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Multicomponent Assembly of 4-Aza-podophyllotoxins: A Fast Entry to Highly Selective and Potent Anti-Leukemic Agents.

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ABSTRACT: The aim of this study was the synthesis and lead structure selection of a best anti-leukemic agent from a library of aza-podophyllotoxin analogues (APTs). To this end, we report a scalable, modified multicomponent reaction using a "sacrificial" aniline partner as a more general route to rapidly construct the pivotal library of 50 APT analogues. Our preliminary structure activity relationship studies for anti-leukemic activity also address the innate toxicity of these compounds against non-malignant cells. As a result, we identified 2 novel compounds **2ca'** and **2jc'** more potent than etoposide **1** (25-60 fold) having high selectivity against the human THP-1 leukemia cell line and a minimal toxicity (IC_{50} of 9.3 ± 0.8 and 19.6 ± 1.4 nM respectively) which represent the best candidates for further pharmacological optimization.

KEYWORDS. Multicomponent reactions; aza-podophyllotoxins; anti-leukemic agents; AML; SAR study; Etoposide.

1. Introduction.

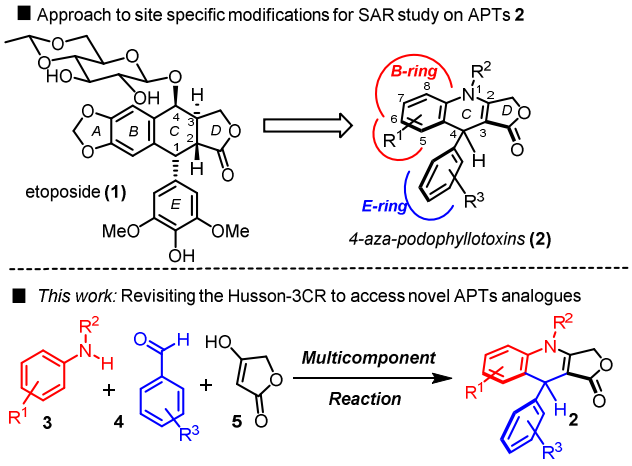
Etoposide **1** (Vepesid®), a synthetic derivative from the natural product podophyllotoxin (PT), is highly prescribed as an anticancer drug for the treatment of several human malignancies such as testicular and small-cell lung cancers.[1] More recently, **1** was also evaluated and validated as constituent of a third line regimen in the treatment of relapsed acute myelogenous leukemia (AML).[2] This semisynthetic drug exerts a strong chemotherapeutic effect by increasing levels of covalent topoisomerase II-cleaved DNA complexes.[3] Although these complexes are common intermediates in the DNA strand passage reaction, higher concentrations of such complexes are responsible for mutagenic and cell death pathways. On the other hand, the 4-aza-structural analogues of PT, namely 4-aza-2,3-didehydropodophyllotoxins (APTs) **2**, have also shown important biological activity as insecticides, anticancer and vascular-disrupting agents and hold a great potential in developing novel therapeutics due to their apparent straightforward preparation in comparison to **1**.[4,5]

To increase antitumor efficacy of the APT analogues **2** while reducing systemic side effects and poor water solubility issues found in the parent molecules podophyllotoxin and etoposide **1**, site-specific modifications expanding the APT-chemotype's chemical space were used to determine the pharmacophores responsible for the optimal bioactivity. Herein are presented our efforts to develop a practical multicomponent reaction to access modified APT analogues **2** for a structure activity relationship (SAR) study, based on bioassays against leukemia and pancreas cancer cell lines.

2. Synthetic strategy to assemble the chemical library.

In multicomponent reactions (MCRs), three or more reagents are combined to synthesize complex small-molecules containing structural contributions from each reaction component.[6] These one-pot processes are especially useful for the rapid construction of polycyclic heterocycles, which can be further decorated to rapidly generate a portfolio of analogues from a targeted chemotype. Therefore, MCRs are powerful maneuvers to rapidly construct large libraries of biologically active molecules with high degree of complexity while minimizing the number of synthetic operations.[7]

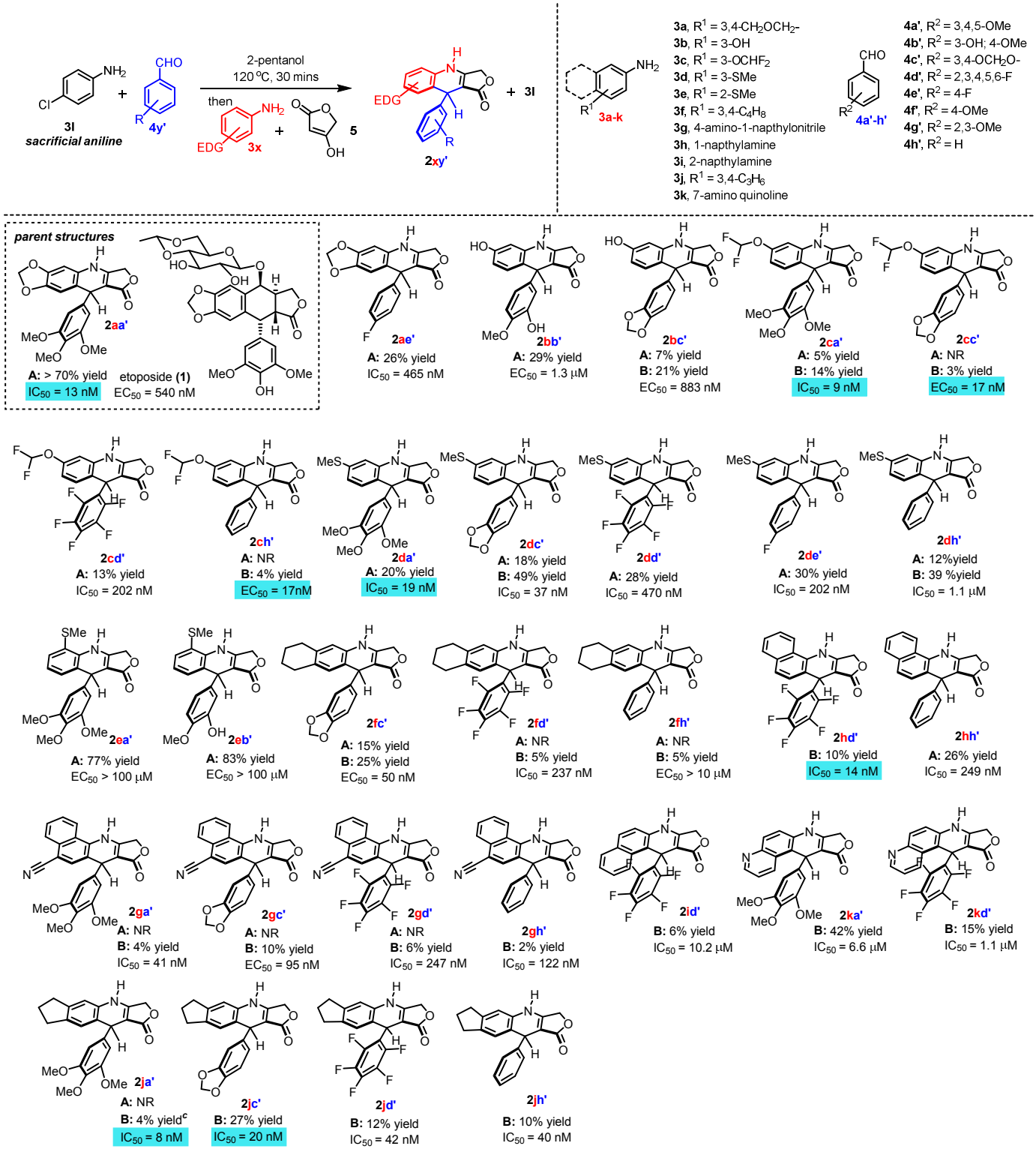
Scheme 1. MCR tactic to the 4-aza-podophyllotoxin chemotype



In 2000, Husson and Giorgi-Renault reported the extremely straightforward synthesis of APTs **2** via a unique 3-component reaction (Husson-3CR) which entails the condensation of a

tetronic acid **5** with variously substituted anilines **3** and aldehydes **4** (Scheme 1).[8] This unique transformation (Husson-3CR) enables the synthesis of podophyllotoxin's aza-analogues in one-pot while site-specific modifications are achieved through the modification of anilines or aldehydes partners utilized. This strategy offers facile modifications of the nitrogen substitution (R^2), as well as numerous possibilities for positional and functional modifications of the APT chemotype on the B-ring (R^1) and the E-ring (R^3). Recently, scientists at Bayer Cropscience prepared a large library of APTs (~140 compounds) and reported a lack of synthetic efficiency using the typical Husson-3CR leading them to advance an

Scheme 2. Synthesis [a-c] and biological evaluation [d] of a library of novel 4-aza-podophyllotoxins (APTs) **2xy'**



[a] ***Only novel analogues are presented above***.[12] Conditions. **Method A**: typical Husson-3CR with an equimolar ratio of tetronic acid **5**, aniline **3a-k** and benzaldehyde **4a'-h'** under Ar was stirred at 120 °C in 2-pentanol (1 mL) for 1 hour; **Method B** using the sacrificial aniline **3l**: aniline **3l** (1.0 equiv.) and benzaldehyde **4a'-h'** (1.0 equiv.) was stirred under Ar at 120 °C in 2-pentanol (1 mL) for 2 hours, followed by addition of tetronic acid **5** (1.0 equiv.) for 10 mins and finally aniline **3a-k** (1.0 equiv.), the resulting mixture was refluxed for an additional 30 mins. [b] Yields refer to pure products **2xy'** isolated after recrystallization of crude material in ethanol. [c] Compound **2ja'** was isolated as over-oxidized quinolines and reduced in a second step; overall yield for both steps is reported above. [d] The reported IC_{50} or EC_{50} values are against the human THP-1 leukemia cell line.

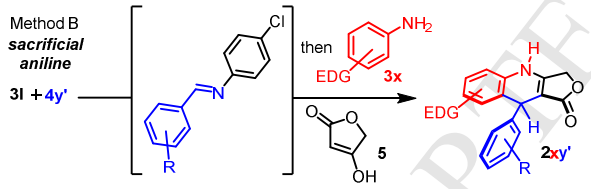
improved one-pot procedure.[9] Several other groups also reported their efforts in optimizing the original Husson-3CR,[10] but a general synthetic method still remains to be found.

3. Results and discussion.

3.1. Chemistry.

Starting a new medicinal chemistry program, we recently revisited the Husson-3CR to prepare new structural variations of APTs **2** in a more efficient manner. Our mechanistic inquiries lead us to identify an improved protocol in which an external sacrificial component was added to the typical Husson-3CR (Schemes 2 & Table 1).[11] In this new process, the electron deficient 'sacrificial' aniline **3l** is first engaged with aldehyde **4y'** to efficiently generate a reactive imine intermediate (observed by ^{1}H NMR) which then undergo condensation with the tetronic acid **5** and the desirable aniline partners **3x** in a more orderly fashion to ultimately deliver the APT molecules **2xy'** in higher yields (up to three fold). Our variant of the Husson-3CR is highlighted by more sequential protocol in which the 'sacrificial' aniline **3l** is extruded during the later stage of condensations/dehydration steps and replaced by the desired aniline partners **3a-k** in the final product. The 'sacrificial' aniline **3l** plays therefore a transient role in the cascade mechanism and is fully regenerated at the end of the reaction. Using this protocol (Scheme 2, ***Method B***), several compounds **2bc'**, **2ca'**, **2dc'**, **2dh'** and **2fc'** were synthesized in higher yields (3-49% yields after recrystallization) in comparison to the typical Husson-3CR (Scheme 2, ***Method A***). More importantly, compounds **2cc'**, **2ch'**, **2fd'**, **2fh'**, **2ga'**, **2gc'**, **2gd'**and **2ja'**, which are inaccessible via the typical Husson-3CR, can now be prepared using the 'sacrificial' aniline strategy albeit in low yields and enter the library for biological screening (Table 1).[12] Overall, we were able demonstrate modest improvements for the MCR, but the practicality of this method enabled us to build rapidly a larger library of 34 new APTs **2xy'**.[12]

Table 1. Comparison of the Husson-3CR and modified-3CR

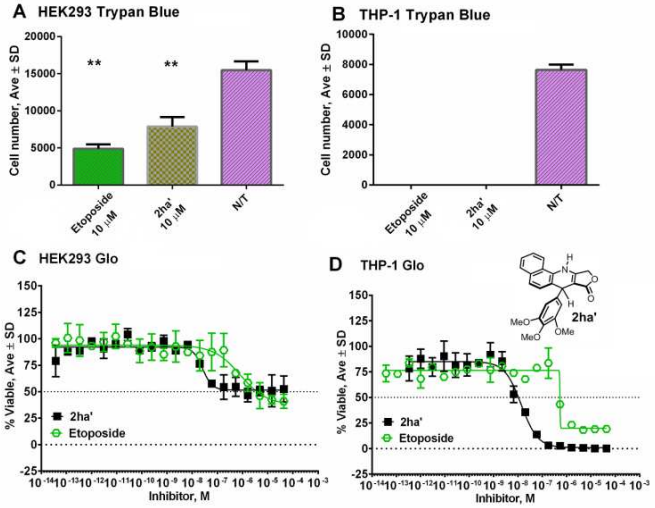


Compound	Yield (%)		Compound	Yield (%)	
	Method A	Method B		Method A	Method B
2bc'	7	21	**2fc'**	15	25
2ca'	5	14	**2fd'**	NR	5
2cc'	NR	3	**2fh'**	NR	5
2ch'	NR	4	**2ga'**	NR	4
2dc'	18	49	**2gc'**	NR	10
2dh'	12	39	**2gd'**	NR	6

3.2. SAR studies.

Having established the optimal reaction conditions to construct the library (50 compounds in total), we extended our investigation to the structure activity relationship (SAR) studies (Table 2). To this aim, each APT compound **2** from the medium-sized library was tested for effect on viability of human THP-1 leukemia, pancreatic cancer PSN-1 and human embryonic kidney HEK293 cells and compared with the two reference compounds **2aa'** and **1** (inset Scheme 2). HEK293 cells are used routinely in drug discovery to ascertain general cytotoxic profile in the early stage of analogues' development.[13] Additionally, acute kidney injury[14] was often reported in patients being treated with **1** which makes HEK293 cells a reasonable choice for this assay. Etoposide **1** was utilized as a pharmacological assay control. Previous IC_{50} values reported for **1** with THP-1 cells vary widely (*e.g.* 8.5 µM [15a], 855 nM [15b]) which suggests that its apparent potency could depend on the type of assay or to etoposide's poor solubility in the assay media. In our hands, we obtained a similar EC_{50} value of 540 ± 42 nM in the assay with THP-1 cells. Closer examination of dose response curves revealed differential effect of **1** and test compound **2ha'** on THP-1 cells as compared to PSN-1 and HEK293 cells (Fig. 1).[12] Overall, compound **2ha'** exhibited complete response towards THP-1 cells (0-100% viability) whereas dose response curves for viability of PSN-1 and HEK293 cells exhibited partial effect (either 50-80% or > 50% residual viability at highest concentration tested). These data suggested **2ha'** may have a different set of molecular targets that affect cell viability of THP-1 cells as compared to PSN-1 and HEK293 cells thus enhancing **2ha'** selectivity as anti-leukemic agent. Furthermore, a full range response in case of THP-1 suggests that **2ha'** causes cell death (cytocidal effect), whereas a partial response in case of PSN-1 and HEK293 cells suggested that **2** only inhibit cell growth (cytostatic effect) in the time frame of the viability assay (72 hours). A similar effect was previously reported for a series of structurally related *N*-hydroxyethyl-4-aza-didehydropodophyllotoxin derivatives.[16] To test this hypothesis, viability assay were performed in the format that allows for cells count (Fig. 1). As shown in Fig. 1, 10 µM of **1** and compound **2ha'** resulted in approximately 70-50% cell death in case of HEK293 cells (Panel A) and 100% cell death in case of THP-1 cells (Panel B) which is in general agreement with CellTiter Glo® viability assay results (Panels C & D). Etoposide exhibited incomplete cell death (~75%) in case of CellTiter Glo® viability assay (Panel D) which could be due to the solubility issues and differences in compound delivery method (pin tool in case of CellTiter Glo® viability assay and pipettor in case Trypan Blue assay). Overall, most APT derivatives **2** from the library may retain a similar mechanism of action as **1** and act *via* the same molecular targets (*i.e.* topoisomerase II) likely responsible for the potent anti-leukemic activity.

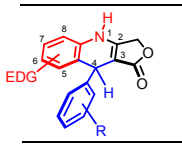
Figure 1. Anti-leukemic activity: Comparison of CellTiter Glo® and Trypan Blue viability assay using etoposide **1** and **2ha'**.[a]

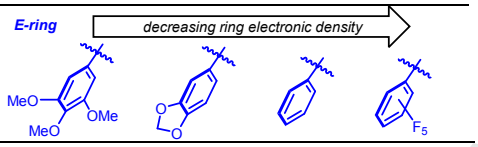


[a] **Panel A**: HEK293 in Trypan Blue assay; **Panel B**: THP-1 in Trypan Blue assay; **Panel C:** HEK293 in CellTiter Glo® assay; **Panel D:** THP-1 in CellTiter Glo® assay.

Selective site modifications of the APT chemotype **2** were first examined on the **E-ring** by looking at the electronic nature of the substituents decorating the ring (Table 2, analysis by columns). These results are mostly in agreement with the previous reports mentioning that electron donating groups (ethers) at the 3,4 or 3,4,5-positions of the E-ring (columns 1 & 2) enhanced the biological (antitumoral) activity of the ATPs **2**.[17] Exception being for compounds **2ch'** and **2hd'**, all the APT analogues bearing either no substitution or being pentafluorinated on the E-ring (columns 3 & 4) tend to be considerably less potent against the two cancer cell lines tested in our study. Furthermore, we decided to correlate these results with the substitution pattern of the **B-ring** (Table 2, analysis by rows). As previously reported, the dioxolane unit on the **B-ring** is an effective pharmacophore of the APTs skeleton as demonstrated by the IC_{50} values ranging from 12 ± 1 to 287 ± 24 nM (row 1).[4a] More surprisingly, the presence of a thioether at the C8-position or a free hydroxyl at the C7-position of the B-ring disturbs entirely the abilities of APTs to inhibit their biological target (IC_{50} > 123 nM to 100 µM, rows 4 & 5). Replacing the free hydroxyl group at C7 (from compounds **2ba'** or **2bc'**) by a difluoromethyl ether or a methyl thioether (rows 2 & 3) enhanced drastically the APTs biological activity affording some of the most active molecules from the present library (*e.g* . compounds **2ca'**, **2cc'**, **2ch'** and **2da'**)

Table 2. SAR study [a,b,c]



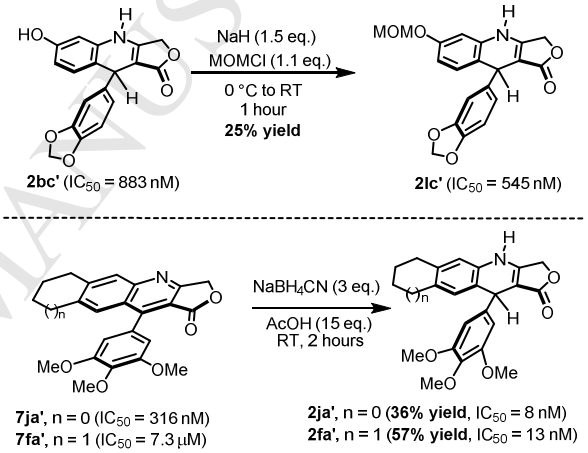


B-ring				
1	**2aa'** IC_{50} = 13 nM[a] EC_{50} = 22 nM[b] EC_{50} > 100 µM[c]	**2ac'** IC_{50} = 49 nM[a] EC_{50} = 180 nM[b] EC_{50} > 50 µM[c]	**2ah'** IC_{50} = 103 nM[a] EC_{50} = 191 nM[b] EC_{50} = 133 nM[c]	**2ad'** IC_{50} = 269 nM[a] EC_{50} = 1 µM[b] EC_{50} > 100 µM[c]
2	**2ca'** IC_{50} = 9 nM[a] EC_{50} = 24 nM[b] EC_{50} > 100 µM[c]	**2cc'** EC_{50} = 17 nM[a] EC_{50} = 88 nM[b] EC_{50} > 50 µM[c]	**2ch'** EC_{50} = 17 nM[a] EC_{50} = 40 nM[b] EC_{50} > 50 µM[c]	**2cd'** EC_{50} = 202 nM[a] EC_{50} = 549 nM[b] EC_{50} > 100 µM[c]
3	**2da'** IC_{50} = 19 nM[a] EC_{50} = 66 nM[b] EC_{50} > 100 µM[c]	**2dc'** EC_{50} = 37 nM[a] EC_{50} = 57 nM[b] EC_{50} > 100 µM[c]	**2dh'** IC_{50} = 1 µM[a] EC_{50} = 2 µM[b] EC_{50} > 100 µM[c]	**2dd'** IC_{50} = 470 nM[a] EC_{50} = 745 nM[b] EC_{50} > 100 µM[c]
4	**2ba'** IC_{50} = 123 nM[a] EC_{50} = 473 nM[b] EC_{50} > 100 µM[c]	**2bc'** EC_{50} = 883 nM[a] EC_{50} > 100 µM[b] EC_{50} > 100 µM[c]	---	---
5	**2ea'** EC_{50} > 100 µM[a] EC_{50} > 100 µM[b] EC_{50} > 100 µM[c]	---	---	---
6	**2ha'** IC_{50} = 13 nM[a] EC_{50} = 60 nM[b] EC_{50} > 50 µM[c]	**2hc'** IC_{50} = 11 nM[a] EC_{50} = 17 nM[b] EC_{50} > 100 µM[c]	**2hh'** IC_{50} = 249 nM[a] EC_{50} = 222 nM[b] EC_{50} > 100 µM[c]	**2hd'** IC_{50} = 17 nM[a] EC_{50} = 24 nM[b] EC_{50} > 500 µM[c]
7	**2ga'** IC_{50} = 41 nM[a] EC_{50} = 71 nM[b] EC_{50} > 100 µM[c]	**2gc'** EC_{50} = 95 nM[a] EC_{50} = 213 nM[b] EC_{50} > 100 µM[c]	**2gh'** IC_{50} = 122 nM[a] EC_{50} = 121 nM[b] EC_{50} > 100 µM[c]	**2gd'** IC_{50} = 247 nM[a] EC_{50} = 968 nM[b] EC_{50} = 3 µM[c]
8	**2ia'** IC_{50} = 631 nM[a] EC_{50} = 4.5 µM[b] EC_{50} > 100 µM[c]	**2ic'** EC_{50} = 107 nM[a] EC_{50} = 1.6 µM[b] EC_{50} > 50 µM[c]	**2ih'** IC_{50} = 58 nM[a] EC_{50} = 144 nM[b] EC_{50} > 50 µM[c]	**2id'** IC_{50} = 10.2 µM[a] EC_{50} > 50 µM[b] EC_{50} > 50 µM[c]

The reported IC_{50} or EC_{50} values are against: [a] human THP-1 leukemia cell line, [b] pancreas cancer PSN-1 cell line and [c] non-malignant kidney HEK293 cell line.

Likely due to similar electronic factors, the aminonaphtyl-APTs derivatives substituted via a C7-C8 linkage (rows 6 & 7) are much more potent than the APTs having a C5-C6 linkage (row 8). Overall, the SAR study suggests the hypothesis that an electron rich moiety placed at the APTs' (**B-ring**) would greatly enhanced potency against leukemic cancer cell lines. To confirm these results, the relatively less active APT **2bc'** bearing a free hydroxyl group was etherified to the corresponding MOM-derivative **2lc'** in 25 % yield (Scheme 3). As expected, APT **2lc'** demonstrated much more potency against the THP-1 cell line (EC_{50} = 545 ± 52 nM), activity very similar to the reference **1** (EC_{50} = 540 ± 42 nM). Finally, the two aromatic quinoline derivatives **7ja'** and **7fa'** (IC_{50} = 316 ± 32 nM and 7.3 ± 0.7 µM respectively) obtained from the modified-3CR were reduced to the corresponding APTs **2ja'** and **2fa'** which demonstrated extremely high potency (IC_{50} = 8 ± 0.7 nM and 13 ± 1 nM respectively; Scheme 3). The 3-dimensional structure of dihydro-quinoline **2fa'** is primarily accountable for the increase in potency (560 fold) in comparison to the parent aromatic quinoline **7fa'**.

