.54 Hz, 1H), 6.84 (s, 2H), 7.30 (d, *J* = 15.72 Hz, 1H), 7.34 (d, *J* = 8.70 Hz, 2H), 7.59 (dd, *J* = 5.42, 1.30 Hz, 1H), 7.68 (d, *J* = 15.72 Hz, 1H), 8.02 (d, *J* = 8.70 Hz, 2H), 8.81 (d, *J* = 5.34 Hz, 1H), 9.24 (d, *J* = 1.22 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.4, 40.7, 43.1, 49.8, 56.4, 61.2, 65.0, 67.8, 105.9, 120.9, 121.5, 126.4, 129.4, 130.2, 130.5, 133.1, 135.3, 138.3, 140.8, 145.9, 153.6, 157.5, 157.8, 168.9, 176.3, 177.3, 189.9; HRMS (NSI): Calcd for $C_{32}H_{30}N_3O_8$ $[M+H]^+$ 584.2033, found 584.2027.

(E)-8,9-Dihydroxy-2-(4-(3-(4-(methylthio)phenyl)acryloyl)phenyl)-4-(pyrimidin-4-yl)-3a,4,7,7a-tetrahydro-1H-4,7-ethano-isoindole-1,3(2H)-dione

PmDUD20S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.52 (s, 3H), 3.56-3.60 (m, 1H), 3.74 (dd, *J* = 8.54, 2.90 Hz, 1H), 3.90-3.95 (m, 3H), 6.52 (dd, *J* = 8.62, 6.48 Hz, 1H), 6.61 (d, *J* = 8.39 Hz, 1H), 7.23-7.28 (m, 2H), 7.32-7.36 (m, 2H), 7.40 (d, *J* = 15.72 Hz, 1H), 7.52-7.56 (m, 2H), 7.59 (dd, *J* = 5.49, 1.37 Hz, 1H), 7.75 (d, *J* = 15.72 Hz, 1H), 8.02-8.05 (m, 2H), 8.81 (d, *J* = 5.34 Hz, 1H), 9.24 (d, *J* = 1.37 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.1, 38.3, 40.6, 43.1, 49.6, 65.0, 67.6, 120.7, 120.8, 126.0, 126.3, 128.9, 129.2, 130.3, 131.1, 133.1, 135.2, 138.2, 142.8, 145.1, 157.4, 157.7, 168.7, 176.2, 177.1, 189.5; HRMS (NSI): Calcd for $C_{30}H_{26}N_3O_5S$ $[M+H]^+$ 540.1593, found 540.1588.

(E)-8,9-Dihydroxy-2-(4-(3-(3-nitrophenyl)acryloyl)phenyl)-4-(pyrimidin-4-yl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3(2H)-dione

PmDUD21S (*endo-syn*): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.56-3.60 (m, 1H), 3.76 (dd, *J* = 8.39, 2.90 Hz, 1H), 3.91-3.95 (m, 3H), 6.53 (dd, *J* = 8.70, 6.41 Hz, 1H), 6.62 (d, *J* = 8.54 Hz, 1H), 7.38 (d, *J* = 8.70 Hz, 2H), 7.55-7.66 (m, 3H), 7.82 (d, *J* = 15.72 Hz, 1H), 7.91 (d, *J* = 7.78 Hz, 1H), 8.06-8.10 (m, 2H), 8.27 (ddd, *J* = 8.20, 2.25, 0.99 Hz, 1H), 8.50 (t, *J* = 1.91 Hz, 1H), 8.82 (d, *J* = 5.49 Hz, 1H), 9.25 (d, *J* = 1.37 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.3, 40.6, 43.2, 49.6, 65.0, 67.6, 120.8, 122.5, 124.2, 124.9, 126.5, 129.4, 130.1, 130.3, 133.1, 134.4, 135.8, 136.5, 137.3, 142.4, 148.8, 157.5, 157.7, 168.7, 176.1, 177.1, 188.6; HRMS (NSI): Calcd for $C_{29}H_{23}N_4O_7$ $[M+H]^+$ 539.1566, found 539.1561.

Methyl-(E)-3-(4-(8,9-dihydroxy-4-(1H-imidazol-1-yl)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-ethanoisoindol-2-yl)phenyl)acrylate