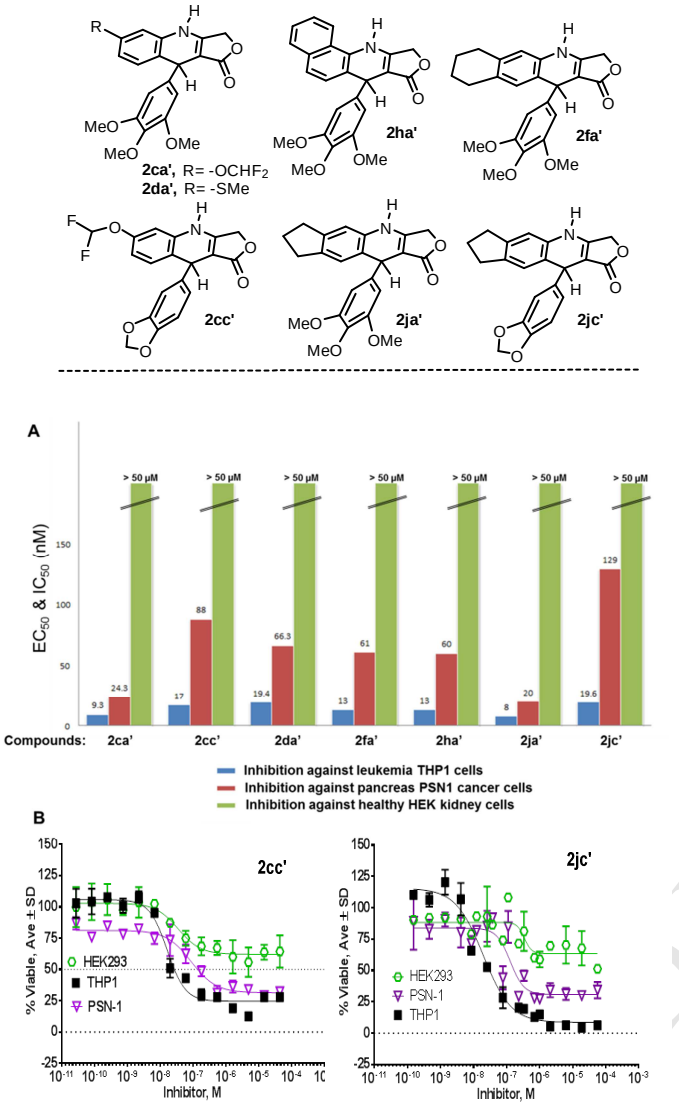
Scheme 3. Modulations of the APTs pharmacophores [a]



[a] The reported IC_{50} values are against the human THP-1 leukemia cell line.

In this collection of 50 APT molecules, 18 compounds have been identified to inhibit the growth of THP-1 leukemia cells with IC_{50} < 50 nM.[12] Moreover, six of them are novel structures (**2ca'**, **2cc'**, **2ch'**, **2da'**, **2jc'** and **2hd'**) presenting a potent activity against leukemia with IC_{50} < 20 nM. Figure 2 depicts a comparison of the seven most potent compounds (**2ca'**, **2cc'**, **2da'**, **2fa'**, **2ha'**, **2ja'** and **2jc'**) in terms of bioactivity and selectivity against three different cell lines (non-malignant, leukemia and pancreas cancer cells). These compounds are more cytotoxic against leukemia while maintaining a low toxicity against the non-malignant cells HEK293 (EC_{50} > 50 µM). **1** remains one of the most selective compound tested to date with 10 fold difference between cancer cell lines. However some novel compounds such as **2cc'** and **2jc'** also exert a comparable selectivity (4 and 7 fold respectively). As shown in Fig. 2 (Panel B), compounds **2cc'** (EC_{50} = 17 ± 2 nM) and **2jc'** (IC_{50} = 20 ± 1 nM) have a similar *in vitro* biological profile against leukemia cells, but **2jc'** uniquely displays a full dose response curve of inhibition (100%) when reaching low micromolar concentrations.

Figure 2. Lead compounds having high potency and selectivity against THP-1 leukemia cells.[a,b]

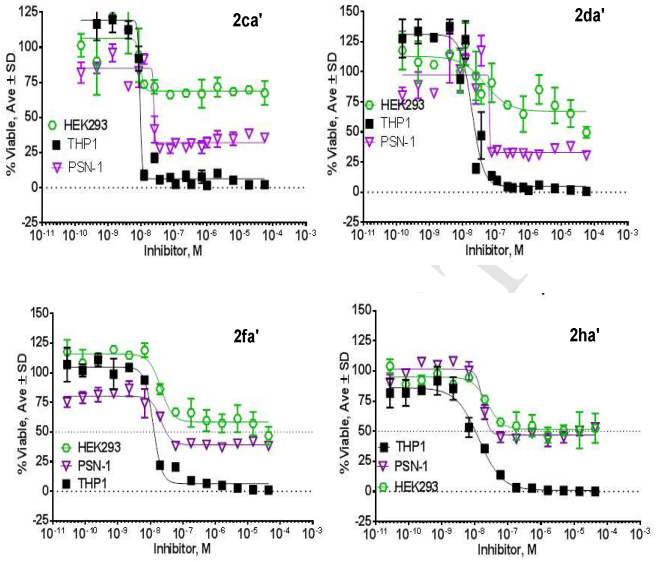


[a] EC_{50} values are reported for HEK293 and PSN-1 cells. **Panel A:** selectivity chart for the 7 best APT compounds of the library. [b] **Panel B:** Compound comparison showing high selectivity and potency.

In addition to compound **2jc'**, four other APTs **2ca'**, **2da'**, **2fa'** and **2ha'** with IC_{50} < 20 nM are showing a curve of inhibition with full response at sub-micromolar concentrations (Fig. 3). From these bioactivity plots, compound **2ca'** is the most potent against leukemia as shown by the sharp curve of activity (Hill slope coefficient) leading to an IC_{50} = 9.3 ± 0.8 nM and an IC_{90} < 20 nM. Three other compounds **2da'**, **2fa'** and **2ha'** are also showing important activity, with **2ha'** being the second most potent and selective compound of the series with an IC_{50} = 13.0 ± 2.0 nM.

Etoposide **1** exhibits poor physicochemical properties for a drug, likely causing its modest solubility in water (clogP =1.16) and absorption.[18] In fact, **1** disobeys three of the five Lipinski's rules[19] with a high molecular weight (MW of 588.6 DA > 500 DA) a large number of hydrogen-bond acceptor sites (HBA(12) > 10) and a topological polar surface area of 160.83 Å^2 (TPSA > 140 Å^2).

Figure 3. Best potencies against THP-1 leukemia cells for the APTs **2ca'**, **2da'**, **2fa'** and **2ha'**



Our approach to the APT-library diversity was to maintain a low toxicity against non-malignant cells and improve the compounds Absorption, Distribution, Metabolism and Excretion properties (ADME) in comparison to the reference drug **1**.[20] For this purpose, specific physicochemical parameters have been calculated: clogP values (clogP < 3-5)[21] to enhance absorption and cell permeation, topological polar surface area values (60-75 Å^2< TPSA in Å^2<140 Å^2)[22] to maintain a good availability and transport of the molecules and finally a direct correlation was proposed with the numbers of hydrogen-bond acceptor and donors sites (HBA+HBD < 12) to potentially increase the number of available binding sites and enhance chemical interactions with the cellular biological target.[23] The calculated physicochemical values for the seven most active compounds (see Fig. 2) were in optimal range according to the literature.[23] However, these data did not follow a clear trend (3.0 < clogP < 3.8; 57 Å^2< TPSA < 75 Å^2; HBA+HBD <7) which could afford a satisfactory explanation to interpret the changes in biological activity.[12] Interestingly, by expanding these calculations to specific series of compounds (C, D and E-rings constant), a trend can be observed between the biological activity (IC_{50} values against leukemia) and the compounds lipophilicity. For example, in the series of compounds **2bc'**, **2lc'**, **2dc'**, **2cc'**, **2hc'** and **2jc'**, the lipophilicity increased substantially (clogP = 2.0, 2.2, 3.0, 3.1, 3.3 and 3.35) which translated into an increase in cell permeation and to the observed bioactivity (corresponding IC_{50} values of 883, 540, 37, 17, 50 nM). The changes of substituents at C7 from a free-phenol to various ethers and thioethers resulted in a large improvement in cytotoxicity which could likely be related to a combination of two factors: an increased in binding ability (–$OCHF_2$ and –SCH_3)[24] and/or a diminished oxidative metabolism of degradation.[25] Indeed, the increase in lipophilicity resulting from the ether appendages (*e.g.* **2bc'** vs **2lc'** and **2cc'**) at C7 may favor a better cell permeation without affecting the binding properties of the small-molecules. Therefore we could conclude that lead compounds such as **2ca'** and **2cc'** may present an interesting balance between a relatively high lipophilicity to facilitate cell permeation (clogP ~ 3.0) while presenting a relatively

large TPSA (TPSA ~ 70 Å^2) to maintain an important binding affinity to the cellular target.

As shown in this SAR study, both factors of physicochemical properties and positional pattern of substitution of the APT-molecules are important to develop novel analogues with high cytotoxicity. Overall, several pharmacophores have been identified with the 3,4-dioxolane and the 3,4,5-trimethoxy substitutions of the **E-ring** and the fused 6,7-cyclopentanyl, 7,8-phenyl, 7-methylthioether or 7-difluoromethylether on the **B-ring**. Single substitution at C5 or C8 were extremely delicate and generally resulted in largely diminish bioactivity.

4. Conclusions.

In summary, we modified the Husson-3CR and create a scalable and practical protocol to synthesize a series of 50 analogues of the etoposide aglycon, namely the 4-aza-2,3didehydropodophyllotoxin (APTs **2**).[12] This improved Husson-3CR enabled the rapid structure-guided optimization of the APT chemotype providing the basis for our SAR study. From the designed library of analogues, 18 compounds (of which 9 are novel structures) demonstrated a greater potency against the human THP-1 leukemia cell line (IC_{50} < 50 nM) than **1** (EC_{50} = 540 ± 42 nM). Moreover, three potent derivatives **2ca'**, **2ha'** and **2jc'** have been identified to be highly selective against leukemia cells with low nanomolar antiproliferative activity (IC_{50} of 9.3 ± 0.8, 13.0 ± 2.0 and 19.6 ± 1.4 nM respectively) while preserving a low toxicity profile against the non-malignant cells tested. In addition, the most potent analogues possess good physicochemical properties and specific substitution patterns on the B-ring either with an heteroatomic ether chain at C7 or a carbocyclic fused appendage at the C6-C7 or C7-C8 which were therefore identified as important pharmacological sites. Finally, the synthetic MOM-derivative **2lc'** displays a comparable anti-leukemic activity and selectivity to etoposide **1** against leukemia and a lower toxicity. From the knowledge gained in this study on the APT tricyclic core, a lead compound **2ca'** may potentially be further modified to develop more soluble derivatives as well as prodrug analogues. Studies along these lines are in progress.

ASSOCIATED CONTENT

*S Supporting Information

Experimental procedures, characterization data, biological profile, and NMR spectra of all new APT-molecules **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of AH, DM and SPR. / All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

APT: 4-aza-2,3-didehydropodophyllotoxins; MCR: multicomponent reaction .

5. EXPERIMENTAL SECTION

5.1. Chemistry.

Reactions were performed in flame-dried glassware under a positive pressure of argon. Yields refer to spectroscopically pure compounds. Analytical TLC was performed on 0.25 mm glass backed 60Å F-254 TLC plates (Silicycle, Inc.). The plates were visualized by exposure to UV light (254 nm) and developed by a solution of cerium-ammonium-molybdate in water/sulfuric acid and heat. Flash chromatography was performed using 200-400 mesh silica gels (Silicycle, Inc.). Infrared spectra were recorded on a Nicolet IS5 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Mercury400 (400 MHz) spectrometer and are reported in ppm using solvent as an internal standard (DMSO-d_6 at 2.50 ppm). NMR spectra were performed using standard parameter and data are reported as: (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz, integration). ^{13}C NMR spectra were recorded on Varian Mercury400 (100 MHz) spectrometer. Chemical shifts are reported in ppm, with solvent resonance employed as the internal standard (DMSO-d_6 at 39.5 ppm). Melting points were determined using Digimelt digital melting point apparatus. The low resolution mass spectra were performed on Agilent 1200 series HPLC system/6120 single quadrupole MSD (electrospray ionization; ESI) with dual detector (PDA and ELSD). A purity of at least 95% was obtained for all the compounds by means of chromatography, crystallization, or recrystallization. This level of purity was established by LC/MS on a Agilent 1200 series HPLC based on the ELSD and UV chromatograms (λ= 360 nm) using a linear gradient, water + 0.1% formic acid and MeCN + 0.1% formic acid, at 60:40 (0 min) to 0:100 (20 min) and a flow rate of 0.8 mL/min; Retention times are reported for the lead compounds (R_t). Accurate mass (High resolution HR-MS) was obtained from University of Florida using Agilent 6210 TOF instrument.

Novel aza-podophyllotoxins **2** were synthesized using either the typical Husson-3CR (method A) or using a modified-3CR enabled by a sacrificial aniline (method B).[12] Compounds **2aa'** [4f,8b,9,10i], **2ac'** [4f], **2ae'** [10i], **2af'** [1b,4f,9,10i], **2ag'** [9], **2ah'** [4f,9], **2ba'** [8b,11], **2fa'** [4e,4f], **2ha'** [4e], **2hc'** [10i], **2ia'** [4e,8b], **2ic'** [4f,10c], **2ih'** [10c], **2kc'** [10d] and **2kh'** [10d] were previously reported by others. Only biological activity was reported for Compound **2ja'** [4a]. Compounds **2bc'**, **2ca'**, **2dc'**, **2dh'** and **2fc'** have been previously reported and fully characterized by us (^{1}H, ^{13}C NMRs, IR, melting points and HRMS).[11]

General procedure A: A round bottom flask was charged under argon with aniline **3** (1.0 equiv.), aldehyde **4** (1.0 equiv.), and tetronic acid **5** (1.0 equiv.) in 2-pentanol or ethanol [0.3 M]. Reaction was refluxed (at 125 °C or 80 °C respectively) for 1 hour, then the solvent was evaporated under vacuum and the crude product purified by recrystallization in ethanol or by flash chromatography on silica gel.

General procedure B: A two neck round bottom flask under argon equipped with a condenser was charged with aldehyde **4** (1.0 equiv.) and 4-chloroaniline **3l** (1.1 equiv.) in 2-pentanol [0.3 M] and stirred at reflux for two hours. The tetronic acid **5** (1.1 equiv.) in 2-pentanol (minimum amount) was then added at reflux. After another 10 mins at reflux, the third component aniline **3** (1.0 equiv.) was added neat. The reaction mixture was refluxed

for an additional 30 mins. All volatiles were evaporated under vacuum and the crude product purified by recrystallization in ethanol or by flash chromatography on silica gel.

9-(benzo[d][1,3]dioxol-5-yl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (2ac'). Compound **2ac'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 1,3-benzodioxole-5-carbaldehyde **4c'** (150 mg, 1.0 mmol, 1.0 equiv.), 3,4-(methylenedioxy)aniline **3a** (137 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was filtered and washed with cold ethanol (3x 2.5 mL) to obtain compound **2ac'** in a pure form (171 mg, 0.5 mmol, 49% yield). Light brown solid; $\mathbf{R}_f$ = 0.29 (acetone/hexanes; 35/65); **m.p.** > 260 °C; **IR** νmax (neat): 3224, 3095, 1712, 1642, 1562, 1481, 1248, 1235, 1038, 1016, 933, 780 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.87 (bs, 1H), 6.83 – 6.75 (m, 1H), 6.73 (s, 1H), 6.65 (dd, *J* = 7.7, 1.5 Hz, 1H), 6.59 (s, 1H), 6.51 (s, 1H), 5.97 – 5.87 (m, 4H), 4.97 (d, *J* = 15.8 Hz, 1H), 4.83 (s, 1H), 4.83 (d, *J* = 15.8 Hz, 1H); **LC-MS (ESI)**: *m/z* 374.0 (M+Na).

9-(perfluorophenyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (2ad'). Compound **2ad'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using pentafluorobenzaldehyde **4d'** (490 mg, 2.5 mmol, 1.0 equiv.), 3,4-(methylenedioxy)aniline **3a** (343 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The crude product was filtered and washed with cold ethanol (3x 2.5 mL) to obtain compound **2ad'** (99 mg, 0.3 mmol, 10% yield). White solid; $\mathbf{R}_f$ = 0.30 (acetone/hexanes; 35/65); **m.p.** >260 °C; **IR** νmax (neat): 3230, 1727, 1650, 1498, 1481, 1190, 1040, 1020, 990, 942, 835, 761 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.12 (bs, 1H), 6.54 (s, 1H), 6.51 (s, 1H), 5.96 (s, 1H), 5.93 (s, 1H), 5.43 (s, 1H), 4.90 (d, *J* = 16.0 Hz, 1H), 4.85 (d, *J* = 16.0 Hz, 1H); **LC-MS (ESI)**: *m/z* 398.0 (M+H), 420.0 (M+Na).

9-(4-methoxyphenyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (2af'). Compound **2af'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 4-methoxybenzaldehyde **4f'** (340 mg, 2.5 mmol, 1.0 equiv.), 3,4-(methylenedioxy)aniline **3a** (343 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The crude product was filtered and sonicated in dichloromethane (4 mL) for 15 minutes, followed by centrifugation at 5,800 rpm for 10 minutes (x3) to obtain pure compound **2af'** (444 mg, 1.3 mmol, 53% yield). Pale yellow solid; $\mathbf{R}_f$ = 0.19 (acetone/hexanes; 35/65); **m.p.** 136-137 °C; **IR** νmax (neat): 3222, 3157, 3092, 1711, 1641, 1560, 1502, 1478, 1192, 1016, 831 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.84 (bs, 1H), 7.09 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 6.54 (s, 1H), 6.51 (s, 1H), 5.95 (s, 1H), 5.89 (s, 1H), 4.92 (d, *J* = 15.5 Hz, 1H), 4.85 (s, 1H), 4.83 (d, *J* = 15.5 Hz, 1H), 3.69 (s, 3H); **LC-MS (ESI)**: *m/z* 338.0 (M+H).

6-hydroxy-9-(4-hydroxy-3-methoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2bb'). Compound **2bb'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-hydroxy-4-methoxybenzaldehyde **4b'** (381 mg, 2.5 mmol, 1.0 equiv.) 3-aminophenol **3b** (273 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The product was filtered off the crude reaction mixture and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2bb'** (235 mg, 0.72 mmol, 29% yield). Off-white solid; $\mathbf{R}_f$ = 0.24 (acetone/hexanes; 45/55); **m.p.** > 260 °C; **IR** νmax (neat): 3446, 3377, 3231, 1724, 1646, 1622, 1496, 1152, 1026, 1018, 770 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.84 (bs, 1H), 9.43 (s, 1H), 8.75 (s, 1H), 6.84 (d, *J* = 8.1 Hz, 1H), 6.79 (d, *J* = 1.6 Hz, 1H), 6.62 (d, *J* = 8.1 Hz, 1H), 6.46 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.33 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.32 (d, *J* = 1.6 Hz, 1H), 4.92 (d, *J* = 15.6 Hz, 1H), 4.82 (d, *J* = 15.6 Hz, 1H), 4.76 (s, 1H), 3.70 (s, 3H); **LC-MS (ESI)**: *m/z* 326.1 (M+H).

6-(difluoromethoxy)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one 2ca'. Compound **2ca'** was prepared accordingly to the *general* ***procedure B*** *for the one pot sequential synthesis* using 4-chloroaniline **3l** (72 mg, 0.56 mmol, 1.1 equiv.), 3-difluoromethoxy aniline **3c** (81 mg, 0.51 mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (100 mg, 0.51 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.56 mmol, 1.1 equiv.), in 2-pentanol (1.7 mL + 0.4 mL). The crude product was purified by column chromatography using isocratic solvent system of 20% acetone in hexanes system to obtain pure compound **2ca'** (30 mg, 0.06 mmol, 14% yield). Compound **2ca'** was also prepared accordingly to the *general procedure A for the one pot synthesis* using the exact same quantities and was purified by column chromatography using isocratic solvent system of 20% acetone in hexanes system to obtain pure compound **2ca'** (5 mg, 0.01 mmol, 2% yield). White powder, $\boldsymbol{R_f}$ = 0.22 (Hexanes/acetone 65 / 35); **m.p.**>260 °C; **IR** νmax (neat): 3246, 1727, 1644, 1548, 1492, 1174 cm^{-1};**^{1}H NMR** (400 MHz, DMSO) δ (ppm):10.13 (s, 1H), 7.19 (s, 1H), 7.18 (t, *J* = 73.9 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.69 (s, 1H), 6.50 (s, 2H), 5.18 – 4.78 (m, 3H), 3.70 (s, 6H), 3.60 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.9, 158.5, 152.8 (2C), 150.0, 142.4, 137.3, 136.1, 132.1, 121.3, 116.3 (t, *J* = 278.6 Hz), 113.2, 106.2, 104.9 (2C), 95.7, 65.1, 59.9, 55.8 (2C), 30.7; **LC-MS (ESI)**: R_t = 5.1 mins, *m/z* 420.0 (M+H); **HR-MS (ESI)**: *m/z* Calcd for $[C_{21}H_{19}F_2NO_6+H]^+$420.1253, Found 420.1250 (-0.7 ppm).

9-(benzo[d][1,3]dioxol-5-yl)-6-(difluoromethoxy)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2cc'). Compound **2cc'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 1,3-benzodioxole-5-carbaldehyde **4c'** (150 mg, 1.0 mmol, 1.0 equiv.), 3-(difluoromethoxy) benzamine **3c** (159 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The oily crude product was taken in methanol (2.5 mL) and the precipitate was filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2cc'** (11 mg, 0.03 mmol, 3% yield). Off-white crystals; $\mathbf{R}_f$ = 0.41 (acetone/hexanes; 40/60); **m.p.** > 260 °C; **IR** νmax (neat): 3322, 1728, 1641, 1611, 1482, 1109, 1040, 1011, 798 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.15 (bs, 1H), 7.17 (t, *J* = 74.0 Hz, 1H), 7.09 (d, *J* = 8.5 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 1.6 Hz, 1H), 6.72 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.69 – 6.67 (m, 2H), 5.94 (d, 2.0 Hz, 2H), 4.93 (s, 1H), 4.93 (d, 15.4 Hz, 1H), 4.87 (d, 15.4 Hz, 1H); **LC-MS (ESI)**: R_t = 7.3 mins, *m/z* 374.0 (M+H), 396.0 (M+Na); **HR-MS (ESI)**: *m/z* Calcd for $[C_{19}H_{13}F_2NO_5+H]^+$ 374.0840, Found 374.0850 (+ 2.7 ppm).

6-(difluoromethoxy)-9-(perfluorophenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2cd'). Compound **2cd'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-(difluoromethoxy)benzamine **3c** (159 mg, 1.0 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was filtered and washed with cold ethanol (3x 2.5 mL) to obtain compound **2cd'** (56 mg, 0.13 mmol, 13% yield). White solid; $\mathbf{R}_f$ = 0.29 (acetone/hexanes; 30/70); **m.p.** > 260 °C; **IR** νmax (neat): 1726, 1644, 1495, 1190, 1170, 1021, 991, 762 cm^{-1};**^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.38 (bs, 1H), 7.23 (t, *J* = 73.7 Hz, 1H), 7.06 (d, *J* = 8.3, 1H), 6.75 (dd, *J* = 8.3, 1.7 Hz, 1H), 6.71 (d, *J* = 2.0 Hz, 1H), 5.56 (s, 1H), 4.96 (d, *J* = 16.1, 1H), 4.90 (d, *J* = 16.1, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.4, 159.1, 150.7, 138.1 (2C),

131.9 (3C), 117.7, 116.2 (t, *J* = 258.6 Hz), 113.4 (3C), 106.3 (2C), 92.2, 65.4, 28.7; **LC-MS (ESI)**: *m/z* 420.0 (M+H), 442.0 (M+Na);

6-(difluoromethoxy)-9-phenyl-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2ch'). Compound **2ch'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (140 mg, 1.1 mmol, 1.1 equiv.), benzaldehyde **4h'** (106 mg, 1.0 mmol, 1.0 equiv.), 3-(difluoromethoxy) benzamine **3c** (159 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The oily crude product was taken in methanol (2.5 mL) and the precipitate was filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ch'** (12 mg, 0.4 mmol, 4% yield). Metallic white crystals; $\mathbf{R}_f$ =0.35 (70/30 acetone/hexanes); **m.p.** > 260 °C; **IR** vmax (neat): 3231, 1723, 1636, 1617, 1495, 1118, 1012, 746, 696 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.16 (bs, 1H), 7.18 (t, *J* = 74.0 Hz, 1H), 7.28-7.07 (m, 6H), 6.73-6.69 (m, 2H), 5.01 (s, 1H), 5.00 (d, *J* = 15.8 Hz, 1H), 4.89 (d, *J* = 15.8 Hz, 1H); **LC-MS (ESI)**: *m/z* 368.0 (M+K).

6-(methylthio)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2da'). Compound **2da'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-methylthioaniline **3d** (138 mg, 1.0 mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2da'** (78 mg, 0.2 mmol, 20% yield). White crystals; $\mathbf{R}_f$ = 0.25 (acetone/hexanes; 35/65); **m.p.** > 260 °C; **IR** vmax (neat): 3249, 2359, 2341, 1725, 1635, 1483, 1128, 1008, 750 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.00 (bs, 1H), 7.07 (d, *J* = 8.1 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.77 (s, 1H), 6.49 (s, 2H), 5.02 (d, *J* = 15.7 Hz, 1H), 4.91 (s, 1H), 4.87 (d, *J* = 15.7 Hz, 1H), 3.70 (s, 6H), 3.60 (s, 3H), 2.42 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm) 172.0, 158.7, 152.8 (2C), 142.6, 137.3, 136.5, 136.1, 131.1, 121.1, 120.7, 112.9, 104.9 (2C), 95.4, 65.1, 59.9, 55.9 (2C), 39.3, 14.6; **LC-MS (ESI)**: R_t = 6.5 mins, *m/z* 400.0 (M+1), 422.0 (M+Na); **HR-MS (ESI)**: *m/z* Calcd for $[C_{21}H_{21}NO_5S+H]^+$ 400.1219, Found 400.1225 (+ 2.4 ppm).

6-(methylthio)-9-(perfluorophenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2dd'). Compound **2dd'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-methylthioaniline **3d** (138 mg, 1.0 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2dd'** (112 mg, 0.28 mmol, 28% yield). Off-white solid; $\mathbf{R}_f$ = 0.30 (acetone/hexanes; 30/70); **m.p.** > 260 °C; **IR** vmax (neat): 3213, 1719, 1637, 1518, 1499, 1486, 1211, 1022, 992, 955, 916 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.26 (bs, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.82 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.77 (d, *J* = 1.7 Hz, 1H), 5.51 (s, 1H), 4.96 (d, *J* = 16.0 Hz, 1H), 4.91 (d, *J* = 16.0 Hz, 1H), 2.44 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.5, 159.3 (2C), 138.7 (2C), 137.2 (2C), 130.6 (2C), 120.8 (2C), 117.3, 112.8 (2C), 91.7, 65.4, 28.8, 14.4; **LC-MS (ESI):** *m/z* 422.0 (M+Na).

9-(4-fluorophenyl)-6-(methylthio)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2de'). Compound **2de'** was prepared accordingly to the *general procedure A for the one pot synthesis* using 3-methylthioaniline **3d** (138 mg, 1.0 mmol, 1.0 equiv.), 4-fluorobenzaldehyde **4e'** (124 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2de'** (98 mg, 0.3 mmol, 30% yield). White solid; $\mathbf{R}_f$ = 0.29 (acetone/hexanes; 30/70); **m.p.** > 260 °C; **IR** vmax (neat): 3240, 1711, 1639, 1606, 1532, 1486, 1211, 1022, 920, 851 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.10 (bs, 1H), 7.22 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.07 (t, *J* = 8.8 Hz, 2H), 6.95 (d, *J* = 8.0 Hz, 1H), 6.80 (dd, *J* = 10.1, 1.7 Hz, 2H), 5.00 (s, 1H),4.97 (d, *J* = 15.8 Hz, 1H), 4.87 (d, *J* = 15.8 Hz, 1H), 2.42 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.1, 160.9 (d, *J* = 240.0 Hz,), 158.6, 143.1 (d, *J* = 3.0 Hz), , 137.6, 136.7, 131.3, 129.5 (d, *J* = 8.0 Hz, 2C), 121.1, 120.8, 115.2 (d, *J* = 21.0 Hz, 2C), 113.0, 95.6, 65.3, 38.3, 14.6; **LC-MS (ESI)**: *m/z* 328.0 (M+H), 350.0 (M+Na).

9-(2,3-dimethoxyphenyl)-6-(methylthio)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2dg'). Compound **2dg'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-methylthioaniline **3d** (123 µL, 1.0 mmol, 1.0 equiv.), 2,3-dimethoxybenzaldehyde **4g'** (166 mg, 1.0 mmol, 1.0 equiv.), and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was purified by recrystallization in ethanol (2.5 mL), then filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2dg'**(66 mg, 0.2 mmol, 18% yield). White powder; $\mathbf{R}_f$ = 0.21 (acetone/hexanes; 30/70); **m.p**. > 260 °C; **IR** vmax (neat): 1721, 1636, 1526, 1483, 1351, 1225, 1078, 1011, 740 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.98 (bs, 1H), 6.94 (t, *J* = 7.9 Hz, 1H), 6.87 – 6.81 (m, 2H), 6.75 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.72 (d, *J* = 1.9 Hz, 1H), 6.64 (dd, *J* = 7.7, 1.1 Hz, 1H), 5.25 (s, 1H), 4.96 (d, *J* = 15.4 Hz, 1H), 4.87 (d, *J* = 15.4 Hz, 1H), 3.78 (s, 3H), 3.73 (s, 3H), 2.40 (s, 3H); **LC-MS (ESI)**: *m/z*: 370.0 (M+H), 392.0 (M+Na).

5-(methylthio)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2ea'). Compound **2ea'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3,4,5-trimethoxybenzaldehyde **4a'** (491 mg, 2.5 mmol, 1.0 equiv.), 2-methylthioaniline **3e** (348 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The crude product was purified by column chromatography on silica gel using an isocratic solvent system of acetone and dichloromethane (20:80) to obtain pure compound **2ea'** (771 mg, 1.93 mmol, 77% yield). Red solid; $\mathbf{R}_f$ = 0.29 (acetone/hexanes; 45/55); **m.p.** > 260 °C; **IR** vmax (neat): 2929, 1734, 1669, 1589, 1455, 1418, 1230, 1121, 1005, 770 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.09 (d, *J* = 2.1 Hz, 1H), 6.86 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.60 (d, *J* = 8.3 Hz, 1H) 6.59 (s, 2H), 4.86 (s, 1H), 4.67 (s, 2H), 3.67 (s, 6H), 3.62 (s, 3H), 2.24 (s, 3H); **LC-MS (ESI)**: *m/z* 418.1 ($M+H_2O+H$);

9-(4-hydroxy-3-methoxyphenyl)-5-(methylthio)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2eb'). Compound **2eb'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-hydroxy-4-methoxybenzaldehyde **4b'** (380 mg, 2.5 mmol, 1.0 equiv.), 2-methylthioaniline **3e** (348 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The crude product was purified by column chromatography on silica gel using an isocratic solvent system of acetone and dichloromethane (30:70) to obtain pure compound **2eb'** (733 mg, 2.1 mmol, 83% yield). Deep red solid; $\mathbf{R}_f$ = 0.26 (acetone/hexanes; 45/55); **m.p.** > 260 °C; **IR** vmax (neat): 2926, 1609, 1512, 1427, 1268, 1229, 1124, 1031, 779, 687 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 8.75 (bs, 1H), 7.06 (d, *J* = 2.0 Hz, 1H), 6.85 – 6.81 (m, 2H), 6.68 – 6.56 (m, 3H), 5.07 (bs, 1H), 4.82 (s, 1H), 4.64 (s, 2H), 3.65 (s, 3H), 2.23 (s, 3H); **LC-MS (ESI)**: *m/z* 356.2 (M+H).

11-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydrobenzo[g]furo[3,4-b]quinolin-1(3H)-one (7fa'). Compound **7fa'** was prepared accordingly to the *general* ***procedure B***

for the sequential one pot synthesis using 4-chloroaniline **3l** (140 mg, 1.1 mmol, 1.1 equiv.), 5,6,7,8-tetrahydronaphthalen-2-amine **3f** (147 mg, 1.0 mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was taken in methanol (2.5 mL) and the precipitate was filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **7fa'** (25 mg, 0.06 mmol, 6% yield). White solid; $\mathbf{R_f}$ = 0.38 (ethyl acetate/hexanes; 1:1); **m.p.** > 260 °C; **IR** νmax (neat): 2936, 1768, 1599, 1579, 1445, 1412, 1121, 1039, 1029, 706, 687 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.89 (bs, 1H), 7.60 (s, 1H), 6.77 (s, 2H), 5.46 (s, 2H), 3.79 (s, 3H), 3.77 (s, 6H), 3.03 (t, *J* = 5.2 Hz, 2H), 2.90 (t, *J* = 5.3 Hz, 2H), 1.84 – 1.75 (m, 4H); **LC-MS (ESI)**: *m/z* 406.0 (M+H).

11-(3,4,5-trimethoxyphenyl)-4,6,7,8,9,11-hexahydrobenzo[g]furo[3,4-b]quinolin-1(3H)-one (2fa'). To a suspension of compound **7fa'** (21 mg, 0.05 mmol, 1.0 equiv.) in glacial acetic acid (0.6 mL, 15.0 equiv.) was added sodium cyanoborohydride (10 mg, 0.2 mmol, 3.0 equiv.) in one portion at room temperature and the reaction mixture was stirred for 4 hours. The reaction mixture was poured into an ice cold water (2 mL) and the newly formed precipitate was filtered and finally washed with cold ethanol (2x 1 mL) to obtain pure compound **2fa'** (12 mg, 0.03 mmol, 57% yield). White powder; $\mathbf{R_f}$ = 0.3 (hexanes/acetone 40 / 60); **m.p.** > 260 °C; **IR** νmax (neat): 1734, 1658, 1320, 1223, 1011, 750 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.85 (bs, 1H), 6.81 (s, 1H), 6.59 (s, 1H), 6.47 (s, 2H), 4.99 (d, *J* = 15.7 Hz, 1H), 4.85 (d, *J* = 15.8 Hz, 1H), 4.85 (s, 1H), 3.70 (d, *J* = 1.3 Hz, 6H), 3.60 (d, *J* = 1.5 Hz, 3H), 2.64 – 2.62 (m, 2H), 2.54 - 2.50 (m, 2H), 1.65-1.62 (m, 4H); **LC-MS (ESI)**: R_t = 7.9 mins, *m/z* 408.0 (M+H); **HR-MS (ESI)**: *m/z* Calcd for $[C_{24}H_{25}NO_5+H]^+$ 408.1811, Found 408.1795 (- 1.5 ppm). Other spectral data match with the previous report from literature. [4e,4f]

11-(perfluorophenyl)-4,6,7,8,9,11-hexahydrobenzo[g]furo[3,4-b]quinolin-1(3H)-one (7fd'). Compound **2fd'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 5,6,7,8-tetrahydro-2-naphthylamine **3f** (75 mg, 0.5 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2fd'** (12 mg, 0.03 mmol, 5% yield). Off-white solid; $\mathbf{R_f}$ = 0.35 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1724, 1638, 1467, 1021, 987, 946 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.12 (bs, 1H), 6.65 (s, 1H), 6.60 (s, 1H), 5.43 (s, 1H), 4.91 (d, *J* = 15.8 Hz, 1H), 4.87 (d, *J* = 15.8 Hz, 1H), 2.61 - 2.65 (m, 2H), 2.50 – 2.53 (m, 2H), 1.62 – 1.67 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.7, 159.3, 137.0 (2C), 134.1, 132.2 (2C), 130.1 (3C), 118.2, 116.1 (3C), 90.8, 65.3, 28.9 28.5, 28.1, 22.7, 22.6; **LC-MS (ESI)**: *m/z* 408.0 (M+H), 430.0 (M+Na).

11-phenyl-4,6,7,8,9,11-hexahydrobenzo[g]furo[3,4-b]quinolin-1(3H)-one. Compound **2fh'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 5,6,7,8-tetrahydro-2-naphthylamine **3f** (147 mg, 1.0 mmol, 1.0 equiv.), benzaldehyde **4h'** (106 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2fh'** (16 mg, 0.05 mmol, 5% yield). Light brown solid; $\mathbf{R_f}$ = 0.41 (acetone/hexanes; 35/65); **m.p.** >260 °C; **IR** νmax (neat): 1754, 1593, 1487, 1429, 1143, 1040, 1028, 699 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.88 (bs, 1H), 7.25 (t, *J* = 7.6 Hz, 2H), 7.20 – 7.10 (m, 3H), 6.70 (s, 1H), 6.60 (s, 1H), 4.95 (d, *J* =16.1 Hz, 1H), 4.90 (s, 1H), 4.85 (d, *J* = 16.1 Hz, 1H), 3.38 (s, 2H), 2.63 (s, 2H), 1.65 (s, 4H). **LC-MS (ESI)**: *m/z* 318.0 (M+H);

8-oxo-7-(3,4,5-trimethoxyphenyl)-7,8,10,11-tetrahydrobenzo[h]furo[3,4-b]quinoline-5-carbonitrile (2ga'). Compound **2ga'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.), 4-amino-1-naphthonitrile **3g** (168 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ga'** (17 mg, 0.04 mmol, 4% yield). Pale yellow solid; $\mathbf{R_f}$ = 0.29 (acetone/hexanes; 40/60); **m.p.** >260 °C; **IR** νmax (neat): 3367, 1741, 1676, 1595, 1456, 1331, 1118, 1029, 998, 749 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.68 (bs, 1H), 8.40 – 8.35 (m, 1H), 8.14 – 8.01 (m, 3H), 7.95 (s, 1H), 7.81 (dd, *J* = 6.3, 3.3 Hz, 2H), 5.69 (s, 1H), 5.18 (d, *J* = 16.2 Hz, 1H), 5.13 (d, *J* = 16.2 Hz, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.71 (s, 3H); **LC-MS (ESI)**: *m/z* 451.0 (M+Na).

7-(benzo[d][1,3]dioxol-5-yl)-8-oxo-7,8,10,11-tetrahydrobenzo[h]furo[3,4-b]quinoline-5-carbonitrile (2gc'). Compound **2gc'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 1,3-benzodioxole-5-carbaldehyde **4c'** (150 mg, 1.0 mmol, 1.0 equiv.), 4-amino-1-naphthonitrile **3g** (168 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2gc'** (50 mg, 0.13 mmol, 10% yield). Light brown solid; **m.p.** >260 °C; **IR** νmax (neat): 2256, 1714, 1611, 1383, 1329, 1027, 920, cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.68 (bs, 1H), 8.37 (dd, *J* = 6.4, 3.2 Hz, 1H), 8.03 (dd, *J* = 6.7, 3.1 Hz, 1H), 7.88 (s, 1H), 7.81 (dd, *J* = 4.5, 1.8 Hz, 2H), 6.88 (s, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.76 (dd, *J* = 8.0, 1.8 Hz, 1H), 5.95 (d, *J* = 6.2 Hz, 2H), 5.16 (s, 1H), 5.14 (d, *J* = 15.5 Hz, 1H), 5.01 (d, *J* = 15.5 Hz, 1H); **LC-MS (ESI; negative mode)**: *m/z* 381.0 (M-H).

8-oxo-7-(perfluorophenyl)-7,8,10,11-tetrahydrobenzo[h]furo[3,4-b]quinoline-5-carbonitrile (2gd'). Compound **2gd'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 4-amino-1-naphthalenecarbonitrile **3g** (86 mg, 0.5 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2gd'** (14 mg, 0.03 mmol, 6% yield). White solid; $\mathbf{R_f}$ = 0.30 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1592, 1444, 1364, 1205, 983, 764, 703, 685 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.90 (bs, 1H), 8.37 (d, *J* = 7.2 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.91 – 7.77 (m, 2H), 7.76 (s, 1H), 5.71 (s, 1H), 5.14 (d, *J* = 15.5 Hz, 1H), 4.87 (d, *J* = 15.5 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm):171.1, 158.9, 137.0 (2C), 135.3, 131.8, 129.6 (2C), 128.0 (2C), 124.9, 122.2 (2C), 121.9 (2C), 117.6, 115.4, 103.1 (2C), 94.8, 66.2, 29.5; **LC-MS (ESI)**: *m/z* 429.1 (M+H).

7-(3,4,5-trimethoxyphenyl)-7,11-dihydrobenzo[h]furo[3,4-b]quinolin-8(10H)-one (2ha'). Compound **2ha'** was prepared accordingly to the *general* ***procedure B*** *for the one pot sequential synthesis* using 4-chloroaniline **3l** (128 mg, 1.1 mmol, 1.1 equiv.), 4-amino-1-naphthalenecarbonitrile **3h** (143 mg, 1.0 mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.1 mmol, 1.1 equiv.), in

2-pentanol (3.30 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ha'** (110 mg, 0.27 mmol, 27% yield). White powder; **IR** νmax (neat): 1721, 1657, 1535, 1125, 1021, 996, 763 cm^{-1}; $\mathbf{R_f}$ = 0.21 (hexanes/ethyl acetate 40 / 60); **^{1}H NMR** (400 MHz, DMSO) δ (ppm): 10.23 (bs, 1H), 8.21 (d, *J* = 8.6 Hz, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.66 – 7.52 (m, 2H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 6.55 (s, 2H), 5.14 (s, 1H), 5.12 (d, *J* = 16.0 Hz, 1H), 4.99 (d, *J* = 16.0 Hz, 1H), 3.68 (s, 6H), 3.59 (s, 3H). **LC-MS (ESI)**: R_t = 7.7 mins, *m/z* 404.0 (M+H). **HR-MS (ESI)**: *m/z* Calcd for $[C_{24}H_{21}NO_5+H]^+$ 404.1498, Found 404.1495 (- 0.5 ppm). Other spectral data match with the previous report from literature. [4e]

7-(perfluorophenyl)-7,11-dihydrobenzo[h]furo[3,4-b]quinolin-8(10H)-one (2hd'). Compound **2hd'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 4-amino-1-naphthalenecarbonitrile **3h** (73 mg, 0.5 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'**(100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2hd'** (21 mg, 0.05 mmol, 10% yield). Light brown solid; $\mathbf{R_f}$ = 0.28 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1729, 1644, 1499, 1107, 992, 951, 757 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.47 (bs, 1H), 8.21 (d, *J* = 8.6 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 5.75 (s, 1H), 5.06 (d, *J* = 15.5 Hz, 1H), 5.01 (d, *J* = 15.5 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.6, 160.0, 133.0 (2C), 131.9, 128.4(3C), 127.4, 126.7,126.6 (2C), 123.5, 122.4 (2C), 120.9 (2C), 115.4, 92.6, 65.9, 30.1; **LC-MS (ESI)**: *m/z* 426.0 (M+Na).

10-(3,4,5-trimethoxyphenyl)-3,6,7,8-tetrahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (7ja'). Compound **7ja'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (140 mg, 1.1 mmol, 1.1 equiv.), 5-aminoindane **3j** (133 mg, 1.0mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ja'** (47.1 mg, 0.1 mmol, 12% yield). White solid; $\mathbf{R_f}$ = 0.3 (ethyl acetate/hexanes; 1:1); **m.p.** >260 °C; **IR** νmax (neat): 2960, 1764, 1579, 1440, 1455, 1248, 1119, 1027, 1006, 718, 690 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.98 (s, 1H), 7.68 (s, 1H), 6.77 (s, 2H), 5.46 (s, 2H), 3.80 (s, 3H), 3.77 (s, 6H), 3.12 (t, *J* = 7.3 Hz, 2H), 3.02 (t, *J* = 7.3 Hz, 2H), 2.10 (p, *J* = 7.4 Hz, 2H); **LC-MS (ESI)**: *m/z* 392.0 (M+H).

10-(3,4,5-trimethoxyphenyl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (2ja'). To a suspension of compound **7ja'** (25 mg, 0.1 mmol, 1.0 equiv.) in glacial acetic acid (0.7 mL, 15.0 equiv.) was added with sodium cyanoborohydride (11 mg, 0.2 mmol, 3.0 equiv.) in one portion at room temperature and the reaction mixture was stirred for 4 hours. The reaction mixture was poured into an ice cold water (2 mL) and the newly formed precipitate was filtered and finally washed with cold ethanol (2x 1 mL) to obtain pure compound **2ja'** (9 mg, 0.02 mmol, 36% yield). White powder; $\mathbf{R_f}$ = 0.3 (hexanes/acetone 40 / 60); **m.p.** >260 °C; **IR** νmax (neat): 1742, 1657, 1479, 1324, 1223, 1140, 1011, 748, 676 cm^{-1};**^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.87 (s, 1H), 6.97 (s, 1H), 6.78 (s, 1H), 6.49 (s, 2H), 5.00 (d, *J* = 15.9 Hz, 1H), 4.90 (s, 1H), 4.86 (d, *J* = 15.9 Hz, 1H), 3.70 (s, 6H), 3.60 (s, 3H), 2.77 (t, *J* = 6.8 Hz, 2H), 2.70 – 2.68 (m, 2H), 2.04 – 1.86 (m, 2H). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.2, 158.7, 152.8 (2C), 143.2, 143.1 138.6, 136.0, 134.3, 125.9, 122.3, 111.9, 104.9 (2C), 94.7, 65.0, 59.9, 55.8 (2C), 39.9, 32.0, 31.7, 25.2; **LC-MS (ESI)**: *m/z* 394.0 (M+1), 416.0 (M+Na); **HR-MS (ESI)**: *m/z* Calcd for $[C_{23}H_{23}NO_5+H]^+$ 394.1654, Found 394.1685 (+ 7.9 ppm).

10-(benzo[d][1,3]dioxol-5-yl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (2jc'). Compound **2jc'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 1,3-benzodioxole-5-carbaldehyde **4c'** (150 mg, 1.0 mmol, 1.0 equiv.), 5-aminoindan **3j** (133 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was then filtered and rinsed with ethanol (3 x 2.5 mL) to obtain pure compound **2jc'** (93 mg, 0.3 mmol, 27% yield). Pale yellow solid; $\mathbf{R_f}$ = 0.21 (acetone/hexanes; 35/65); **m.p.** >260 °C; **IR** νmax (neat): 3259, 3177, 3120, 1712, 1635, 1621, 1541, 1484, 1228, 1199, 1034, 1015 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.89 (bs, 1H), 6.88 (s, 1H), 6.76 (s, 1H), 6.75 (d, *J* = 29.2 Hz, 2H), 6.65 (d, *J* = 8.9 Hz, 1H), 5.93 (d, *J* = 2.9 Hz, 2H), 4.90 (d, *J* = 15.6 Hz, 1H), 4.87 (s, 1H), 4.84 (d, *J* = 15.6 Hz, 1H), 2.76 (s, 2H), 2.68 (d, *J* = 7.2 Hz, 2H), 2.05 – 1.76 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.3, 158.6, 147.3, 145.6, 143.3, 141.7, 138.8, 134.4, 126.0, 122.6, 120.5, 112.0, 108.2 (2C), 108.0, 100.9, 94.9, 65.1, 32.1, 31.7, 25.3; **LC-MS (ESI)**: R_t = 8.9 mins, *m/z* 348.0 (M+H), 370.0 (M+Na); **HR-MS (ESI)**: *m/z* Calcd for $[C_{21}H_{17}NO_4+H]^+$ 348.1236, Found 348.1244 (+ 2.3 ppm).

10-(perfluorophenyl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (2jd'). Compound **2jd'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 5-aminoindan **3j** (68 mg, 0.5 mmol, 1.0 equiv.), Pentafluorobenzaldehyde **4d'** (100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2jd'** (23 mg, 0.02 mmol, 12% yield). Off-White solid; $\mathbf{R_f}$ = 0.34 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1728, 1642, 1499, 1328, 1190, 1107, 991, 951, 776, 756 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.15 (bs, 1H), 6.82 (s, 1H), 6.79 (s, 1H), 5.50 (s, 1H), 4.89 (d, *J* = 15.6 Hz, 1H), 4.84 (d, *J* = 15.6 Hz, 1H), 2.80 - 2.70 (m, 2H),2.68 - 2.62 (m, 2H), 2.02 – 1.87 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.7, 159.4, 144.5 (2C), 139.3 (2C), 135.0, 125.4 (3C), 118.6, 112.2 (3C), 90.8, 65.3, 32.1, 31.6, 29.4, 25.2; **LC-MS (ESI)**: *m/z* 394.1 (M+H).

10-phenyl-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (2jh'). Compound **2jh'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140 mg, 1.1 mmol, 1.1 equiv.), benzaldehyde **4h'** (106 mg, 1.0 mmol, 1.0 equiv.), 5-aminoindan **3j** (133 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2jh'** (30 mg, 0.1 mmol, 10% yield). Off-white solid; $\mathbf{R_f}$ = 0.31 (acetone/hexanes; 35/65); **m.p.** >260 °C; **IR** νmax (neat): 3253, 3176, 3118, 1711, 1635, 1624, 1542, 1019, 701 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.92 (bs, 1H), 7.64 – 7.43 (m, 3H), 7.31 – 7.08 (m, 3H), 6.82 (d, *J* = 35.1 Hz, 1H), 5.49 (s, 1H), 4.96 (d, *J* = 15.6 Hz, 1H), 4.86 (d, *J* = 15.6 Hz, 1H), 3.14 – 2.96 (m, 2H), 2.78 – 2.63 (m, 2H), 2.20 – 1.88 (m, 2H); **LC-MS (ESI)**: *m/z* 304.1 (M+H), 326.1 (M+Na).