ImDUD10S (*endo-syn*): ^{1}H NMR (500 MHz, Acetone-d_6): δ 3.36 (dtd, *J* = 6.37, 3.07, 3.07, 1.22 Hz, 1H), 3.75 (s, 5H), 3.96 (dd, *J* = 8.62, 3.13 Hz, 1H), 4.24 (d, *J* = 8.39 Hz, 1H), 6.49 (dd, *J* = 8.77, 6.48 Hz, 1H), 6.57 (d, *J* = 16.02 Hz, 1H), 6.72 (d, *J* = 8.85 Hz, 1H), 6.90-6.97 (m, 1H), 7.25-7.29 (m, 2H), 7.41 (t, *J* = 1.30 Hz, 1H), 7.67 (d, *J* = 16.02 Hz, 1H), 7.72-7.77 (m, 2H), 7.98 (t, *J* = 1.07 Hz, 1H); ^{13}C NMR (125 MHz, Acetone-d_6): δ 30.7, 39.6, 41.3, 41.8, 51.8, 65.6, 68.0, 118.9, 119.8, 126.0, 128.0, 129.0, 129.3, 132.3, 133.4, 135.0, 135.2, 137.6, 144.1, 167.3, 175.7, 177.7; HRMS (NSI): Calcd for $C_{23}H_{22}N_3O_6$ $[M+H]^+$ 436.1508, found 436.1503.

Cell proliferation assay (MTT assay)

The procedure of Mosmann[22] was modified as follows. A suspension (120 µl) of L929 cells (mouse fibroblast) was added into each well of a 96-well micro-titre plate (MTP). The test compounds, dissolved in either DMSO or DMSO/Methanol (1/1, v/v), were serially diluted and the final compound dilutions and the solvent controls (60 µl) were transferred to the cells in two replicates to reach final concentrations in the range 37 µg/mL to 0.2 ng/mL. After 5 days of incubation at 37 °C, the metabolic activity of the cells was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Twenty µl of MTT (0.5 mg/mL) in PBS was added to the cells. After 2h of incubation the micro-titre plates were centrifuged (1744 x g) and the medium was removed by tapping. The precipitate was washed with 100 µl of PBS and the formazan crystals were dissolved by adding 100 µl of acidic isopropanol (0.4% hydrochloric acid). The absorbance of the colored solution was measured in a plate reader at 595 nm.

Cellular impedance measurement

A. Experimental procedure:[24] For time-dependent cell response profiling, 60 µl of medium was added to each well of a 96 well plate equipped with gold electrodes (E-96 plate, Roche/Acea Biosciences). A background measurement was recorded prior to the the addition of 120 µL of a suspension of L-929 cells (100000 cells/mL). After each step, the E-96 Plate was incubated for 30 min at RT and then placed on the reader in the incubator for continuous recording of impedance as reflected by the cell index. After 24 h of incubation the cells were treated with the test compounds dissolved in DMSO/MeOH (1/1, v/v). The same mixture of solvent was also used as solvent control in the E-96 plate. Compound quantities corresponding to their IC_{90} values in the MTT assay were added to the cells in triplicates in volumes of 1-3 µl. The impedance measurement was run for 5 days.[24]

B. Data analysis: The time-dependent cellular response profiles (TCRP) were recorded by the Roche RTCA software. The statistical programming language R, Version 2.12.2 (R Development Core Team, 2011) was used for the execution of data mining and processing. The raw cell index (CI) data provided by RTCA software was normalized by dividing the cell indices for each time point after the compound addition, by the cell index at the reference point as suggested by Abassi and co-workers.[23] The last measurement recorded before the addition of compounds was used as the reference point. Only the readings after compound addition were considered for subsequent analysis. The outliers out of triplicates were detected and removed by the median polish method. Cubic smoothing splines fitting was used to reduce the dimensionality of the data set and to approximate the TCRPs.[25]

Spline basis coefficients were used as descriptors to construct the distance matrix which was used for hierarchical cluster analysis to construct dendrograms. The distance matrix provided numerical values (Euclidean distance) to assess similarities between reference compounds with known biological activity and test compounds. These numerical values were used to select the compounds for further biological activity assays. A heatmap was generated which displays the Z-transformed values of the 22 descriptors which correspond to the basis spline coefficients. Hierarchical cluster analysis was done for the reference compounds together with the test compounds.

Proteasome inhibition assay

All contents supplied in the commercial kit (Promega) were used in the assay according to the instructions provided.[29] All solid substrates or reagents supplied were dissolved in the appropriate buffer solution. Each reaction including solvent control was done in duplicate. KB 3–1 cells (50 µl) were seeded into a 96 well white walled MTP with 15,000 cells/well. Cells were incubated for 2 h at 37 °C. Test compounds (1-2 µl), dissolved in DMSO/MeOH (1/1, v/v) were added to the cells, and the cells were incubated for another 2h at 37 °C. The MTP was allowed to equilibrate to RT. The proteasome reagent (50 µl) was added to the cells and the contents of the wells were mixed on a plate shaker for 2 minutes, followed by the incubation for 20 minutes at RT. Subsequently, the luminescence of each well was measured with a plate reader.

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An Approach to "Escape from Flatland": Chemo-enzymatic Synthesis and Biological Profiling of a Library of Bridged Bicyclic Compounds

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A small library of 78 bridged bicyclic compounds were synthesized via a chemo-enzymatic pathway. Biological evaluation suggested that rigid spherical scaffolds are useful to enhance the success rate of compound libraries for drug development.