11-(3,4,5-trimethoxyphenyl)-8,11-dihydrofuro[3,4-b][4,7]phenanthrolin-10(7H)-one (2ka'). Compound **2ka'** was prepared accordingly to the *general* ***procedure B*** *for the sequen-*

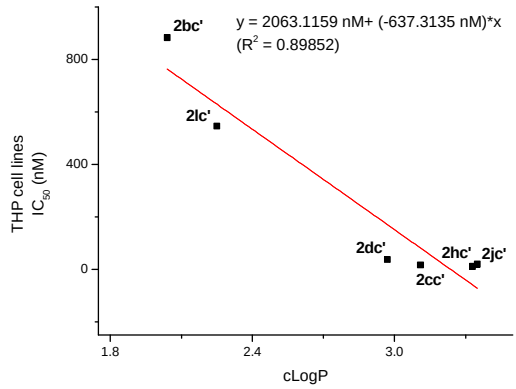
tial one pot synthesis using 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.), 6-aminoquinoline **3k** (144 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ka'** (168 mg, 0.4 mmol, 42% yield). Yellow solid; $\mathbf{R}_f$ = 0.19 (acetone/hexanes; 40/60); **m.p.** >260 °C; **IR** νmax (neat): 3182, 1721, 1643, 1541, 1322, 1228, 1205, 1113, 999, 819 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.46 (bs, 1H), 8.68 (d, *J* = 4.0 Hz, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 7.95 (d, *J* = 9.0 Hz, 1H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.39 (dd, *J* = 8.6, 4.2 Hz, 1H), 6.49 (s, 2H), 5.67 (s, 1H), 5.00 (d, *J* = 15.8 Hz, 1H), 4.91 (d, *J* = 15.8 Hz, 1H), 3.60 (s, 6H), 3.54 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.1, 157.3, 152.8 (2C), 148.0, 145.7, 141.4, 135.9, 135.2, 131.6, 130.0, 127.2, 121.9, 121.2, 114.8, 105.1 (2C), 97.0, 65.2, 59.9, 55.8 (2C), 36.7; **LC-MS (ESI)**: *m/z* 405.1 (M+H).

11-(perfluorophenyl)-8,11-dihydrofuro[3,4-b][4,7]phenanthrolin-10(7H)-one (2kd'). Compound **2kd'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 4-amino-1-naphthalenecarbonitrile **3k** (74 mg, 0.5 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2kd'** (30 mg, 0.03 mmol, 15% yield). Off-White solid; $\mathbf{R}_f$ = 0.25 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1746, 1500, 1209, 1021, 1007, 990, 832 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.73 (bs, 1H), 8.73 (d, *J* = 4.2 Hz, 1H), 7.96 (d, *J* = 9.1, 1H), 7.95 (d, *J* = 9.1, 1H), 7.52 - 7.50 (m, 2H), 6.14 (s, 1H), 5.06 (d, *J* = 15.8 Hz, 1H), 5.00 (d, *J* = 15.8 Hz, 1H); **LC-MS (ESI)**: *m/z* 405.0 (M+H).

9-(benzo[d][1,3]dioxol-5-yl)-6-(methoxymethoxy)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2lc'). To a flame dried and argon purged 10 mL round-bottom flask, NaH (60% weight in mineral oil) (2 mg, 0.05 mmol, 1.5 equiv.) was suspended in DMF (200 µL, [0.15 M]) at 0 °C. Compound **2bc'** (11 mg, 0.03 mmol, 1.0 equiv.) followed by chloromethylmethyl ether (3 mg, 0.033 mmol, 1.1 equiv.) were then added at 0 °C. The reaction mixture was stirred under argon for an hour at room temperature and then poured into cold water (1 mL), extracted with ethyl acetate (3 x 3 mL) and the combined organic extracts were dried over sodium sulfate. Removal of solvents under reduced pressure afforded the crude product which was further purified by column chromatography on silica gel by using an isocratic solvent system (hexanes/ethyl acetate; 70:30) to obtain pure compound **2lc'** (3 mg, 0.01 mmol, 24% yield). White powder; $\mathbf{R}_f$ = 0.30 (hexanes/acetone 40 / 60); **m.p.** >260 °C; **IR** νmax (neat): 3357, 1728, 1621, 1610, 1485, 1109, 1035, 798 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.99 (bs, 1H), 6.95 (d, *J* = 8.2 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.72 (s, 1H), 6.65 (d, *J* = 7.9 Hz, 1H), 6.58 (d, *J* = 7.8 Hz, 2H), 5.93 (d, *J* = 3.4 Hz, 2H), 5.20 – 5.04 (m, 2H), 4.96 (d, *J* = 15.6 Hz, 1H), 4.87 (s, 1H), 4.84 (d, *J* = 15.6 Hz, 1H), 3.35 (s, 3H); **LC-MS (ESI)**: *m/z* 368.0 (M+H).

5.2. Calculated physicochemical properties.

Partition coefficient values (cLogP), hydrogen-bond acceptor and donors sites at physiological pH (HBA and HBD) and the topological polar surface area (TPSA in $Å^2$) were calculated for all APT molecules from the library, using the Plexus discovery software from the Plexus Suite 9.0 which is a web-based software package that integrate the ChemAxon's structure-based property calculations application.[12] A meaningful selected data set of these properties is presented in the supporting information. All the calculated physicochemical values for these compounds (cLogP < 3.7; 56 $Å^2$< TPSA <79 $Å^2$; HBA+HBD<7) were in acceptable range according to the literature.[20-23] Although the calculated physicochemical values could not be directly correlated to the biological activity and IC_{50} values (against leukemia), a trend can be observed. For example, in the series of compounds **2bc'**, **2lc'**, **2dc'**, **2cc'**, **2hc'** and **2fc'**, the lipophilicity increased drastically (cLogP = 2.0, 2.2, 3.0, 3.1, 3.3 and 3.35) which also correspond to an increase in cell permeation and in biological activity (corresponding IC_{50} values of 883, 545, 37, 17, 11 and 50 nM). The increase in lipophilicity resulting from the ether appendages at C7 (*e.g.* **2bc'** vs **2lc'** and **2cc'**) may well favor a better cell permeation without affecting the binding properties of the small-molecules. The substitution change from a free-phenol at C7 to various ethers and thioethers resulted in a large improvement of cytotoxicity which could be related to the nature of the substituents (increased binding abilities for $–OCHF_2$ and SCH_3) and possibly to a diminished oxidative metabolism of degradation. Therefore we could conclude that lead compounds such as **2ca'** and **2cc'** may present an interesting balance between a relatively high lipophilicity to facilitate cell permeation (cLogP ~ 3.0) while presenting a relatively large TPSA (TPSA ~ 70 $Å^2$) to maintain an important binding affinity to the cellular target.



Plot of IC_{50} results as function of clogP values for a series of ATP molecules: **2bc'**, **2lc'**, **2dc'**, **2cc'**, **2hc'** and **2jc'**.

Based on these physicochemical properties, the APT molecules synthesized in this study demonstrated high solubility, a favorable cell permeability and an important level of biological validation, therefore these small-molecules should have the appropriate structural and electronic features (high TPSA and count of HBA+HBD) to induce a high binding affinity to the biological target. The degree of interaction or affinity of the APT molecules and the biological target, leading to a potent activity on leukemia THP-1 cells, can be related mostly to the stereoelectronic pattern of substitution on the B-ring.

5.3. Biological evaluation.

CellTiter-Glo® cell viability assays. Test compounds were solubilized in 100% DMSO and added to polypropylene 384 well plates (Greiner cat# 781280). 1,250 of THP-1, PSN-1, or HEK293 cells were plated in 384-well plates in 8 µl of serum-free media (RPMI-1640 for THP-1 and PSN-1, EMEM for HEK293). Test compounds and **1** (pharmacological assay control) were prepared as 10-point, 1:3 serial dilutions starting at 10 mM, then added to the cells using the pin tool mounted on Biomek NX^P. Plates were

incubated for 72 h at 37°C, 5% CO_2 and 95% RH. After incubation, 8 µL of CellTiter-Glo® (Promega cat#: G7570) were added to each well, and incubated for 15 min at room temperature. Luminescence was recorded using a Biotek Synergy H4 multimode microplate reader. Viability was expressed as a percentage relative to wells containing media only (0%) and wells containing cells treated with 1% DMSO only (100%). Three parameters were calculated on a per-plate basis: (a) the signal-to-background ratio (S/B); (b) the coefficient for variation [CV; CV = (standard deviation/mean) x 100)] for all compound test wells; and (c) the Z'-factor. [26] The IC_{50} value of the pharmacological control (**1**, LC Laboratories # E-4488) was also calculated to ascertain the assay robustness. Each dose response curve was examined and curve type was determined according to Inglese et al., [18]. In cases where a complete response was observed (viability < 20%, both asymptotes present) the IC_{50} value derived from GraphPad software was used. In cases of partial response (viability 20-50%, both asymptotes present) the lowest concentration that induced > 50% viability loss was assigned as EC_{50} value.[27] In cases where the highest efficacy observed was > 50% viability, IC_{50} value was assigned as " > highest concentration tested" (*e.g.*, > 100 µM).

Trypan Blue cell viability assays. 10,000 of THP-1 or HEK293 cells were plated in 96-well plates in 100 µl of serum-free media (RPMI-1640 for THP-1 and EMEM for HEK293). 10 µM of Etoposide (**1**) and compound **2ha'** were added to the corresponding wells of 96-well plates. Plates were incubated for 72 h at 37°C, 5% CO_2 and 95% RH. After incubation, cells were harvested using standard tissue culture techniques, Trypan Blue was added and cell counts and viability were analyzed using Cellometer Auto T4 (Nexcelom Bioscience, Lawrence, MA) according to the manufacturer's instructions.

REFERENCES

(1) (a) Rusch, V. W.; Giroux, D. J.; Kraut, M. J.; Crowley, J.; Hazuka, M.; Winton, T.; Johnson, D. H.; Shulman, L.; Shepherd, F.; Deschamps, C.; Livingston, R. B.; Gandara, D. Induction chemoradiation and surgical resection for superior sulcus non-small-cell lung carcinomas: long-term results of Southwest Oncology Group Trial 9416. *J. Clin. Oncol.* **2007**, *25*, 313-318. (b) For a review on etoposide, see: Hande, K. R. Etoposide: four decades of development of a topoisomerase II inhibitor. *Eur. J. Cancer*, **1998**, *34*, 1514-1521.

(2) (a) Ho, A. D.; Lipp, T.; Ehninger, G.; Illiger, H. J.; Meyer, P.; Freund, M.; Hunstein, W. Combination of mitoxantrone and etoposide in refractory acute myelogenous leukemia-an active and well-tolerated regimen. *J. Clin. Oncol.* **1988**, *6*, 213-217. (b) Milligan, D. W.; Wheatley, K.; Littlewood, T.; Craig, J. I. O.; Burnett, A. K. Fludarabine and cytosine are less effective than standard ADE chemotherapy in high-risk acute myeloid leukemia, and addition of G-CSF and ATRA are not beneficial: results of the MRC AML-HR randomized trial. *Blood* **2006**, *107*, 4614-4622.

(3) Hande, K. R. Clinical applications of anticancer drugs targeted to topoisomerase II. *Biochim. Biophys. Acta*, **1998**, *1400*, 173-184. (b) Baldwin, E. L.; Osheroff, N. Etoposide, topoisomerase II and cancer. *Curr. Med. Chem. Anticancer Agents* **2005**, *5*, 363-372.

(4) (a) Hitotsuyanagi, Y.; Fukuyo, M.; Tsuda, K.; Kobayashi, M.; Ozeki, A.; Itokawa, H.; Takeya, K. 4-Aza-2,3-dehydro-4-deoxypodophyllotoxins: simple aza-podophyllotoxin analogues possessing potent cytotoxicity. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 315-317. (b) Labruère, R.; Gautier, B.; Testud, M.; Seguin, J.; Lenoir, C.; Desbène-Finck, S.; Helissey, P.; Garbay, C.; Chabot, G. G.; Vidal, M.; Giorgi-Renault; S. Design, synthesis, and biological evaluation of the first podophyllotoxin analogues as potential vascular-disrupting agents. *ChemMedChem.* **2010**, *5*, 2016–2025. (c) Kamal, A.; Suresh, P.; Mallareddy, A.; Kumar, B. A.; Reddy, P. V.; Raju, P.; Tamboli, J. R.; Shaik, T. B. Synthesis of a new 4-aza-2,3-didehydropodophyllotoxin analogues as potent cytotoxic and antimitotic agents. *Bioorg. Med. Chem.* **2011**, *19*, 2349-2358. (d) Shi, F.; Zeng, X.-N.; Zhang, G.; Ma, N.; Jiang, B.; Tu, S. Facile synthesis of new 4-aza-podophyllotoxin analogs via microwave-assisted multicomponent reactions and evaluation of their cytotoxic activity. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7119-7123. (e) Magedov, I. V.; Frolova, L.; Manpadi, M.; Bhoga, U. D.; Tang, H.; Evdokimov, N. M.; George, O.; Hadje, G. K.; Renner, S.; Getlic, M.; Kinnibrugh, T. L.; Fernandes, M. A.; Van, S. S.; Steelant, W. F. A.; Shuster, C. B.; Rogelj, S.; van, O. W. A. L.; Kornienko, A. Anticancer properties of an important drug lead podophyllotoxin can be efficiently mimicked by diverse heterocyclic scaffolds accessible via one-step synthesis. *J. Med. Chem.* **2011**, *54*, 4234-4246. (f) Semenova, M. N.; Kiselyov, A. S.; Tsyganov, D. V.; Konyushkin, L. D.; Firgang, S. I.; Semenov, R. V.; Malyshev, O. R.; Raihstat, M. M.; Fuchs, F.; Stielow, A.; Lantow, M.; Philchenkov, A. A.; Zavelevich, M. P.; Zefirov, N. S.; Kuznetsov, S. A.; Semenov, V. V. Polyalkoxybenzenes from plants. 5. Parsley seed extract in synthesis of azapodophyllotoxins featuring strong tubulin destabilizing activity in the sea urchin embryo and cell culture assays. *J. Med. Chem.* **2011**, *54*, 7138-7149. (g) Chernysheva, N. B.; Tsyganov, D. V.; Philchenkov, A. A.; Zavelevich, M. P.; Kiselyov, A. S.; Semenov, R. V.; Semenova, M. N.; Semenov, V. V. Synthesis and comparative evaluation of 4-oxa- and 4-aza-podophyllotoxins as antiproliferative microtubule destabilizing agents. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2590-2593. (h) Kamal, A.; Tamboli, J. R.; Nayak, V. L.; Adil, S. F.; Vishnuvardhan, M. V. P. S.; Ramakrishna, S. Synthesis of a terphenyl substituted 4-aza-2,3-didehydropodophyllotoxin analogues as inhibitors of tubulin polymerization and apoptosis inducers. *Bioorg. Med. Chem.* **2014**, *22*, 2714-2723.

(5) For a review on synthesis of APTs and biological evaluation, see: Botes, M. G.; Pelly, S. C.; Blackie, M. A. L.; Kornienko, A.; van Otterlo, W. A. L. Synthesis of 4-Azapodophyllotoxins with Anticancer Activity by Multicomponent Reactions. *Chem. Heterocycl. Compd.* **2014**, *50*, 119-138.

(6) For selected reviews on MCR, see: (a) Dömling, A.; Ugi, I. Multicomponent Reactions with Isocyanides. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168-3210. (b) Zhu, J. Recent developments in the isonitrile- based multicomponent synthesis of heterocycles. *Eur. J. Org. Chem.* **2003**, 1133-1144. (c) Ramon, D. J.; Yus, M. Asymmetric multicomponent reactions (AMCRs): the new frontier. *Angew. Chem., Int. Ed.* **2005**, *44*, 1602-1634. (d) Enders, D.; Grondal, C.; Huttl, M. R. M. Asymmetric organocatalytic domino reactions. *Angew. Chem. Int. Ed.* **2007**, *46*, 1570-1581. (e) Touré, B. B.; Hall, D. G. Natural product synthesis using multicomponent reaction strategies. *Chem. Rev.* **2009**, *109*, 4439-4486. (f) Dömling, A.; Wang, W.; Wang, K. Chemistry and biology of multicomponent reactions. *Chem. Rev.* **2012**, *112*, 3083-3135.

(7) For selected reviews on MCR for the synthesis of large libraries of bioactive small-molecules, see: (a) Dömling, A. Recent advances in isocyanide-based multicomponent chemistry. *Curr. Opin. Chem. Biol.* **2002**, *6*, 306-313. (b) Sahn, J. J.; Granger, B. A.; Martin, S. F. Evolution of a strategy for preparing bioactive small-molecules by sequential multicomponent assembly processes, cyclizations, and diversification. *Org. Biomol. Chem.* **2014**, *12*, 7659-7672. (c) Zarganes-Tzitzikas, T.; Dömling, A. Modern Multicomponent Reactions for better Drug Syntheses. *Org. Chem. Front.* **2014**, *1*, 834-837.

(8) (a) Husson, H.-P.; Giorgi-Renault, S.; Tratrat, C.; Atassi, G.; Pierre, A.; Renard, P.; Pfeiffer, B. Dihydrofuro[3,4-b]quinolin-l-one compounds. French patent no. 99.14771, Nov 24, **1999**. Extension no. 00403255.3, Nov 22, **2000**; (b) Tratrat, C.; Giorgi-Renault, S.; Husson, H.-P. A multicomponent reaction for the one-pot synthesis of 4-aza-2,3-didehydropodophyllotoxin and derivatives. *Org. Lett.* **2002**, *4*, 3187-3189.

(9) Frackenpohl, J.; Adelt, I.; Antonicek, H.; Arnold, C.; Behrmann, P.; Blaha, N.; Boehmer, J.; Gutbrod, O.; Hanke, R.; Hohmann, S.; Van, H. M.; Loesel, P.; Malsam, O.; Melchers, M.; Neufert, V.; Peschel, E.; Reckmann, U.; Schenke, T.; Thiesen, H.-P.; Velten, R.; Vogelsang, K.; Weiss, H.-C. Insecticidal heterolignans--tubuline

polymerization inhibitors with activity against chewing pests. *Bioorg. Med. Chem.* **2009**, *17*, 4160-4184.

(10) (a) Tu, S.; Zhang, Y.; Zhang, J.; Jiang, B.; Jia, R.; Zhang, J.; Ji, S. A simple procedure for the synthesis of 4- aza- podophyllotoxin derivatives in water under microwave irradiation conditions. *Synlett* **2006**, 2785-2790. (b) Labruère, R.; Desbène-Finck, S.; Helissey, P.; Giorgi-Renault, S. Design and Effective Synthesis of the First 4- Aza-2, 3- didehydropodophyllotoxin Rigid Aminologue: A N-Methyl-4-[(3, 4, 5- trimethoxyphenyl) amino)]-1, 2-dihydroquinoline-lactone. *J. Org. Chem.* **2008**, *73*, 3642-3645. (c) Kozlov, N. G.; Bondarev, S. L.; Kadutskii, A. P.; Basalaeva, L. I.; Pashkovskii, F. S. Tetronic acid in reaction with aromatic aldehydes and 2- naphthylamine. Investigation of fluorescent and nonlinear- optical characteristics of compounds obtained. *Russ. J. Org. Chem.* **2008**, *44*, 1031-1037. (d) Shi, F.; Zhou, D.; Tu, S.; Shao, Q.; Li, C.; Cao, L. An efficient microwave-assisted synthesis furo[3,4-*b*]-[4,7] phenanthroline and indeno[2,1-*b*][4,7]phenanthroline derivatives in water. *J. Heterocycl. Chem.* **2008**, *45*, 1065-1070. (e) Madec, D.; Mingoia, F.; Prestat, G.; Poli, G. N-Substituted tetronamides as ambident nucleophilic building blocks for the synthesis of new 4- aza- 2, 3- didehydropodophyllotoxins. *Synlett* **2008**, 1475-1478. (f) Kozlov, N. G.; Agabekov, V. E.; Bondarev, S. L.; Basalayeva, L. I.; Kadutskiu, A. P. Synthesis of carbonyl containing heterocyclic compounds and their fluorescent and nonlinear optical properties. *Dokl. Nats. Akad. Nauk. Belarusi.* **2009**, *53*, 64-67. (g) Tu, S.; Wu, S.; Yan, S.; Hao, W.; Zhang, X.; Cao, X.; Han, Z.; Jiang, B.; Shi, F.; Xia, M.; Zhou, J. Design and microwave-assisted synthesis of naphtho[2, 3- f] quinoline derivatives and their luminescent properties. *J. Comb. Chem.* **2009**, *11*, 239-242. (h) Kumar, A.; Alegria, A. E. Synthesis of Novel Functionalized 4-Aza-2,3-Didehydropodophyllotoxin Derivatives with Potential Antitumor Activity. *J. Heterocycl. Chem.* **2010**, *47*, 1275-1282. (i) Shi, C.; Wang, J.; Chen, H.; Shi, D. Regioselective synthesis and in vitro anticancer activity of 4-aza-podophyllotoxin derivatives catalyzed by L-proline. *J. Comb. Chem.* **2010**, *12*, 430-434. (j) Shi, F.; Zhang, S.; Wu, S.-S.; Gao, Y.; Tu, S.-J. A diversity-oriented synthesis of pyrazolo[4,3-f]quinoline derivatives with potential bioactivities via microwave-assisted multi-component reactions. *Mol. Diversity* **2011**, *15*, 497-505. (k) Peng, J.-H.; Jia, R.-H.; Ma, N.; Zhang, G.; Wu, F.-Y.; Cheng, C.; Tu, S.-J. A facile and expeditious microwave- assisted synthesis of furo[3, 4- b] indeno[2, 1- f] quinolin- 1- one derivatives via multicomponent reaction. *J. Heterocycl. Chem.* **2013**, *50*, 899-902. (l) Aillerie, A.; De Talance, V. L.; Moncomble, A.; Bousquet, T.; Pelinski, L. Enantioselective organocatalytic partial transfer hydrogenation of lactone-fused quinolones. *Org. Lett.* **2014**, *16*, 2982-2985. (m) see also reference [4e].

(11) Jeedimalla, N.; Johns, J.; Roche, S. P. Mechanistic investigation and implications of a sacrificial aniline for the tandem cascade synthesis of 4-aza-podophyllotoxin analogues. *Tetrahedron Lett.* **2013**, *54*, 5845-5848.

(12) For full experimental details, description of the entire library of APTs **2** synthesis and biological evaluation, refer to the supporting information.

(13) (a) Cao, X.; Lee, Y. T.; Holmqvist, M.; Lin, Y.; Ni, Y.; Mikhailov, D.; Zhang, H.; Hogan, C.; Zhou, L.; Lu, Q.; Digan, M. E.; Urban, L.; Erdemli, G. Cardiac Ion Channel Safety Profiling on the IonWorks Quattro Automated Patch Clamp System. *ASSAY Drug Dev. Techn.* **2010**, *8*, 766-780. (b) Zang, R.; Li, D.; Tang, I. C.; Wang, J.; Yang, S.-T. Cell-Based Assays in High-Throughput Screening for Drug Discovery. *Int. J. Biotechnol. Wellness Ind.* **2012**, *1*, 31-51.

(14) DiRocco, D. P.; Bisi, J., Roberts, P.; Strum, J.; Wong, K. K.; Sharpless, N.; Humphreys, B. D. CDK4/6 inhibition induces epithelial cell cycle arrest and ameliorates acute kidney injury. *Am. J. Physiol. Renal Physiol.* **2014**, 4, 379-388.

(15) (a) Sung, E. S.; Kim, A.; Park, J. S.; Chung, J.; Kwon, M. H.; Kim, Y. S. Histone deacetylase inhibitors synergistically potentiate death receptor 4-mediated apoptotic cell death of human T-cell acute lymphoblastic leukemia cells. *Apoptosis.* **2010**, *15*, 1256-1269. (b) Yao, Q.; Weigel, B.; Kersey, J. Synergism between etoposide and 17-AAG in leukemia cells: critical roles for Hsp90, FLT3, topoisomerase II, Chk1, and Rad51. *Clin. Cancer Res.* **2007**, *13*, 1591-1600.

(16) Kumar, A.; Kumar, V.; Alegria, A. E.; Malhotra, S. V. N-hydroxyethyl-4-aza-didehydropodophyllotoxin derivatives as potential antitumor agents. *Eur. J. Pharm. Sci.* **2011**, *44*, 21-26.

(17) (a) Smith, N. A.; Byl, J. A. W.; Mercer, S. L.; Deweese, J. E.; Osheroff, N. Etoposide quinone is a covalent poison of human topoisomerase IIβ. *Biochemistry.* **2014**, *53*, 3229-3236. (b) see also reference [4a].

(18) Hu, C.; Lancaster, C. S.; Zuo, Z.; Hu, S.; Chen, Z.; Rubnitz, J. E.; Baker, S. D.; Sparreboom, A. Inhibition of OCTN2-Mediated Transport of Carnitine by Etoposide. *Mol. Cancer Ther.* **2012**, *11*, 921-929.

(19) (a) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235-249. (b) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **2012**, *64*, 4-17.

(20) Nassar, A-E. F.; Kamel, A. M.; Clarimont, C. Improving the decision-making process in the structural modification of drug candidates: enhancing metabolic stability. *Drug Discov. Today* **2004**, *9*, 1020-1028.

(21) (a) Gaillard, P.; Carrupt, P.-A.; Testa, B.; Boudon, A. Molecular Lipophilicity Potential, a tool in 3D QSAR: Method and applications. *J. Comput. Aided Mol. Des.* **1994**, *8*, 83-96. (b) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings1. *Adv. Drug Delivery Rev*. **2001**, *46*, 3-26. (c) Congreve, M.; Carr, R.; Murray, C.; Jhoti, H. A 'Rule of Three' for fragment-based lead discovery? *Drug Discov. Today* **2003**, *8*, 876-877. (d) Hann, M. M.; Keserü, G. M. Finding the sweet spot: the role of nature and nurture in medicinal chemistry. *Nat. Rev. Drug Discov*. **2012**, *11*, 355-365.

(22) (a) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J. Med. Chem.* **2002**, *45*, 2615-2623. (b) Kelder, J.; Grootenhuis, P. J.; Bayada, D.; Delbressine, L. C.; Ploemen, J.-P. Polar Molecular Surface as a Dominating Determinant for Oral Absorption and Brain Penetration of Drugs. *Pharm. Res.* **1999**, *16*, 1514-1519. (c) Palm, K.; Luthman, K.; Ungell, A.-L.; Strandlund, G.; Artursson, P. Correlation of drug absorption with molecular surface properties. *J. Pharm. Sci.* **1996**, *85*, 32-39.

(23) (a) Abad-Zapatero, C. Ligand efficiency indices for effective drug discovery. *Expert Opin. Drug Discov*. **2007**, *2*, 469-488. (b) Abad-Zapatero, C.; Champness, E. J.; Segall, M. D. Alternative variables in drug discovery: promises and challenges. *Future Med. Chem.* **2014**, *6*, 577-593.

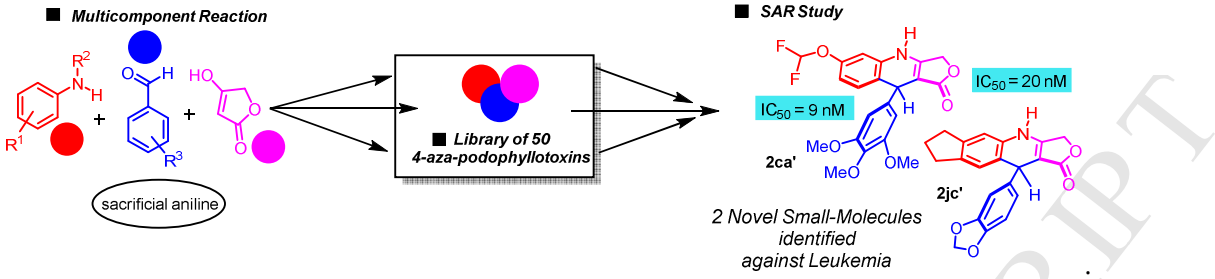
(24) Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of Fluorine in Medicinal Chemistry. *J. Med. Chem.* **2015**, *58 In Print* **DOI:** 10.1021/acs.jmedchem.5b00258.

(25) Nassar, A.-E. F.; Kamel, A. M.; Clarimont, C. Improving the decision-making process in the structural modification of drug candidates: enhancing metabolic stability. *Drug Discov. Today* **2004**, *9*, 1020-1028.

(26) Zhang, J.-H; Chung, T. D. Y.; Oldenburg, K. R. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J. Biomol. Screen*. **1999**, *4*, 67-73.

(27) Inglese, J.; Auld, D. S.; Jadhav, A.; Johnson, R. L.; Simeonov, A.; Yasgar, A.; Zheng, W.; Austin, C. P. Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc. Natl. Acad. Sci. U S A* **2006**, *103*, 11473-11478.

GRAPHICAL ABSTRACT



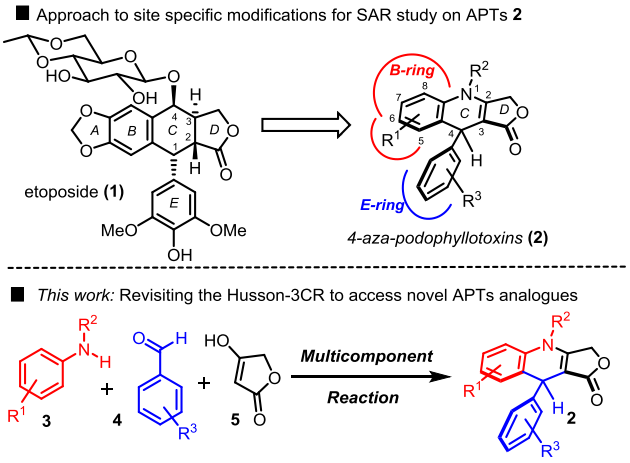
HIGHLIGHTS

- The modified Husson-3CR reported herein (multicomponent reaction) using a sacrificial aniline enabled the rapid construction of a library of podophyllotoxin aza-analogues.
- 7 Compounds (**2ca'**, **2cc'**, **2da'**, **2fa'**, **2ha'**, **2ja'** and **2jc'**) have been identified to have potent (low nano-molar activity) and selective activity against leukemia while preserving a minimal toxicity against no-malignant cells.
- From this study, 2 novel lead small-molecules **2ca'**and **2jc'** were identified to be more potent than the reference drug etoposide (25-60 fold) against leukemia.
- Our preliminary structure activity relationship studies demonstrated the importance of several pharmaco-phore features on the azapodophyllotoxin B- and E-rings to enhance antitumoral activity.

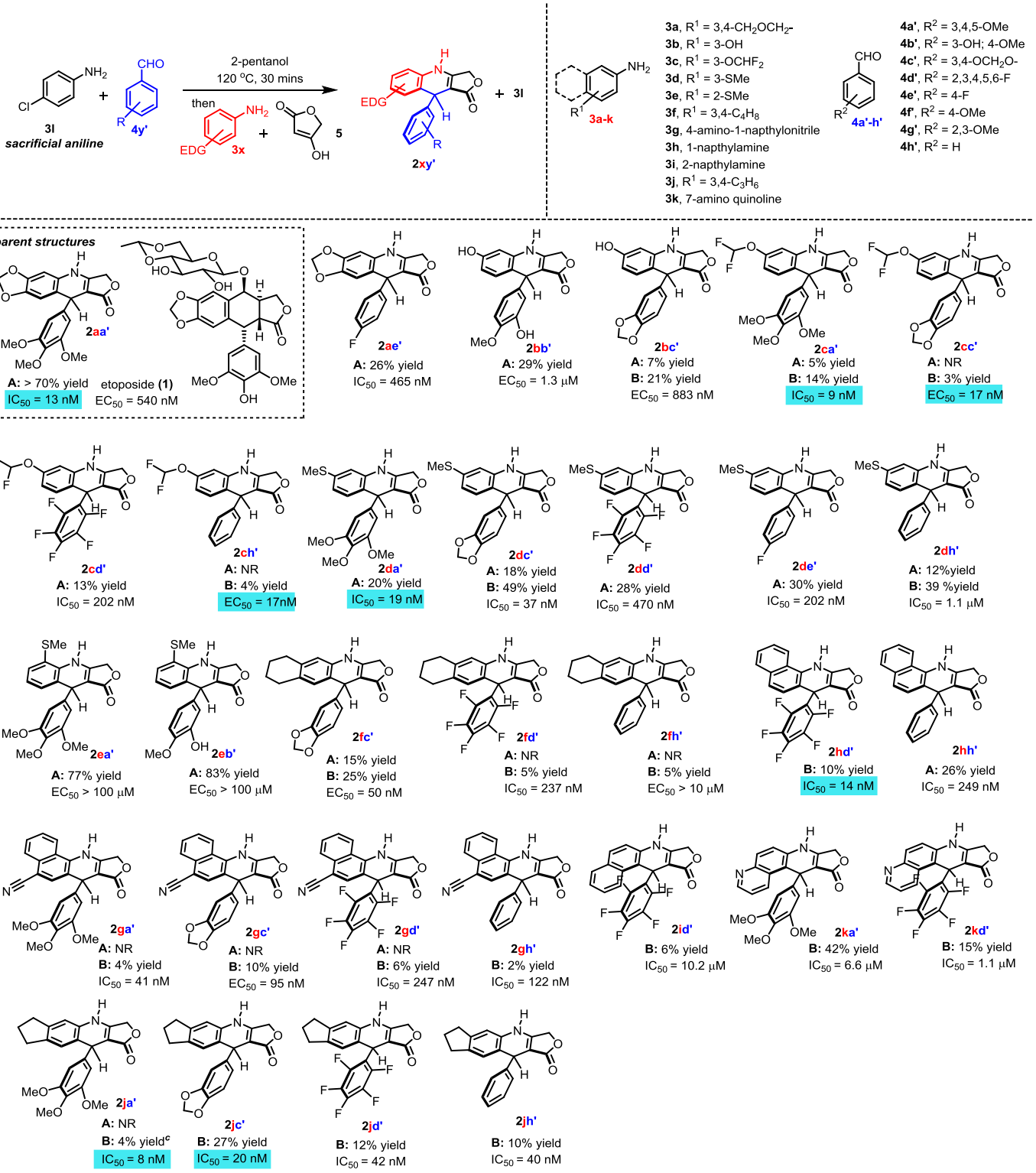
Multicomponent Assembly of 4-Aza-podophyllotoxins: A Fast Entry to Highly Selective and Potent Anti-Leukemic Agents.

All Graphics

Scheme 1. MCR tactic to the 4-aza-podophyllotoxin chemotype

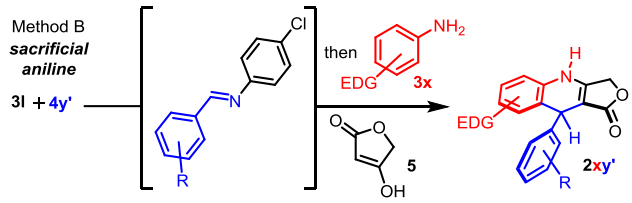


Scheme 2. Synthesis [a-c] and biological evaluation [d] of a library of novel 4-aza-podophyllotoxins (APTs) **2xy'**



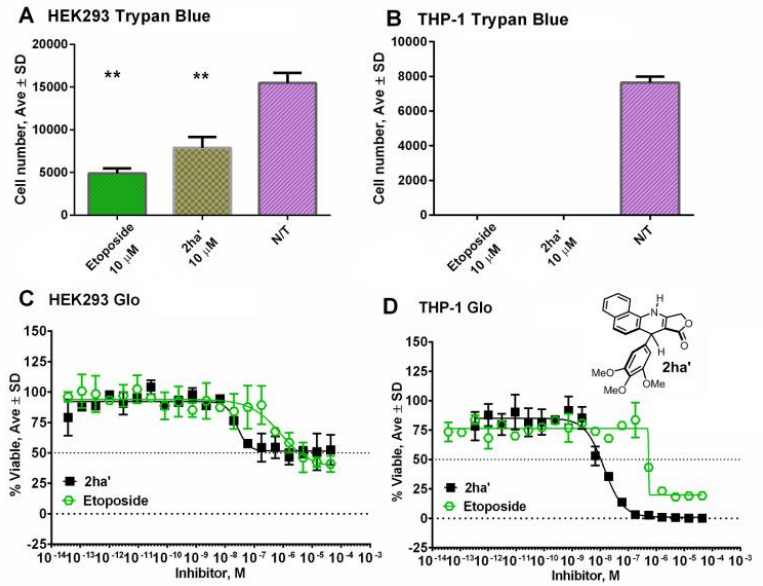
[a] ***Only novel analogues are presented above***.[12] Conditions. **Method A**: typical Husson-3CR with an equimolar ratio of tetronic acid **5**, aniline **3a-k** and benzaldehyde **4a'-h'** under Ar was stirred at 120 °C in 2-pentanol (1 mL) for 1 hour; **Method B** using the sacrificial aniline **3l**: aniline **3l** (1.0 equiv.) and benzaldehyde **4a'-h'** (1.0 equiv.) was stirred under Ar at 120 °C in 2-pentanol (1 mL) for 2 hours, followed by addition of tetronic acid **5** (1.0 equiv.) for 10 mins and finally aniline **3a-k** (1.0 equiv.), the resulting mixture was refluxed for an additional 30 mins. [b] Yields refer to pure products **2xy'** isolated after recrystallization of crude material in ethanol. [c] Compound **2ja'** was isolated as over-oxidized quinolines and reduced in a second step; overall yield for both steps is reported above. [d] The reported IC_{50} or EC_{50} values are against the human THP-1 leukemia cell line.

Table 1. Comparison of the Husson-3CR and modified-3CR



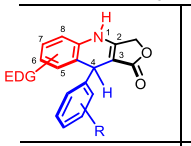
Compound	Yield (%)		Compound	Yield (%)	
	Method A	Method B		Method A	Method B
2bc'	7	21	**2fc'**	15	25
2ca'	5	14	**2fd'**	NR	5
2cc'	NR	3	**2fh'**	NR	5
2ch'	NR	4	**2ga'**	NR	4
2dc'	18	49	**2gc'**	NR	10
2dh'	12	39	**2gd'**	NR	6

Figure 1. Anti-leukemic activity: Comparison of CellTiter Glo® and Trypan Blue viability assay using etoposide (**1**) and **2ha'**.[a]

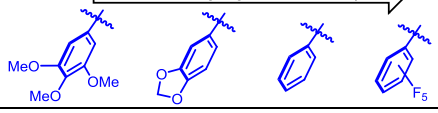


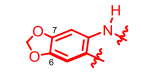
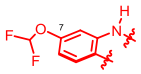
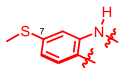
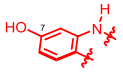
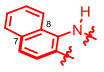
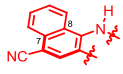
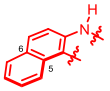
[a] **Panel A**: HEK293 in Trypan Blue assay; **Panel B**: THP-1 in Trypan Blue assay; **Panel C:** HEK293 in CellTiter Glo® assay; **Panel D:** THP-1 in CellTiter Glo® assay.

Table 2. SAR study [a,b,c]



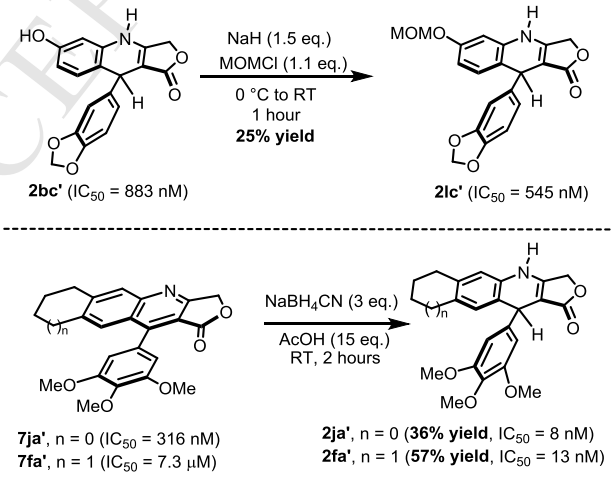
E-ring — *decreasing ring electronic density*



	B-ring				
1		**2aa'** IC_{50} = 13 nM[a] EC_{50} = 22 nM[b] EC_{50} > 100 μM[c]	**2ac'** IC_{50} = 49 nM[a] EC_{50} = 180 nM[b] EC_{50} > 50 μM[c]	**2ah'** IC_{50} = 103 nM[a] EC_{50} = 191 nM[b] EC_{50} = 133 nM[c]	**2ad'** IC_{50} = 269 nM[a] EC_{50} = 1 μM[b] EC_{50} > 100 μM[c]
2		**2ca'** IC_{50} = 9 nM[a] EC_{50} = 24 nM[b] EC_{50} > 100 μM[c]	**2cc'** EC_{50} = 17 nM[a] EC_{50} = 88 nM[b] EC_{50} > 50 μM[c]	**2ch'** EC_{50} = 17 nM[a] EC_{50} = 40 nM[b] EC_{50} > 50 μM[c]	**2cd'** EC_{50} = 202 nM[a] EC_{50} = 549 nM[b] EC_{50} > 100 μM[c]
3		**2da'** IC_{50} = 19 nM[a] EC_{50} = 66 nM[b] EC_{50} > 100 μM[c]	**2dc'** IC_{50} = 37 nM[a] EC_{50} = 57 nM[b] EC_{50} > 100 μM[c]	**2dh'** IC_{50} = 1 μM[a] EC_{50} = 2 μM[b] EC_{50} > 100 μM[c]	**2dd'** IC_{50} = 470 nM[a] EC_{50} = 745 nM[b] EC_{50} > 100 μM[c]
4		**2ba'** IC_{50} = 123 nM[a] EC_{50} = 473 nM[b] EC_{50} > 100 μM[c]	**2bc'** EC_{50} = 883 nM[a] EC_{50} > 100 μM[b] EC_{50} > 100 μM[c]	---	---
5		**2ea'** EC_{50} > 100 μM[a] EC_{50} > 100 μM[b] EC_{50} > 100 μM[c]	---	---	---
6		**2ha'** IC_{50} = 13 nM[a] EC_{50} = 60 nM[b] EC_{50} > 50 μM[c]	**2hc'** IC_{50} = 11 nM[a] EC_{50} = 17 nM[b] EC_{50} > 100 μM[c]	**2hh'** IC_{50} = 249 nM[a] EC_{50} = 222 nM[b] EC_{50} > 100 μM[c]	**2hd'** IC_{50} = 17 nM[a] EC_{50} = 24 nM[b] EC_{50} > 500 μM[c]
7		**2ga'** IC_{50} = 41 nM[a] EC_{50} = 71 nM[b] EC_{50} > 100 μM[c]	**2gc'** EC_{50} = 95 nM[a] EC_{50} = 213 nM[b] EC_{50} > 100 μM[c]	**2gh'** IC_{50} = 122 nM[a] EC_{50} = 121 nM[b] EC_{50} > 100 μM[c]	**2gd'** IC_{50} = 247 nM[a] EC_{50} = 968 nM[b] EC_{50} = 3 μM[c]
8		**2ia'** IC_{50} = 631 nM[a] EC_{50} = 4.5 μM[b] EC_{50} > 100 μM[c]	**2ic'** EC_{50} = 107 nM[a] EC_{50} = 1.6 μM[b] EC_{50} > 50 μM[c]	**2ih'** IC_{50} = 58 nM[a] EC_{50} = 144 nM[b] EC_{50} > 50 μM[c]	**2id'** IC_{50} = 10.2 μM[a] EC_{50} > 50 μM[b] EC_{50} > 50 μM[c]

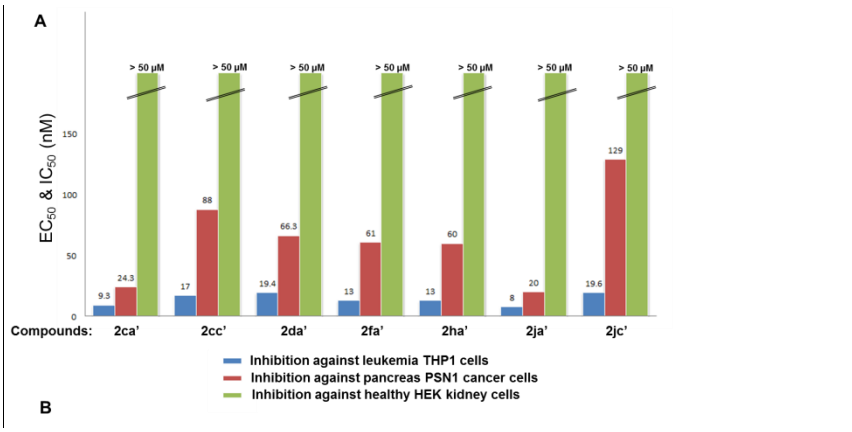
The reported IC_{50} or EC_{50} values are against: [a] the human THP-1 leukemia cell line, [b] the pancreas cancer PSN-1 cell line and [c] the non-malignant kidney HEK293 cell line.

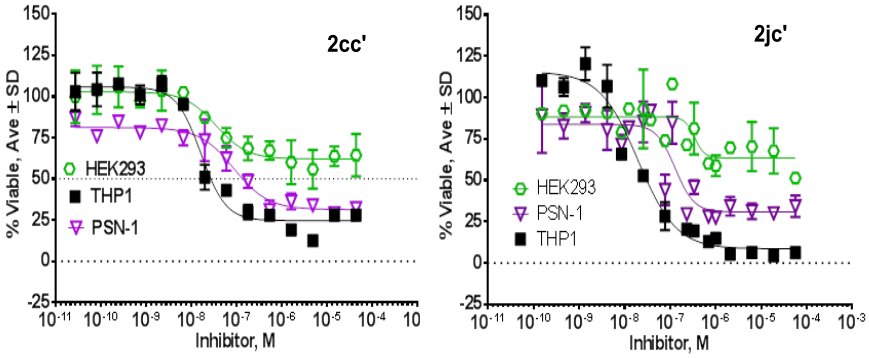
Scheme 3. Modulations of the APTs pharmacophores [a]



[a] The reported IC_{50} values are against the human THP-1 leukemia cell line.

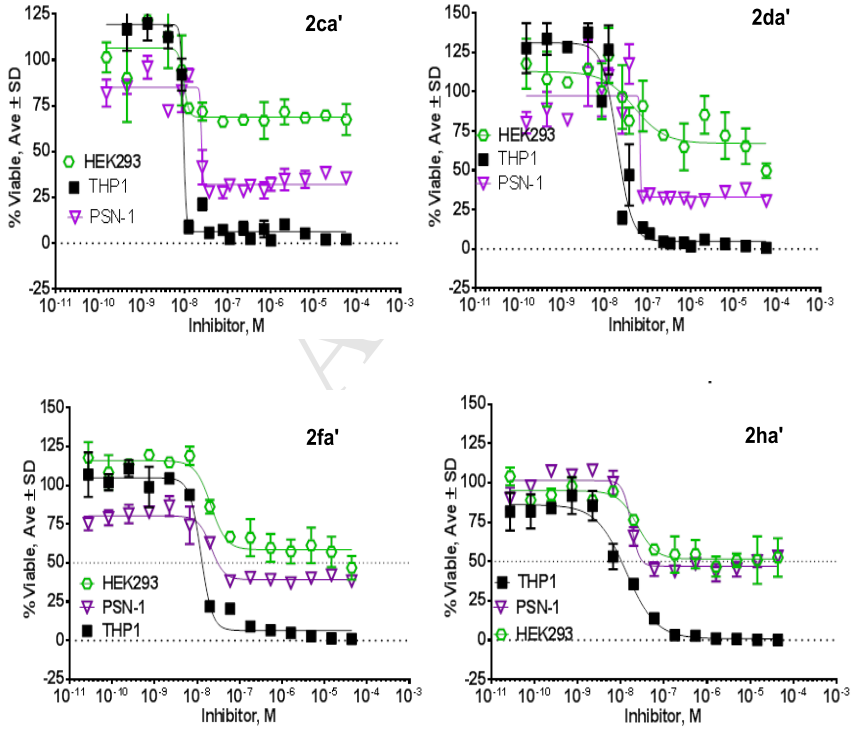
Figure 2. Lead compounds having high potency and selectivity against THP-1 leukemia cells.[a,b]



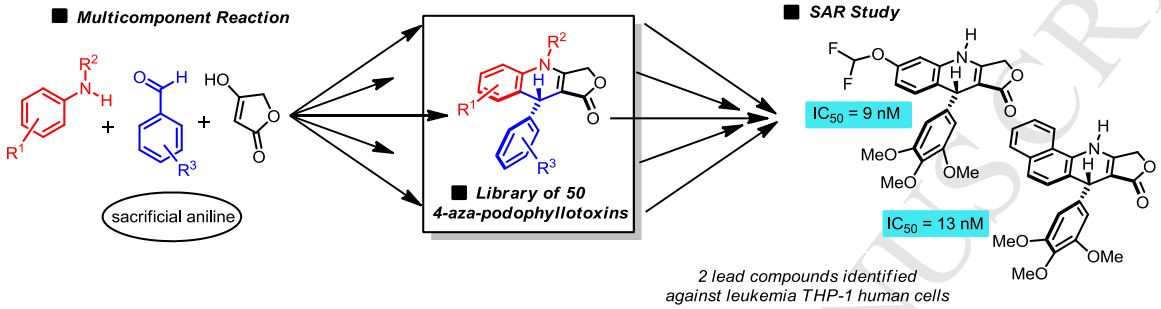


[a] EC_{50} values are reported for HEK293 and PSN-1 cells. **Panel A:** selectivity chart for the 7 best APT compounds of the library. [b] **Panel B:** Compound comparison showing high selectivity and potency.

Figure 3. Best potencies against THP-1 leukemia cells for the APTs **2ca'**, **2da'**, **2fa'** and **2ha'**



Graphical abstract



Multicomponent Assembly of 4-Aza-podophyllotoxins: A Fast Entry to Highly Selective and Potent Anti-Leukemic Agents

Nagalakshmi Jeedimalla, Madison Flint, Lyndsay Smith, Alberto Haces, Dmitriy Minond* and Stéphane P. Roche*

Table of Contents

I. I. GENERAL INFORMATION

A. *Instrumentation and Methods*

Reactions were performed in flame-dried glassware under a positive pressure of argon. Yields refer to chromatographically and spectroscopically pure compounds.

Analytical TLC was performed on 0.25 mm glass backed 60Å F-254 TLC plates (Silicycle, Inc.). The plates were visualized by exposure to UV light (254 nm) and developed by a solution of cerium-ammonium-molybdate in water/sulfuric acid and heat. Flash chromatography was performed using 200-400 mesh silica gels (Silicycle, Inc.).

Infrared spectra were recorded on a Nicolet IS5 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Mercury400 (400 MHz) spectrometer and are reported in ppm using solvent as an internal standard (DMSO-d_6 at 2.50 ppm). NMR spectra were performed using standard parameter and data are reported as: (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz, integration). ^{13}C NMR spectra were recorded on Varian Mercury400 (100 MHz) spectrometer. Chemical shifts are reported in ppm, with solvent resonance employed as the internal standard (DMSO-d_6 at 39.5 ppm). Melting points were determined using Digimelt digital melting point apparatus. The low resolution mass spectra were performed on Agilent 1200 series HPLC system/6120 single quadrupole MSD (electrospray ionization; ESI) with dual detector (PDA and ELSD). A purity of at least 95% was obtained for all the compounds by means of chromatography, crystallization, or recrystallization. This level of purity was established by LC/MS on a Agilent 1200 series HPLC based on the ELSD and UV chromatograms (λ= 360 nm) using a linear gradient, water + 0.1% formic acid and MeCN + 0.1% formic acid, at 60:40 (0 min) to 0:100 (20 min) and a flow rate of 0.8 mL/min; Retention times are reported for the lead compounds (R_t). Accurate mass (High resolution HR-MS) was obtained from University of Florida using Agilent 6210 TOF instrument.

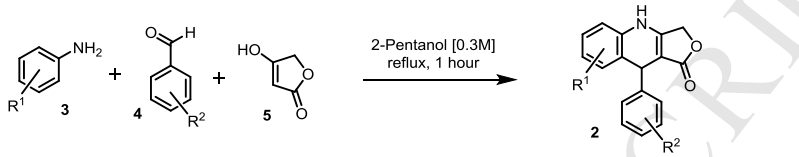
B. *Reagents and Solvents*

All reagents used in the present paper were acquired from Alfa Aesar or Sigma Aldrich. 2-pentanol was purchased from Sigma Aldrich.

II. Experimental procedures and compound characterization

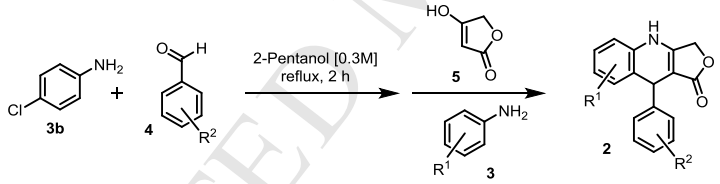
A. General Procedure A for the one pot synthesis of 4-aza-podophyllotoxins.

A round bottom flask was charged under argon with aniline **3** (1.0 equiv.), aldehyde **4** (1.0 equiv.), and tetronic acid **5** (1.0 equiv.) in 2-pentanol or ethanol [0.3 M]. Reaction was refluxed (at 125 °C or 80 °C respectively) for 1 hour, then the solvent was evaporated under vacuum and the crude product purified by recrystallization in ethanol or by flash chromatography on silica gel.



B. General Procedure B for the sequential one pot synthesis of 4-aza-podophyllotoxins.

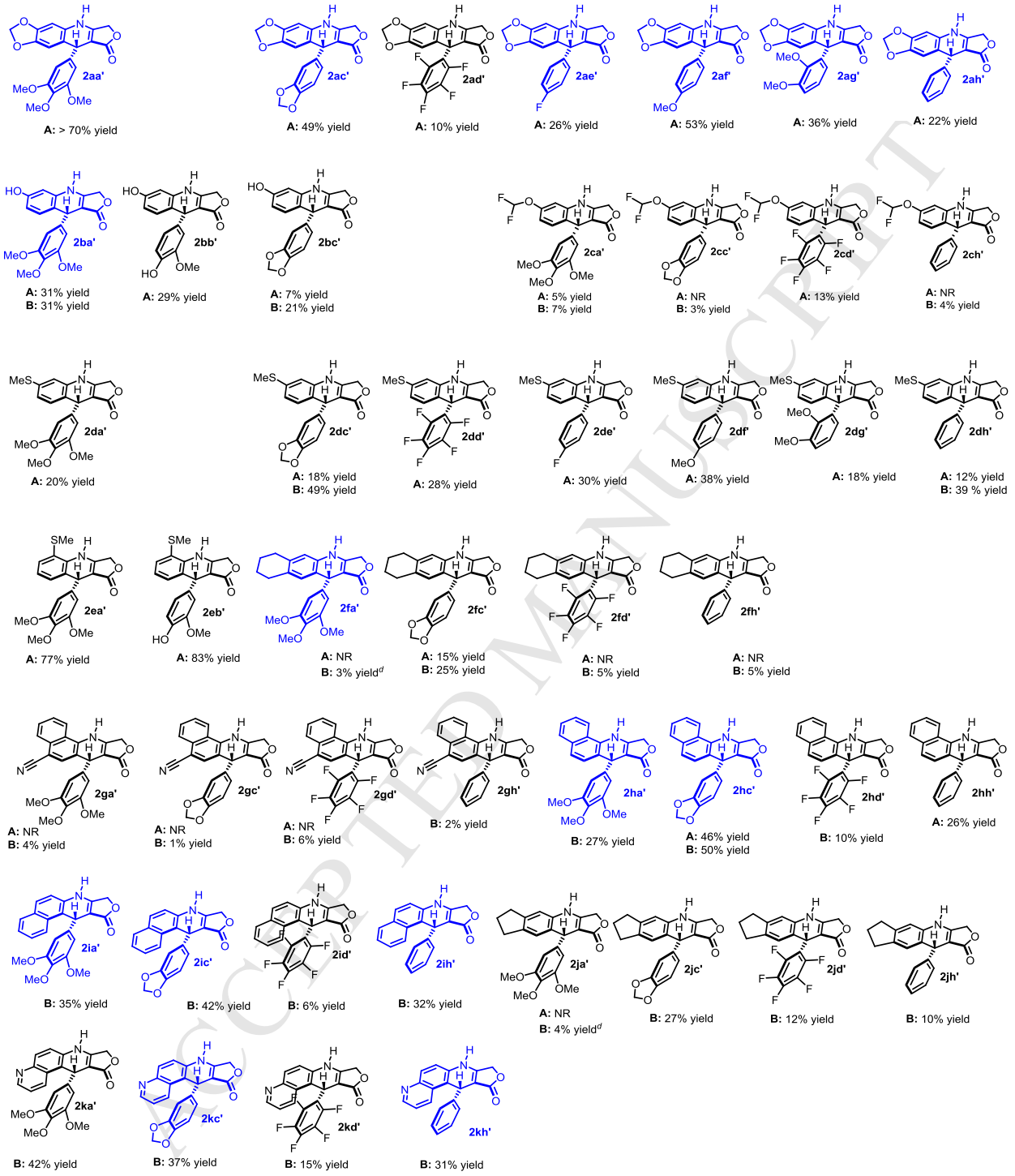
A two neck round bottom flask under argon equipped with a condenser was charged with aldehyde **4** (1.0 equiv.) and 4-chloroaniline **3b** (1.1 equiv.) in 2-pentanol [0.3 M] and stirred at reflux for two hours. The tetronic acid **5** (1.1 equiv.) in 2-pentanol (minimum amount) was then added at reflux. After another 10 mins at reflux, the third component aniline **3** (1.0 equiv.) was added neat. The reaction mixture was refluxed for an additional 30 mins. All volatiles were evaporated under vacuum and the crude product purified by recrystallization in ethanol or by flash chromatography on silica gel.



Compounds **2aa'**,[SI-1a-f] **2ac'**, [SI-1b] **2ae'**, [SI-1c] **2af'**, [SI-1b-d] **2ag'**,[SI-1d] **2ah'**,[SI-1a, b, d] **2ba'**,[SI-1f, SI-2] **2fa'**, [SI-1b, SI-3] **2ha'**,[SI-3] **2hc'**,[SI-1c] **2ia'**,[SI-1f, SI-3] **2ic'**,[SI-1b, SI-4] **2ih'**,[SI-4] **2kc'**,[SI-5] and **2kh'**[SI-5] were previously reported by others. Only biological activity was reported for Compound **2ja'**[SI-6]. Compounds **2bc'**, **2ca'**, **2dc'**, **2dh'** and **2fc'** were reported by us with ^{1}H, ^{13}C NMRs, IR, melting points and HR-MS. [SI-2]

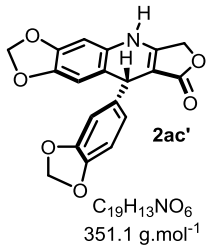
[SI-1] a) Aillerie, A.; Talancé, V. L. d.; Moncomble, A.; Bousquet, T.; Pélinski, L. *Org. Lett.* **2014**, *16*, 2982-2985; b) Semenova, M. N.; Kiselyov, A. S.; Tsyganov, D. V.; Konyushkin, L. D.; Firgang, S. I.; Semenov, R. V.; Malyshev, O. R.; Raihstat, M. M.; Fuchs, F.; Stielow, A.; Lantow, M.; Philchenkov, A. A.; Zavelevich, M. P.; Zefirov, N. S.; Kuznetsov, S. A.; Semenov, V. V. *J. Med. Chem.* **2011**, *54*, 7138-7149; c) Shi, C.; Wang, J.; Chen, H.; Shi, D. *J. Comb. Chem.* **2010**, *12*, 430-434; d) Frackenpohl, J.; Adelt, I.; Antonicek, H.; Arnold, C.; Behrmann, P.; Blaha, N.; Böhmer, J.; Gutbrod, O.; Hanke, R.; Hohmann, S.; Houtdreve, M. v.; Lösel, P.; Malsam, O.; Melchers, M.; Neufert, V.; Peschel, E.; Reckmann, U.; Schenke, T.; Thiesen, H.-P.; Velten, R.; Vogelsang, K.; Weiss, H.-C. *Bioorg. Med. Chem.* **2009**, *17*, 4160-4184; e) Giorgi-Renault. S. *Ann. Pharm. Fr.* **2005**, *63*, 63-68; f) Tratrat, C.; Giorgi-Renault, S.; Husson, H.-P. *Org. Lett.* **2002**, *4*, 3187-3189.
[SI-2]Jeedimalla, N.; Johns, J.; Roche, S. P. *Tetrahedron Lett.* **2013**, *54*, 5845-5848.
[SI-3]Magedov, I. V.; Frolova, L.; Manpadi, M.; Bhoga, U. d.; Tang, H.; Evdokimov, N. M.; George, O.; Hadje Georgiou, K.; Renner, S.; Getlik, M. u.; Kinnibrugh, T. L.; Fernandes, M. A.; Van slambrouck, S.; Steelant, W. F. A.; Shuster, C. B.; Rogelj, S.; van Otterlo, W. A. L.; Kornienko, A. *J. Med. Chem.* **2011**, *54*, 4234-4246.
[SI-4]Kozlov, N. G.; Bondarev, S. L.; Kadutskii, A. P.; Basalaeva, L. I.; Pashkovskii, F. S. *Russ. J. Org. Chem.* **2008,** *44*, 1031-1037.
[SI-5]Shi, F.; Zhou, D.; Tu, S.; Shao, Q.; Li, C.; Cao, L. *J. heterocyc. Chem*. **2008**, *45*, 1065-1070.
[SI-6]Hitotsuyanagi, Y.; Fukuyo, M.; Tsuda, K.; Kobayashi, M.; Ozeki, A.; Itokawa, H.; Takeya, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 315-317.

Scheme SI-1. Synthesis of a library of 50 compounds: 4-aza-2,3didehydropodophyllotoxins (APT) **2**[a-c]



[a]Note. Compounds presented in blue have been reported previously and compounds in black have ben synthesized for the first time in our library. [b] Conditions. **Method A**: *typical Husson-3CR* with an equimolar ratio of tetronic acid **5**, aniline **3a-k** and benzaldehyde **4a'-h'**under N_2 was stirred at 120 ^oC in 2-pentanol (1 mL) for 1 hour; **Method B** *using the sacrificial aniline* **3l***:* aniline **3l** (1.0 equiv.) and benzaldehyde **4a'-h'** (1.0 equiv.) was stirred under N_2 at 120 ^oC in 2-pentanol (1 mL) for 2 hours, followed by addition of tetronic acid **5** (1.0 equiv.) for 10 mins and finally aniline **3a-k** (1.0 equiv.), the resulting mixture was refluxed for an additional 30 mins. [c] Yields refer to pure products **2xy'** isolated after recrystallization of crude material in ethanol. [d] Compounds **2fa'** and **2ja'** were isolated as over-oxidized quinolines and reduced in a second step; overall yields for both steps are reported above.

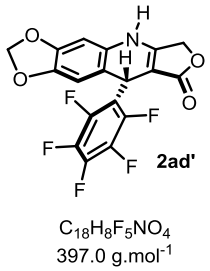
9-(benzo[d][1,3]dioxol-5-yl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (2ac'). Compound



2ac' was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 1,3-benzodioxole-5-carbaldehyde **4c'** (150 mg, 1.0 mmol, 1.0 equiv.), 3,4-(methylenedioxy)aniline **3a** (137 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was filtered and washed with cold ethanol (3x 2.5 mL) to obtain compound **2ac'** in a pure form (171 mg, 0.5 mmol, 49% yield). Light brown solid; $\mathbf{R}_f$ = 0.29 (acetone/hexanes; 35/65); **m.p.** > 260 °C; **IR** νmax (neat): 3224, 3095, 1712, 1642, 1562, 1481, 1248, 1235, 1038, 1016, 933, 780 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.87 (bs, 1H), 6.83 – 6.75 (m, 1H), 6.73 (s, 1H), 6.65 (dd, *J* = 7.7, 1.5 Hz, 1H), 6.59 (s, 1H), 6.51 (s, 1H), 5.97 – 5.87 (m, 4H), 4.97 (d, *J* = 15.8 Hz, 1H), 4.83 (s, 1H), 4.83 (d, *J* = 15.8 Hz, 1H); **LC-MS (ESI)**: *m/z* 374.0 (M+Na);

SMILES: O=C(OC1)C2=C1NC3=CC(OCO4)=C4C=C3C2C5=CC(OCO6)=C6C=C5

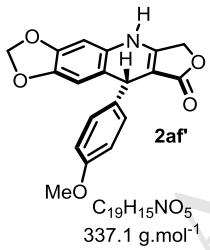
9-(perfluorophenyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (2ad'). Compound **2ad'** was



prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using pentafluorobenzaldehyde **4d'** (490 mg, 2.5 mmol, 1.0 equiv.), 3,4-(methylenedioxy)aniline **3a** (343 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The crude product was filtered and washed with cold ethanol (3x 2.5 mL) to obtain compound **2ad'** (99 mg, 0.3 mmol, 10% yield). White solid; $\mathbf{R}_f$ = 0.30 (acetone/hexanes; 35/65); **m.p.** >260 °C; **IR** νmax (neat): 3230, 1727, 1650, 1498, 1481, 1190, 1040, 1020, 990, 942, 835, 761 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.12 (bs, 1H), 6.54 (s, 1H), 6.51 (s, 1H), 5.96 (s, 1H), 5.93 (s, 1H), 5.43 (s, 1H), 4.90 (d, *J* = 16.0 Hz, 1H), 4.85 (d, *J* = 16.0 Hz, 1H); **LC-MS (ESI)**: *m/z* 398.0 (M+H), 420.0 (M+Na);

SMILES: O=C(OC1)C2=C1NC3=CC(OCO4)=C4C=C3C2C5=C(F)C(F)=C(F)C(F)=C5F

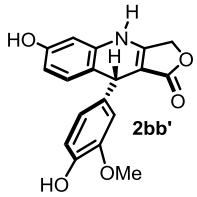
9-(4-methoxyphenyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (2af'). Compound **2af'** was



prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 4-methoxybenzaldehyde **4f'** (340 mg, 2.5 mmol, 1.0 equiv.), 3,4-(methylenedioxy)aniline **3a** (343 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The crude product was filtered and sonicated in dichloromethane (4 mL) for 15 minutes, followed by centrifugation at 5,800 rpm for 10 minutes (x3) to obtain pure compound **2af'** (444 mg, 1.3 mmol, 53% yield). Pale yellow solid; $\mathbf{R}_f$ = 0.19 (acetone/hexanes; 35/65); **m.p.** 136-137 °C; **IR** νmax (neat): 3222, 3157, 3092, 1711, 1641, 1560, 1502, 1478, 1192, 1016, 831 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.84 (bs, 1H), 7.09 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 6.54 (s, 1H), 6.51 (s, 1H), 5.95 (s, 1H), 5.89 (s, 1H), 4.92 (d, *J* = 15.5 Hz, 1H), 4.85 (s, 1H), 4.83 (d, *J* = 15.5 Hz, 1H), 3.69 (s, 3H); **LC-MS (ESI)**: *m/z* 338.0 (M+H);

SMILES: O=C(OC1)C2=C1NC3=CC(OCO4)=C4C=C3C2C5=CC=C(OC)C=C5

6-hydroxy-9-(4-hydroxy-3-methoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2bb'). Compound **2bb'**

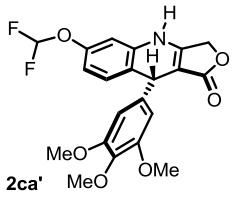


$C_{18}H_{15}NO_5$
325.1 g.mol^{-1}

was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-hydroxy-4-methoxybenzaldehyde **4b'** (381 mg, 2.5 mmol, 1.0 equiv.) 3-aminophenol **3b** (273 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The product was filtered off the crude reaction mixture and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2bb'** (235 mg, 0.72 mmol, 29% yield). Off-white solid; $\mathbf{R}_f$ = 0.24 (acetone/hexanes; 45/55); **m.p.** > 260 °C; **IR** νmax (neat): 3446, 3377, 3231, 1724, 1646, 1622, 1496, 1152, 1026, 1018, 770 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.84 (bs, 1H), 9.43 (s, 1H), 8.75 (s, 1H), 6.84 (d, *J* = 8.1 Hz, 1H), 6.79 (d, *J* = 1.6 Hz, 1H), 6.62 (d, *J* = 8.1 Hz, 1H), 6.46 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.33 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.32 (d, *J* = 1.6 Hz, 1H), 4.92 (d, *J* = 15.6 Hz, 1H), 4.82 (d, *J* = 15.6 Hz, 1H), 4.76 (s, 1H), 3.70 (s, 3H); **LC-MS (ESI)**: *m/z* 326.1 (M+H);

SMILES: O=C(OC1)C2=C1NC3=CC(O)=CC=C3C2C4=CC=C(OC)C(O)=C4

6-(difluoromethoxy)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one 2ca'. Compound

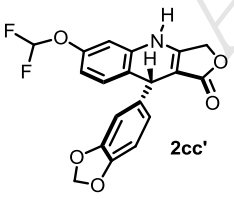


$C_{21}H_{19}F_2NO_6$
MW: 419.4 g.mol^{-1}

2ca' was prepared accordingly to the *general* ***procedure B*** *for the one pot sequential synthesis* using 4-chloroaniline **3l** (72 mg, 0.56 mmol, 1.1 equiv.), 3-difluoromethoxy aniline **3c** (81 mg, 0.51 mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (100 mg, 0.51 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.56 mmol, 1.1 equiv.), in 2-pentanol (1.7 mL + 0.4 mL). The crude product was purified by column chromatography using isocratic solvent system of 20% acetone in hexanes system to obtain pure compound **2ca'** (30 mg, 0.06 mmol, 14% yield). Compound **2ca'** was also prepared accordingly to the *general procedure A for the one pot synthesis* using the exact same quantities and was purified by column chromatography using isocratic solvent system of 20% acetone in hexanes system to obtain pure compound **2ca'** (5 mg, 0.01 mmol, 2% yield). White powder, $\boldsymbol{R}_f$ = 0.22 (Hexanes/acetone 65 / 35); **m.p.**>260 °C; **IR** νmax (neat): 3246, 1727, 1644, 1548, 1492, 1174 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO) δ (ppm):10.13 (s, 1H), 7.19 (s, 1H), 7.18 (t, *J* = 73.9 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.69 (s, 1H), 6.50 (s, 2H), 5.18 – 4.78 (m, 3H), 3.70 (s, 6H), 3.60 (s, 3H).**^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.9, 158.5, 152.8 (2C), 150.0, 142.4, 137.3, 136.1, 132.1, 121.3, 116.3 (t, *J* = 278.6 Hz), 113.2, 106.2, 104.9 (2C), 95.7, 65.1, 59.9, 55.8 (2C), 30.7; **LC-MS (ESI)**: R_t = 5.1 mins, *m/z* 420.0 (M+H); **HR-MS (ESI)**: *m/z* Calcd for $[C_{21}H_{19}F_2NO_6+H]^+$ 420.1253, Found 420.1250 (- 0.7 ppm).

SMILES:COC1=C(OC)C(OC)=CC(C2C(C(OC3)=O)=C3NC4=C2C=CC(OC(F)F)=C4)=C1.

9-(benzo[d][1,3]dioxol-5-yl)-6-(difluoromethoxy)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2cc'). Compound



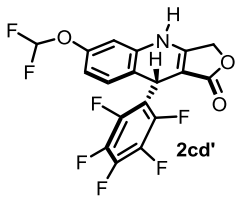
$C_{19}H_{13}F_2NO_5$
MW: 373.1 g.mol^{-1}

2cc' was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 1,3-benzodioxole-5-carbaldehyde **4c'** (150 mg, 1.0 mmol, 1.0 equiv.), 3-(difluoromethoxy) benzamine **3c** (159 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The oily crude product was taken in methanol (2.5 mL) and the precipitate was filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2cc'** (11 mg, 0.03 mmol, 3% yield). Off-white crystals; $\mathbf{R}_f$ = 0.41 (acetone/hexanes; 40/60); **m.p.** > 260 °C; **IR** νmax (neat): 3322,

1728, 1641, 1611, 1482, 1109, 1040, 1011, 798 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.15 (bs, 1H), 7.17 (t, *J* = 74.0 Hz, 1H), 7.09 (d, *J* = 8.5 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 1.6 Hz, 1H), 6.72 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.69 – 6.67 (m, 2H), 5.94 (d, 2.0 Hz, 2H), 4.93 (s, 1H), 4.93 (d, 15.4 Hz, 1H), 4.87 (d, 15.4 Hz, 1H); **LC-MS (ESI)**: R_t = 7.3 mins, *m/z* 374.0 (M+H), 396.0 (M+Na); **HR-MS (ESI)**: *m/z* Calcd for $[C_{19}H_{13}F_2NO_5+H]^+$ 374.0840, Found 374.0850 (+ 2.7 ppm).

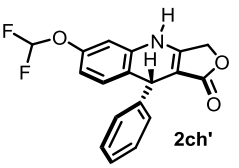
SMILES: O=C(OC1)C2=C1NC(C=C(OC(F)F)C=C3)=C3C2C4=CC(OCO5)=C5C=C4

6-(difluoromethoxy)-9-(perfluorophenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2cd'). Compound **2cd'** was prepared accordingly to the *general **procedure A** for the one pot synthesis* using 3-(difluoromethoxy) benzamine **3c** (159 mg, 1.0 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was filtered and washed with cold ethanol (3x 2.5 mL) to obtain compound **2cd'** (56 mg, 0.13 mmol, 13% yield). White solid; **R_f** = 0.29 (acetone/hexanes; 30/70); **m.p.** > 260 °C; **IR** νmax (neat): 1726, 1644, 1495, 1190, 1170, 1021, 991, 762 cm^{-1};**^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.38 (bs, 1H), 7.23 (t, *J* = 73.7 Hz, 1H), 7.06 (d, *J* = 8.3, 1H), 6.75 (dd, *J* = 8.3, 1.7 Hz, 1H), 6.71 (d, *J* = 2.0 Hz, 1H), 5.56 (s, 1H), 4.96 (d, *J* = 16.1, 1H), 4.90 (d, *J* = 16.1, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.4, 159.1, 150.7, 138.1 (2C), 131.9 (3C), 117.7, 116.2 (t, *J* = 258.6 Hz), 113.4 (3C), 106.3 (2C), 92.2, 65.4, 28.7; **LC-MS (ESI)**: *m/z* 420.0 (M+H), 442.0 (M+Na); **SMILES**: FC(F)OC1=CC=C(C(C2=C(F)C(F)=C(F)C(F)=C2F)C(C(OC3)=O)=C3N4)C4=C1



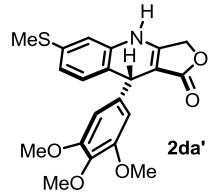
$C_{18}H_8F_7NO_3$
MW: 419.0 g.mol^{-1}

6-(difluoromethoxy)-9-phenyl-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2ch'). Compound **2ch'** was prepared accordingly to the *general **procedure B** for the sequential one pot synthesis* using 4-chloroaniline **3l** (140 mg, 1.1 mmol, 1.1 equiv.), benzaldehyde **4h'** (106 mg, 1.0 mmol, 1.0 equiv.), 3-(difluoromethoxy) benzamine **3c** (159 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The oily crude product was taken in methanol (2.5 mL) and the precipitate was filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ch'** (12 mg, 0.4 mmol, 4% yield). Metallic white crystals; **R_f** =0.35 (70/30 acetone/hexanes); **m.p.** > 260 °C; **IR** νmax (neat): 3231, 1723, 1636, 1617, 1495, 1118, 1012, 746, 696 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.16 (bs, 1H), 7.18 (t, *J* = 74.0 Hz, 1H), 7.28-7.07 (m, 6H), 6.73-6.69 (m, 2H), 5.01 (s, 1H), 5.00 (d, *J* = 15.8 Hz, 1H), 4.89 (d, *J* = 15.8 Hz, 1H); **LC-MS (ESI)**: *m/z* 368.0 (M+K); **SMILES**: FC(F)OC(C=C1)=CC2=C1C(C(C(OC3)=O)=C3N2)C4=CC=CC=C4



$C_{18}H_{13}F_2NO_3$
MW: 329.3 g.mol^{-1}

6-(methylthio)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2da'). Compound **2da'** was prepared accordingly to the *general **procedure A** for the one pot synthesis* using 3-methylthioaniline **3d** (138 mg, 1.0 mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2da'** (78 mg, 0.2 mmol, 20% yield). White

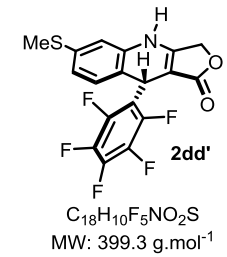


$C_{21}H_{21}NO_5S$
MW: 399.1 g.mol^{-1}

crystals; $\mathbf{R_f}$ = 0.25 (acetone/hexanes; 35/65); **m.p.** > 260 °C; **IR** νmax (neat): 3249, 2359, 2341, 1725, 1635, 1483, 1128, 1008, 750 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.00 (bs, 1H), 7.07 (d, *J* = 8.1 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.77 (s, 1H), 6.49 (s, 2H), 5.02 (d, *J* = 15.7 Hz, 1H), 4.91 (s, 1H), 4.87 (d, *J* = 15.7 Hz, 1H), 3.70 (s, 6H), 3.60 (s, 3H), 2.42 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm) 172.0, 158.7, 152.8 (2C), 142.6, 137.3, 136.5, 136.1, 131.1, 121.1, 120.7, 112.9, 104.9 (2C), 95.4, 65.1, 59.9, 55.9 (2C), 39.3, 14.6; **LC-MS (ESI)**: R_t = 6.5 mins, *m/z* 400.0 (M+1), 422.0 (M+Na); **HR-MS (ESI)**: *m/z* Calcd for $[C_{21}H_{21}NO_5S+H]^+$ 400.1219, Found 400.1225 (+ 2.4 ppm).

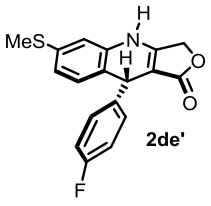
SMILES: O=C(OC1)C2=C1NC3=CC(SC)=CC=C3C2C4=CC(OC)=C(OC)C(OC)=C4

6-(methylthio)-9-(perfluorophenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2dd'). Compound **2dd'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-methylthioaniline **3d** (138 mg, 1.0 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2dd'** (112 mg, 0.28 mmol, 28% yield). Off-white solid; $\mathbf{R_f}$ = 0.30 (acetone/hexanes; 30/70); **m.p.** > 260 °C; **IR** νmax (neat): 3213, 1719, 1637, 1518, 1499, 1486, 1211, 1022, 992, 955, 916 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.26 (bs, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.82 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.77 (d, *J* = 1.7 Hz, 1H), 5.51 (s, 1H), 4.96 (d, *J* = 16.0 Hz, 1H), 4.91 (d, *J* = 16.0 Hz, 1H), 2.44 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.5, 159.3 (2C), 138.7 (2C), 137.2 (2C), 130.6 (2C), 120.8 (2C), 117.3, 112.8 (2C), 91.7, 65.4, 28.8, 14.4; **LC-MS (ESI):** *m/z* 422.0 (M+Na). **SMILES**: CSC1=CC=C(C(C2=C(F)C(F)=C(F)C(F)=C2F)C(C(OC3)=O)=C3N4)C4=C1



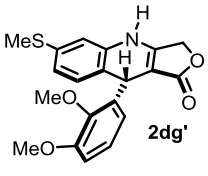
$C_{18}H_{10}F_5NO_2S$
MW: 399.3 g.mol^{-1}

9-(4-fluorophenyl)-6-(methylthio)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2de'). Compound **2de'** was prepared accordingly to the *general procedure A for the one pot synthesis* using 3-methylthioaniline **3d** (138 mg, 1.0 mmol, 1.0 equiv.), 4-fluorobenzaldehyde **4e'** (124 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2de'** (98 mg, 0.3 mmol, 30% yield). White solid; $\mathbf{R_f}$ = 0.29 (acetone/hexanes; 30/70); **m.p.** > 260 °C; **IR** νmax (neat): 3240, 1711, 1639, 1606, 1532, 1486, 1211, 1022, 920, 851 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.10 (bs, 1H), 7.22 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.07 (t, *J* = 8.8 Hz, 2H), 6.95 (d, *J* = 8.0 Hz, 1H), 6.80 (dd, *J* = 10.1, 1.7 Hz, 2H), 5.00 (s, 1H),4.97 (d, *J* = 15.8 Hz, 1H), 4.87 (d, *J* = 15.8 Hz, 1H), 2.42 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.1, 160.9 (d, *J* = 240.0 Hz,), 158.6, 143.1 (d, *J* = 3.0 Hz), , 137.6, 136.7, 131.3, 129.5 (d, *J* = 8.0 Hz, 2C), 121.1, 120.8, 115.2 (d, *J* = 21.0 Hz, 2C), 113.0, 95.6, 65.3, 38.3, 14.6; **LC-MS (ESI)**: *m/z* 328.0 (M+H), 350.0 (M+Na); **SMILES**: CSC1=CC=C(C(C2=CC=C(F)C=C2)C(C(OC3)=O)=C3N4)C4=C1



$C_{18}H_{14}FNO_2S$
MW: 327.1 g.mol^{-1}

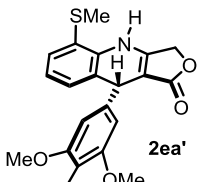
9-(2,3-dimethoxyphenyl)-6-(methylthio)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2dg'). Compound **2dg'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-methylthioaniline **3d** (123 µL, 1.0 mmol, 1.0 equiv.), 2,3-dimethoxybenzaldehyde **4g'** (166 mg, 1.0 mmol, 1.0 equiv.), and tetronic acid **5** (100 mg, 1.0 mmol, 1.0 equiv.) in 2-pentanol (3.3 mL). The crude product was purified by recrystallization in ethanol (2.5 mL), then filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2dg'**(66 mg, 0.2 mmol, 18% yield). White powder; $\mathbf{R}_f$ = 0.21 (acetone/hexanes; 30/70); **m.p**. > 260 °C; **IR** νmax (neat): 1721, 1636, 1526, 1483, 1351, 1225, 1078, 1011, 740 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.98 (bs, 1H), 6.94 (t, *J* = 7.9 Hz, 1H), 6.87 – 6.81 (m, 2H), 6.75 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.72 (d, *J* = 1.9 Hz, 1H), 6.64 (dd, *J* = 7.7, 1.1 Hz, 1H), 5.25 (s, 1H), 4.96 (d, *J* = 15.4 Hz, 1H), 4.87 (d, *J* = 15.4 Hz, 1H), 3.78 (s, 3H), 3.73 (s, 3H), 2.40 (s, 3H); **LC-MS (ESI)**: *m/z*: 370.0 (M+H), 392.0 (M+Na);



$C_{20}H_{19}NO_4S$
MW: 369.4 g.mol^{-1}

SMILES: CSC1=CC=C(C(C2=C(OC)C(OC)=CC=C2)C(C(OC3)=O)=C3N4)C4=C1

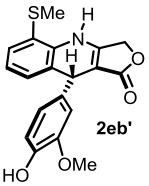
5-(methylthio)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2ea'). Compound **2ea'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3,4,5-trimethoxybenzaldehyde **4a'** (491 mg, 2.5 mmol, 1.0 equiv.), 2-methylthioaniline **3e** (348 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The crude product was purified by column chromatography on silica gel using an isocratic solvent system of acetone and dichloromethane (20:80) to obtain pure compound **2ea'** (771 mg, 1.93 mmol, 77% yield). Red solid; $\mathbf{R}_f$ = 0.29 (acetone/hexanes; 45/55); **m.p.** > 260 °C; **IR** νmax (neat): 2929, 1734, 1669, 1589, 1455, 1418, 1230, 1121, 1005, 770 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.09 (d, *J* = 2.1 Hz, 1H), 6.86 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.60 (d, *J* = 8.3 Hz, 1H) 6.59 (s, 2H), 4.86 (s, 1H), 4.67 (s, 2H), 3.67 (s, 6H), 3.62 (s, 3H), 2.24 (s, 3H); **LC-MS (ESI)**: *m/z* 418.1 (M+H_2O+H);



$C_{21}H_{21}NO_5S$
399.5 g.mol^{-1}

SMILES: O=C(OC1)C2=C1NC3=C(SC)C=CC=C3C2C4=CC(OC)=C(OC)C(OC)=C4

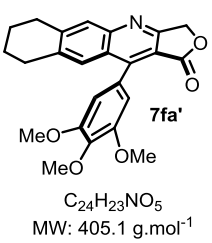
9-(4-hydroxy-3-methoxyphenyl)-5-(methylthio)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2eb'). Compound **2eb'** was prepared accordingly to the *general* ***procedure A*** *for the one pot synthesis* using 3-hydroxy-4-methoxybenzaldehyde **4b'** (380 mg, 2.5 mmol, 1.0 equiv.), 2-methylthioaniline **3e** (348 mg, 2.5 mmol, 1.0 equiv.) and tetronic acid **5** (250 mg, 2.5 mmol, 1.0 equiv.) in ethanol (10 mL). The crude product was purified by column chromatography on silica gel using an isocratic solvent system of acetone and dichloromethane (30:70) to obtain pure compound **2eb'** (733 mg, 2.1 mmol, 83% yield). Deep red solid; $\mathbf{R}_f$ = 0.26 (acetone/hexanes; 45/55); **m.p.** > 260 °C; **IR** νmax (neat): 2926, 1609, 1512, 1427, 1268, 1229, 1124, 1031, 779, 687 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 8.75 (bs, 1H), 7.06 (d, *J* = 2.0 Hz, 1H), 6.85 – 6.81 (m, 2H), 6.68 – 6.56 (m, 3H), 5.07 (bs, 1H), 4.82 (s, 1H), 4.64 (s, 2H), 3.65 (s, 3H), 2.23 (s, 3H); **LC-MS (ESI)**: *m/z* 356.2 (M+H);



$C_{19}H_{17}NO_4S$
355.1 g.mol^{-1}

SMILES: O=C(OC1)C2=C1NC3=C(SC)C=CC=C3C2C4=CC=C(OC)C(O)=C4

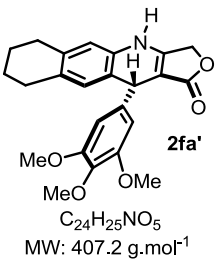
11-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydrobenzo[g]furo[3,4-b]quinolin-1(3H)-one (7fa'). Compound **7fa'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (140 mg, 1.1 mmol, 1.1 equiv.), 5,6,7,8-tetrahydronaphthalen-2-amine **3f** (147 mg, 1.0 mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was taken in methanol (2.5 mL) and the precipitate was filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **7fa'** (25 mg, 0.06 mmol, 6% yield). White solid; $\mathbf{R}_f$ = 0.38 (ethyl acetate/hexanes; 1:1); **m.p.** > 260 °C; **IR** νmax (neat): 2936, 1768, 1599, 1579, 1445, 1412, 1121, 1039, 1029, 706, 687 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.89 (bs, 1H), 7.60 (s, 1H), 6.77 (s, 2H), 5.46 (s, 2H), 3.79 (s, 3H), 3.77 (s, 6H), 3.03 (t, *J* = 5.2 Hz, 2H), 2.90 (t, *J* = 5.3 Hz, 2H), 1.84 – 1.75 (m, 4H); **LC-MS (ESI)**: *m/z* 406.0 (M+H);



$C_{24}H_{23}NO_5$
MW: 405.1 g.mol^{-1}

SMILES: O=C1C2=C(C3=CC(OC)=C(OC)C(OC)=C3)C4=CC5=C(CCCC5)C=C4N=C2CO1

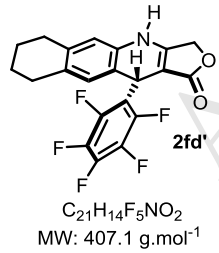
11-(3,4,5-trimethoxyphenyl)-4,6,7,8,9,11-hexahydrobenzo[g]furo[3,4-b]quinolin-1(3H)-one (2fa'). To a suspension of compound **7fa'** (21 mg, 0.05 mmol, 1.0 equiv.) in glacial acetic acid (0.6 mL, 15.0 equiv.) was added with sodium cyanoborohydride (10 mg, 0.2 mmol, 3.0 equiv.) in one portion at room temperature and the reaction mixture was stirred for 4 hours. The reaction mixture was poured into an ice cold water (2 mL) and the newly formed precipitate was filtered and finally washed with cold ethanol (2x 1 mL) to obtain pure compound **2fa'** (12 mg, 0.03 mmol, 57% yield). White powder; $\mathbf{R}_f$ = 0.3 (hexanes/acetone 40 / 60); **m.p.** > 260 °C; **IR** νmax (neat): 1734, 1658, 1320, 1223, 1011, 750 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.85 (bs, 1H), 6.81 (s, 1H), 6.59 (s, 1H), 6.47 (s, 2H), 4.99 (d, *J* = 15.7 Hz, 1H), 4.85 (d, *J* = 15.8 Hz, 1H), 4.85 (s, 1H), 3.70 (d, *J* = 1.3 Hz, 6H), 3.60 (d, *J* = 1.5 Hz, 3H), 2.64 – 2.62 (m, 2H), 2.54 - 2.50 (m, 2H), 1.65-1.62 (m, 4H); **LC-MS (ESI)**: R_t = 7.9 mins, *m/z* 408.0 (M+H); **HR-MS (ESI)**: *m/z* Calcd for $[C_{24}H_{25}NO_5+H]^+$ 408.1811, Found 408.1795 (- 1.5 ppm). Other spectral data match with the previous report from literature.



$C_{24}H_{25}NO_5$
MW: 407.2 g.mol^{-1}

SMILES: O=C(OC1)C2=C1NC3=CC(CCCC4)=C4C=C3C2C5=CC(OC)=C(OC)C(OC)=C5

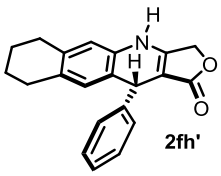
11-(perfluorophenyl)-4,6,7,8,9,11-hexahydrobenzo[g]furo[3,4-b]quinolin-1(3H)-one (2fd'). Compound **2fd'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 5,6,7,8-tetrahydro-2-naphthylamine **3f** (75 mg, 0.5 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2fd'** (12 mg, 0.03 mmol, 5% yield). Off-white solid; $\mathbf{R}_f$ = 0.35 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1724, 1638, 1467, 1021, 987, 946 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.12 (bs, 1H), 6.65 (s, 1H), 6.60 (s, 1H), 5.43 (s, 1H), 4.91 (d, *J* = 15.8 Hz, 1H), 4.87 (d, *J* = 15.8 Hz, 1H), 2.61 - 2.65 (m, 2H), 2.50 – 2.53 (m, 2H), 1.62 – 1.67 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.7, 159.3, 137.0 (2C), 134.1, 132.2



$C_{21}H_{14}F_5NO_2$
MW: 407.1 g.mol^{-1}

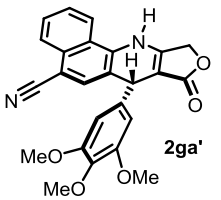
(2C), 130.1 (3C), 118.2, 116.1 (3C), 90.8, 65.3, 28.9 28.5, 28.1, 22.7, 22.6; **LC-MS (ESI)**: *m/z* 408.0 (M+H), 430.0 (M+Na); **SMILES**: O=C(OC1)C2=C1NC3=CC(CCCC4)=C4C=C3C2C5=C(F)C(F)=C(F)C(F)=C5F

11-phenyl-4,6,7,8,9,11-hexahydrobenzo[g]furo[3,4-b]quinolin-1(3H)-one (2fh'). Compound **2fh'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 5,6,7,8-tetrahydro-2-naphthylamine **3f** (147 mg, 1.0 mmol, 1.0 equiv.), benzaldehyde **4h'** (106 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2fh'** (16 mg, 0.05 mmol, 5% yield). Light brown solid; $\mathbf{R_f}$ = 0.41 (acetone/hexanes; 35/65); **m.p.** >260 °C; **IR** νmax (neat): 1754, 1593, 1487, 1429, 1143, 1040, 1028, 699 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.88 (bs, 1H), 7.25 (t, *J* = 7.6 Hz, 2H), 7.20 – 7.10 (m, 3H), 6.70 (s, 1H), 6.60 (s, 1H), 4.95 (d, *J* =16.1 Hz, 1H), 4.90 (s, 1H), 4.85 (d, *J* = 16.1 Hz, 1H), 3.38 (s, 2H), 2.63 (s, 2H), 1.65 (s, 4H). **LC-MS (ESI)**: *m/z* 318.0 (M+H); **SMILES**: O=C(OC1)C2=C1NC3=CC(CCCC4)=C4C=C3C2C5=CC=CC=C5



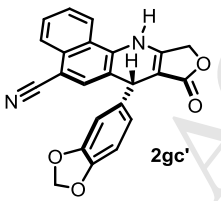
$C_{21}H_{19}NO_2$
MW: 317.1 g.mol^{-1}

8-oxo-7-(3,4,5-trimethoxyphenyl)-7,8,10,11-tetrahydrobenzo[h]furo[3,4-b]quinoline-5-carbonitrile (2ga'). Compound **2ga'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.), 4-amino-1-naphthonitrile **3g** (168 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ga'** (17 mg, 0.04 mmol, 4% yield). Pale yellow solid; $\mathbf{R_f}$ = 0.29 (acetone/hexanes; 40/60); **m.p.** >260 °C; **IR** νmax (neat): 3367, 1741, 1676, 1595, 1456, 1331, 1118, 1029, 998, 749 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.68 (bs, 1H), 8.40 – 8.35 (m, 1H), 8.14 – 8.01 (m, 3H), 7.95 (s, 1H), 7.81 (dd, *J* = 6.3, 3.3 Hz, 2H), 5.69 (s, 1H), 5.18 (d, *J* = 16.2 Hz, 1H), 5.13 (d, *J* = 16.2 Hz, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.71 (s, 3H); **LC-MS (ESI)**: *m/z* 451.0 (M+Na); **SMILES**: N#CC1=C2C(C=CC=C2)=C3C(C(C4=CC(OC)=C(OC)C(OC)=C4)C(C(OC5)=O)=C5N3)=C1



$C_{25}H_{20}N_2O_5$
MW: 428.1 g.mol^{-1}

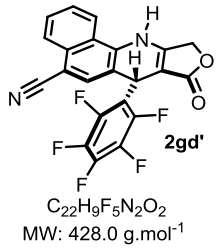
7-(benzo[d][1,3]dioxol-5-yl)-8-oxo-7,8,10,11-tetrahydrobenzo[h]furo[3,4-b]quinoline-5-carbonitrile (2gc'). Compound **2gc'** was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 1,3-benzodioxole-5-carbaldehyde **4c'** (150 mg, 1.0 mmol, 1.0 equiv.), 4-amino-1-naphthonitrile **3g** (168 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2gc'** (50 mg, 0.13 mmol, 10% yield). Light brown solid; **m.p.** >260 °C; **IR** νmax (neat): 2256, 1714, 1611, 1383, 1329, 1027, 920, cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.68 (bs, 1H), 8.37 (dd, *J* = 6.4, 3.2 Hz, 1H), 8.03 (dd, *J* = 6.7, 3.1 Hz, 1H), 7.88 (s, 1H), 7.81 (dd, *J* = 4.5, 1.8 Hz, 2H), 6.88 (s, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.76 (dd, *J* = 8.0, 1.8 Hz, 1H), 5.95 (d, *J* = 6.2 Hz,



$C_{23}H_{14}N_2O_4$
MW: 382.1 g.mol^{-1}

2H), 5.16 (s, 1H), 5.14 (d, *J* = 15.5 Hz, 1H), 5.01 (d, *J* = 15.5 Hz, 1H); **LC-MS (ESI; negative mode)**: *m/z* 381.0 (M-H). **SMILES**: N#CC1=C2C(C=CC=C2)=C3C(C(C4=CC(OCO5)=C5C=C4)C(C(OC6)=O)=C6N3)=C1

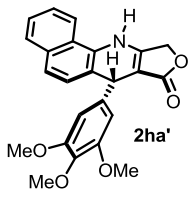
8-oxo-7-(perfluorophenyl)-7,8,10,11-tetrahydrobenzo[h]furo[3,4-b]quinoline-5-carbonitrile (2gd'). Compound



$C_{22}H_9F_5N_2O_2$
MW: 428.0 g.mol^{-1}

2gd' was prepared accordingly to the *general **procedure B** for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 4-amino-1-naphthalenecarbonitrile **3g** (86 mg, 0.5 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'** (100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2gd'** (14 mg, 0.03 mmol, 6% yield). White solid; $\mathbf{R_f}$ = 0.30 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1592, 1444, 1364, 1205, 983, 764, 703, 685 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.90 (bs, 1H), 8.37 (d, *J* = 7.2 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.91 – 7.77 (m, 2H), 7.76 (s, 1H), 5.71 (s, 1H), 5.14 (d, *J* = 15.5 Hz, 1H), 4.87 (d, *J* = 15.5 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm):171.1, 158.9, 137.0 (2C), 135.3, 131.8, 129.6 (2C), 128.0 (2C), 124.9, 122.2 (2C), 121.9 (2C), 117.6, 115.4, 103.1 (2C), 94.8, 66.2, 29.5; **LC-MS (ESI)**: *m/z* 429.1 (M+H);
SMILES: O=C(OC1)C2=C1NC3=C(C=CC=C4)C4=C(C#N)C=C3C2C5=C(F)C(F)=C(F)C(F)=C5F

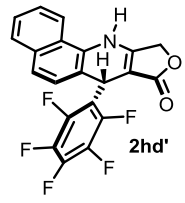
7-(3,4,5-trimethoxyphenyl)-7,11-dihydrobenzo[h]furo[3,4-b]quinolin-8(10H)-one (2ha'). Compound **2ha'** was



$C_{24}H_{21}NO_5$
MW: 403.4 g.mol^{-1}

prepared accordingly to the *general **procedure B** for the one pot sequential synthesis* using 4-chloroaniline **3l** (128 mg, 1.1 mmol, 1.1 equiv.), 4-amino-1-naphthalenecarbonitrile **3h** (143 mg, 1.0 mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (100 mg, 1.1 mmol, 1.1 equiv.), in 2-pentanol (3.30 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ha'** (110 mg, 0.27 mmol, 27% yield). White powder; **IR** νmax (neat): 1721, 1657, 1535, 1125, 1021, 996, 763 cm^{-1}; $\mathbf{R_f}$ = 0.21 (hexanes/ethyl acetate 40 / 60); **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.23 (bs, 1H), 8.21 (d, *J* = 8.6 Hz, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.66 – 7.52 (m, 2H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 6.55 (s, 2H), 5.14 (s, 1H), 5.12 (d, *J* = 16.0 Hz, 1H), 4.99 (d, *J* = 16.0 Hz, 1H), 3.68 (s, 6H), 3.59 (s, 3H). **LC-MS (ESI)**: R_t = 7.7 mins, *m/z* 404.0 (M+H); **HR-MS (ESI)**: *m/z* Calcd for $[C_{24}H_{21}NO_5+H]^+$ 404.1498, Found 404.1495 (- 0.5 ppm). Other spectral data match with the previous report from literature. [SIx] **SMILES**: O=C1OCC2=C1[C@H](C3=CC(OC)=C(OC)C(OC)=C3)C(C=CC4=C5C=CC=C4)=C5N2

7-(perfluorophenyl)-7,11-dihydrobenzo[h]furo[3,4-b]quinolin-8(10H)-one (2hd'). Compound **2hd'** was



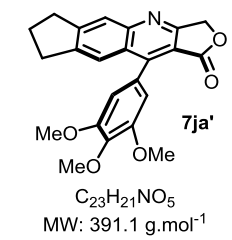
$C_{21}H_{10}F_5NO_2$
MW: 403.1 g.mol^{-1}

prepared accordingly to the *general **procedure B** for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 4-amino-1-naphthalenecarbonitrile **3h** (73 mg, 0.5 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'**(100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2hd'** (21 mg, 0.05 mmol, 10%

yield). Light brown solid; $\mathbf{R_f}$ = 0.28 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1729, 1644, 1499, 1107, 992, 951, 757 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.47 (bs, 1H), 8.21 (d, *J* = 8.6 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 5.75 (s, 1H), 5.06 (d, *J* = 15.5 Hz, 1H), 5.01 (d, *J* = 15.5 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.6, 160.0, 133.0 (2C), 131.9, 128.4(3C), 127.4, 126.7,126.6 (2C), 123.5, 122.4 (2C), 120.9 (2C), 115.4, 92.6, 65.9, 30.1; **LC-MS (ESI)**: *m/z* 426.0 (M+Na);

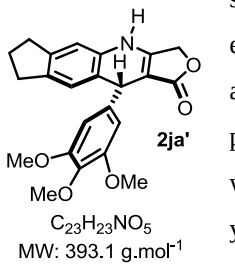
SMILES: O=C(OC1)C2=C1NC3=C(C=CC=C4)C4=CC=C3C2C5=C(F)C(F)=C(F)C(F)=C5F

10-(3,4,5-trimethoxyphenyl)-3,6,7,8-tetrahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (7ja'). Compound **7ja'** was prepared accordingly to the *general **procedure B** for the sequential one pot synthesis* using 4-chloroaniline **3l** (140 mg, 1.1 mmol, 1.1 equiv.), 5-aminoindane **3j** (133 mg, 1.0mmol, 1.0 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **7ja'** (47.1 mg, 0.1 mmol, 12% yield). White solid; $\mathbf{R_f}$ = 0.3 (ethyl acetate/hexanes; 1:1); **m.p.** >260 °C; **IR** νmax (neat): 2960, 1764, 1579, 1440, 1455, 1248, 1119, 1027, 1006, 718, 690 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm**):** 7.98 (s, 1H), 7.68 (s, 1H), 6.77 (s, 2H), 5.46 (s, 2H), 3.80 (s, 3H), 3.77 (s, 6H), 3.12 (t, *J* = 7.3 Hz, 2H), 3.02 (t, *J* = 7.3 Hz, 2H), 2.10 (p, *J* = 7.4 Hz, 2H); **LC-MS (ESI)**: *m/z* 392.0 (M+H); **SMILES**: O=C1C2=C(C3=CC(OC)=C(OC)C(OC)=C3)C4=CC5=C(CCC5)C=C4N=C2CO1



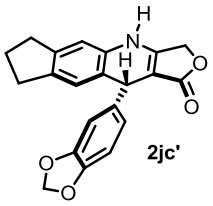
$C_{23}H_{21}NO_5$
MW: 391.1 g.mol^{-1}

10-(3,4,5-trimethoxyphenyl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (2ja'). To a suspension of compound **7ja'** (25 mg, 0.1 mmol, 1.0 equiv.) in glacial acetic acid (0.7 mL, 15.0 equiv.) was added with sodium cyanoborohydride (11 mg, 0.2 mmol, 3.0 equiv.) in one portion at room temperature and the reaction mixture was stirred for 4 hours. The reaction mixture was poured into an ice cold water (2 mL) and the newly formed precipitate was filtered and finally washed with cold ethanol (2x 1 mL) to obtain pure compound **2ja'** (9 mg, 0.02 mmol, 36% yield). White powder; $\mathbf{R_f}$ = 0.3 (hexanes/acetone 40 / 60); **m.p.** >260 °C; **IR** νmax (neat): 1742, 1657, 1479, 1324, 1223, 1140, 1011, 748, 676 cm^{-1};**^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.87 (s, 1H), 6.97 (s, 1H), 6.78 (s, 1H), 6.49 (s, 2H), 5.00 (d, *J* = 15.9 Hz, 1H), 4.90 (s, 1H), 4.86 (d, *J* = 15.9 Hz, 1H), 3.70 (s, 6H), 3.60 (s, 3H), 2.77 (t, *J* = 6.8 Hz, 2H), 2.70 – 2.68 (m, 2H), 2.04 – 1.86 (m, 2H). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.2, 158.7, 152.8 (2C), 143.2, 143.1 138.6, 136.0, 134.3, 125.9, 122.3, 111.9, 104.9 (2C), 94.7, 65.0, 59.9, 55.8 (2C), 39.9, 32.0, 31.7, 25.2; **LC-MS (ESI)**: R_t = 7.0 mins, *m/z* 394.0 (M+1), 416.0 (M+Na); **HR-MS (ESI)**: *m/z* Calcd for $[C_{23}H_{23}NO_5+H]^+$ 394.1654, Found 394.1685 (+ 7.9 ppm). **SMILES**: O=C(OC1)C2=C1NC3=CC(CCC4)=C4C=C3C2C5=CC(OC)=C(OC)C(OC)=C5



$C_{23}H_{23}NO_5$
MW: 393.1 g.mol^{-1}

(R)-10-(benzo[d][1,3]dioxol-5-yl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (2jc').

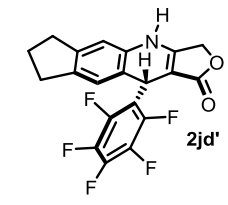


$C_{21}H_{17}NO_4$
MW: 347.1 g.mol^{-1}

Compound **2jc'** was prepared accordingly to the *general **procedure B** for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 1,3-benzodioxole-5-carbaldehyde **4c'** (150 mg, 1.0 mmol, 1.0 equiv.), 5-aminoindan **3j** (133 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was then filtered and rinsed with ethanol (3 x 2.5 mL) to obtain pure compound **2jc'** (93 mg, 0.3 mmol, 27% yield). Pale yellow solid; $\mathbf{R_f}$ = 0.21 (acetone/hexanes; 35/65); **m.p.** >260 °C; **IR** νmax (neat): 3259, 3177, 3120, 1712, 1635, 1621, 1541, 1484, 1228, 1199, 1034, 1015 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.89 (bs, 1H), 6.88 (s, 1H), 6.76 (s, 1H), 6.75 (d, *J* = 29.2 Hz, 2H), 6.65 (d, *J* = 8.9 Hz, 1H), 5.93 (d, *J* = 2.9 Hz, 2H), 4.90 (d, *J* = 15.6 Hz, 1H), 4.87 (s, 1H), 4.84 (d, *J* = 15.6 Hz, 1H), 2.76 (s, 2H), 2.68 (d, *J* = 7.2 Hz, 2H), 2.05 – 1.76 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.3, 158.6, 147.3, 145.6, 143.3, 141.7, 138.8, 134.4, 126.0, 122.6, 120.5, 112.0, 108.2 (2C), 108.0, 100.9, 94.9, 65.1, 32.1, 31.7, 25.3; **LC-MS (ESI)**: R_t = 8.9 mins, *m/z* 348.0 (M+H), 370.0 (M+Na); **HR-MS (ESI)**: *m/z* Calcd for $[C_{21}H_{17}NO_4+H]^+$ 348.1236, Found 348.1244 (+ 2.3 ppm).

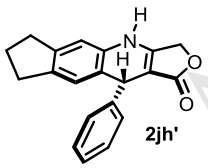
SMILES: O=C(OC1)C2=C1NC(C=C(CCC3)C3=C4)=C4C2C5=CC=C6C(OCO6)=C5

10-(perfluorophenyl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (2jd'). Compound **2jd'** was prepared accordingly to the *general **procedure B** for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 5-aminoindan **3j** (68 mg, 0.5 mmol, 1.0 equiv.), Pentafluorobenzaldehyde **4d'** (100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2jd'** (23 mg, 0.02 mmol, 12% yield). Off-White solid; $\mathbf{R_f}$ = 0.34 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1728, 1642, 1499, 1328, 1190, 1107, 991, 951, 776, 756 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.15 (bs, 1H), 6.82 (s, 1H), 6.79 (s, 1H), 5.50 (s, 1H), 4.89 (d, *J* = 15.6 Hz, 1H), 4.84 (d, *J* = 15.6 Hz, 1H), 2.80 - 2.70 (m, 2H),2.68 - 2.62 (m, 2H), 2.02 – 1.87 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 171.7, 159.4, 144.5 (2C), 139.3 (2C), 135.0, 125.4 (3C), 118.6, 112.2 (3C), 90.8, 65.3, 32.1, 31.6, 29.4, 25.2; **LC-MS (ESI)**: *m/z* 394.1 (M+H); **SMILES**: FC(C(F)=C(F)C(F)=C1F)=C1C2C3=CC4=C(CCC4)C=C3NC5=C2C(OC5)=O



$C_{20}H_{12}F_5NO_2$
MW: 393.1 g.mol^{-1}

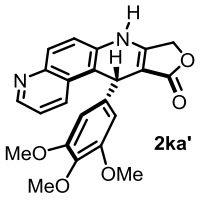
10-phenyl-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (2jh'). Compound **2jh'** was prepared accordingly to the *general **procedure B** for the sequential one pot synthesis using* 4-chloroaniline **3l** (140 mg, 1.1 mmol, 1.1 equiv.), benzaldehyde **4h'** (106 mg, 1.0 mmol, 1.0 equiv.), 5-aminoindan **3j** (133 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1 mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2jh'** (30 mg, 0.1 mmol, 10% yield). Off-white solid; $\mathbf{R_f}$ = 0.31 (acetone/hexanes; 35/65); **m.p.** >260 °C; **IR** νmax (neat): 3253, 3176, 3118, 1711, 1635, 1624, 1542, 1019, 701 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.92 (bs, 1H), 7.64 – 7.43 (m, 3H), 7.31 – 7.08 (m, 3H), 6.82 (d, *J* = 35.1 Hz, 1H), 5.49 (s, 1H), 4.96 (d, *J* = 15.6 Hz,



$C_{20}H_{17}NO_2$
MW: 303.1 g.mol^{-1}

1H), 4.86 (d, *J* = 15.6 Hz, 1H), 3.14 – 2.96 (m, 2H), 2.78 – 2.63 (m, 2H), 2.20 – 1.88 (m, 2H); **LC-MS (ESI)**: *m/z* 304.1 (M+H), 326.1 (M+Na); **SMILES**: O=C(OC1)C2=C1NC(C=C(CCC3)C3=C4)=C4C2C5=CC=CC=C5

11-(3,4,5-trimethoxyphenyl)-8,11-dihydrofuro[3,4-b][4,7]phenanthrolin-10(7H)-one (2ka'). Compound **2ka'**

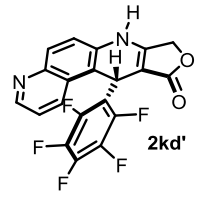


$C_{23}H_{20}N_2O_5$
MW: 404.4 g.mol^{-1}

was prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis using* 4-chloroaniline **3l** (140mg, 1.1 mmol, 1.1 equiv.), 3,4,5-trimethoxybenzaldehyde **4a'** (196 mg, 1.0 mmol, 1.0 equiv.), 6-aminoquinoline **3k** (144 mg, 1.0 mmol, 1.0 equiv.) and tetronic acid **5** (110 mg, 1.1mmol, 1.1 equiv.) in 2-pentanol (3.3 mL). The crude product was recrystallized in ethanol (2.5 mL), then filtered and washed with cold ethanol (3x 2.5 mL) to obtain pure compound **2ka'** (168 mg, 0.4 mmol, 42% yield). Yellow solid; $\mathbf{R_f}$ = 0.19 (acetone/hexanes; 40/60); **m.p.** >260 °C; **IR** νmax (neat): 3182, 1721, 1643, 1541, 1322, 1228, 1205, 1113, 999, 819 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.46 (bs, 1H), 8.68 (d, *J* = 4.0 Hz, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 7.95 (d, *J* = 9.0 Hz, 1H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.39 (dd, *J* = 8.6, 4.2 Hz, 1H), 6.49 (s, 2H), 5.67 (s, 1H), 5.00 (d, *J* = 15.8 Hz, 1H), 4.91 (d, *J* = 15.8 Hz, 1H), 3.60 (s, 6H), 3.54 (s, 3H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.1, 157.3, 152.8 (2C), 148.0, 145.7, 141.4, 135.9, 135.2, 131.6, 130.0, 127.2, 121.9, 121.2, 114.8, 105.1 (2C), 97.0, 65.2, 59.9, 55.8 (2C), 36.7; **LC-MS (ESI)**: *m/z* 405.1 (M+H);

SMILES: O=C(OC1)C2=C1NC(C=CC3=C4C=CC=N3)=C4C2C5=CC(OC)=C(OC)C(OC)=C5

11-(perfluorophenyl)-8,11-dihydrofuro[3,4-b][4,7]phenanthrolin-10(7H)-one (2kd'). Compound **2kd'** was

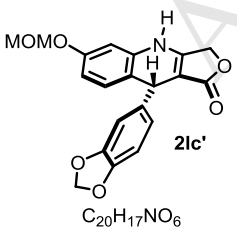


$C_{20}H_9F_5N_2O_2$
MW: 404.3 g.mol^{-1}

prepared accordingly to the *general* ***procedure B*** *for the sequential one pot synthesis* using 4-chloroaniline **3l** (72 mg, 0.6 mmol, 1.1 equiv.), 4-amino-1-naphthalenecarbonitrile **3k** (74 mg, 0.5 mmol, 1.0 equiv.), pentafluorobenzaldehyde **4d'**(100 mg, 0.5 mmol, 1.0 equiv.) and tetronic acid **5** (56 mg, 0.6 mmol, 1.1 equiv.) in 2-pentanol (1.7 mL). The crude product was filtered and washed with cold ethanol (3 x 2.5 mL) to obtain pure compound **2kd'** (30 mg, 0.03 mmol, 15% yield). Off-White solid; $\mathbf{R_f}$ = 0.25 (acetone/hexanes; 30/70); **m.p.** >260 °C; **IR** νmax (neat): 1746, 1500, 1209, 1021, 1007, 990, 832 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 10.73 (bs, 1H), 8.73 (d, *J* = 4.2 Hz, 1H), 7.96 (d, *J* = 9.1, 1H), 7.95 (d, *J* = 9.1, 1H), 7.52 - 7.50 (m, 2H), 6.14 (s, 1H), 5.06 (d, *J* = 15.8 Hz, 1H), 5.00 (d, *J* = 15.8 Hz, 1H); **LC-MS (ESI)**: *m/z* 405.0 (M+H);

SMILES: O=C(OC1)C2=C1NC3=CC=C(N=CC=C4)C4=C3C2C5=C(F)C(F)=C(F)C(F)=C5F

9-(benzo[d][1,3]dioxol-5-yl)-6-(methoxymethoxy)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (2lc'). To a flame

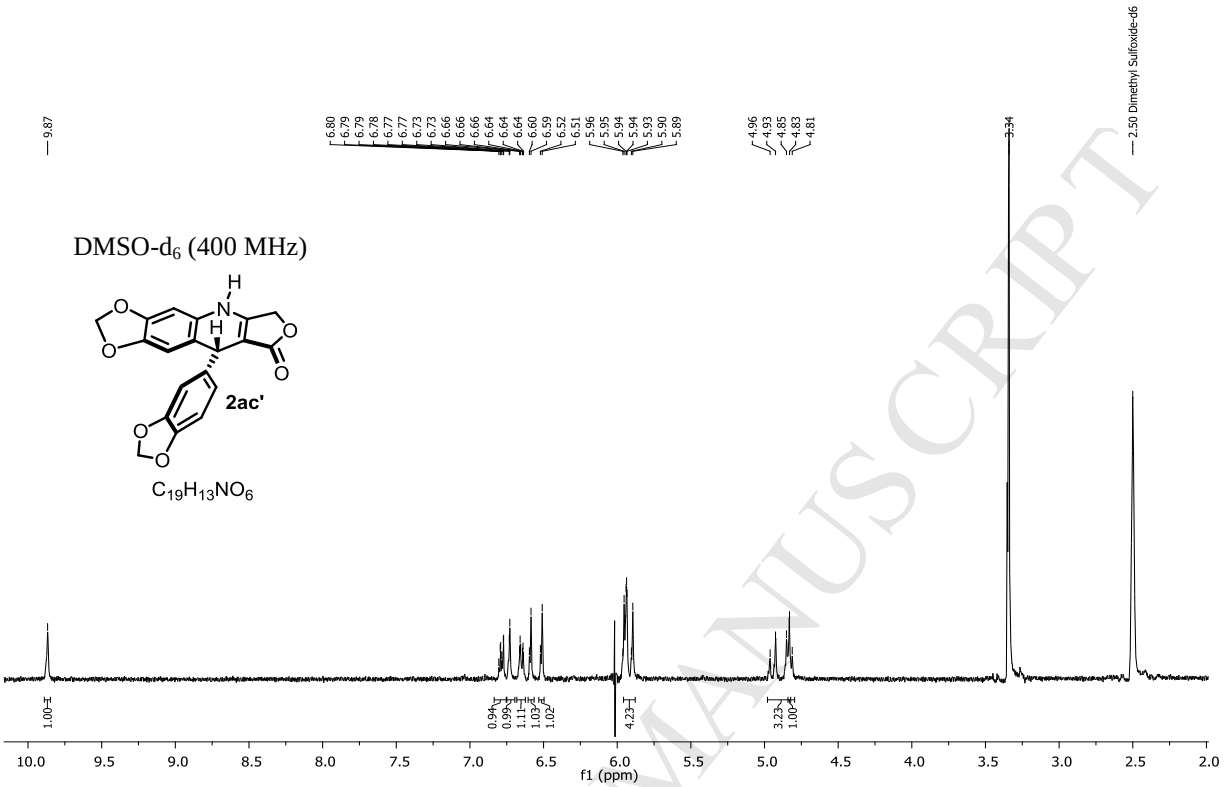


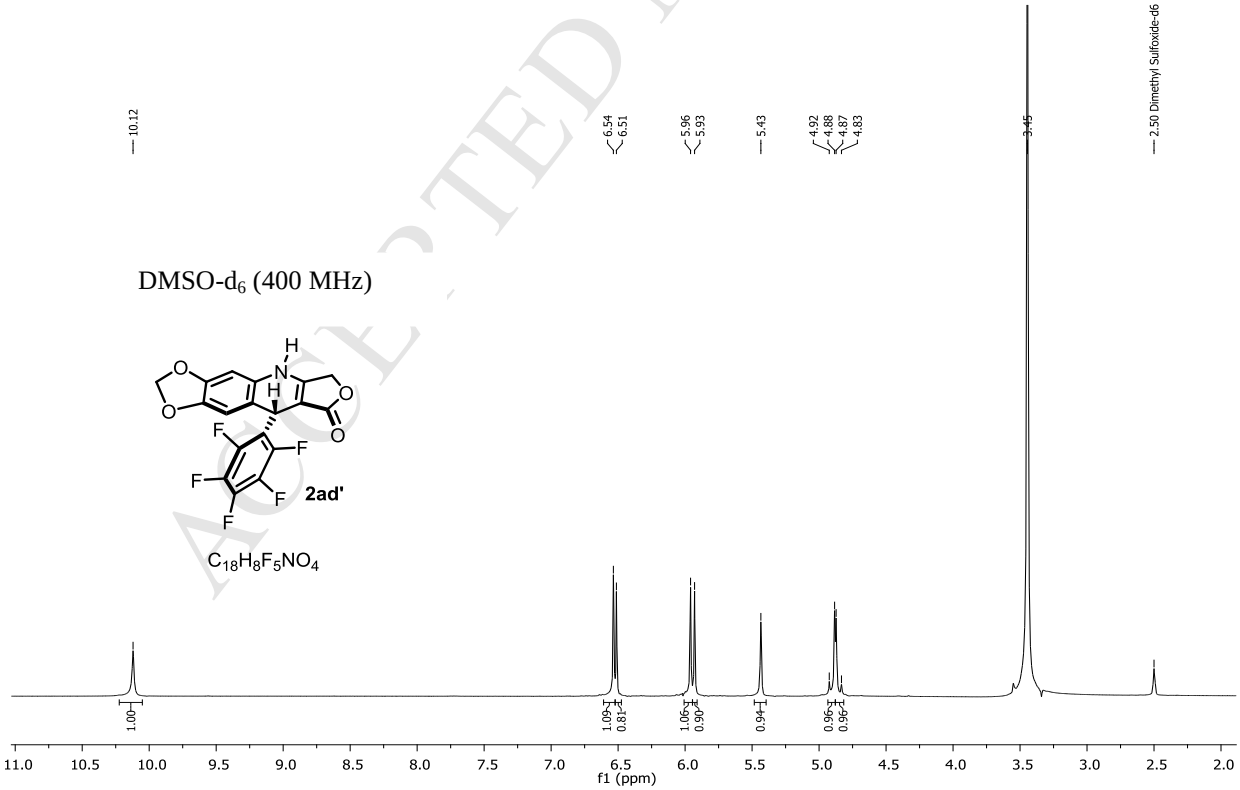
$C_{20}H_{17}NO_6$
MW: 367.1 g.mol^{-1}

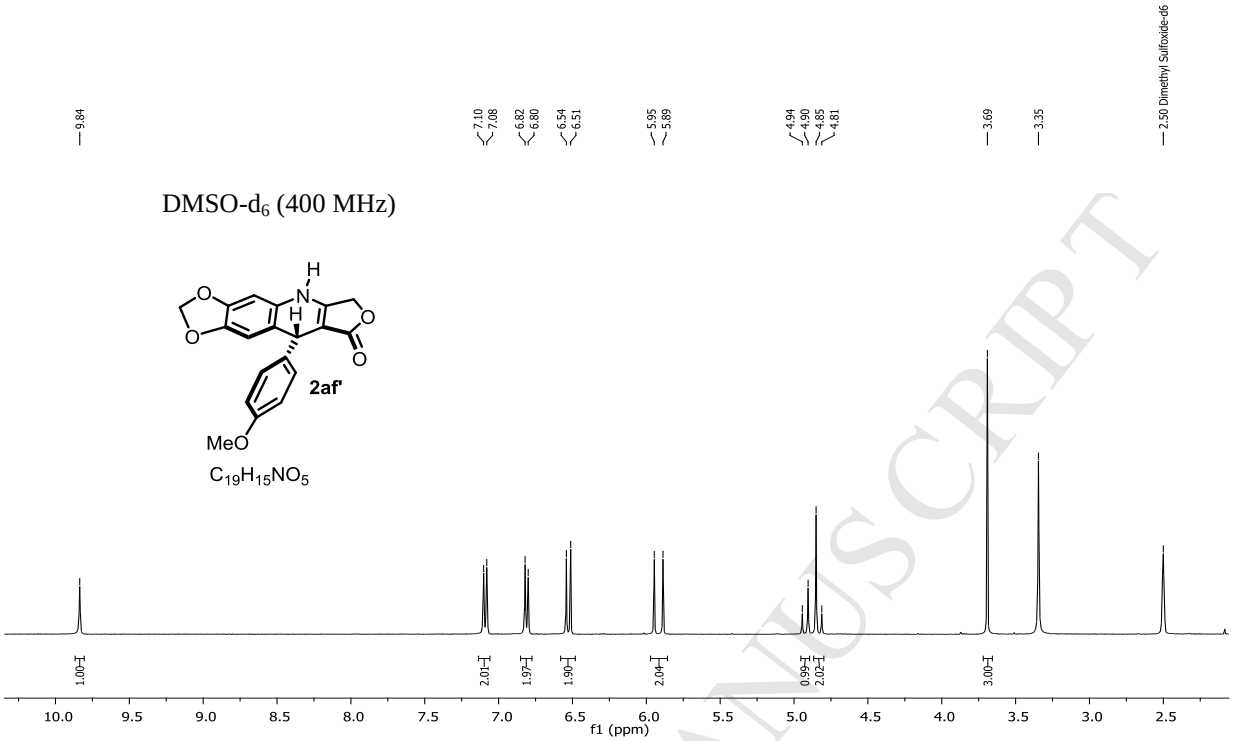
dried and argon purged 10 mL round-bottommed flask was added NaH (2 mg, 0.05 mmol, 1.5 equiv.) in DMF [0.15 M] at 0 °C. Compound **2bc'** (11 mg, 0.03 mmol, 1.0 equiv.) followed by chloromethyl methyl ether (3 mg, 0.03 mmol, 1.1 equiv.) were added. The reaction mixture was stirred for an hour at room temperature and then poured in to an ice cold water (1 mL) and extracted in to ethyl acetate (3X3 mL). Removal of solvents in *vacuo* gave crude product. The crude product was purified by column chromatography on silica gel by using isocratic solvent system (hexanes/ethyl acetate; 70:30) to obtain pure compound **2lc'** (3 mg, 0.01 mmol,

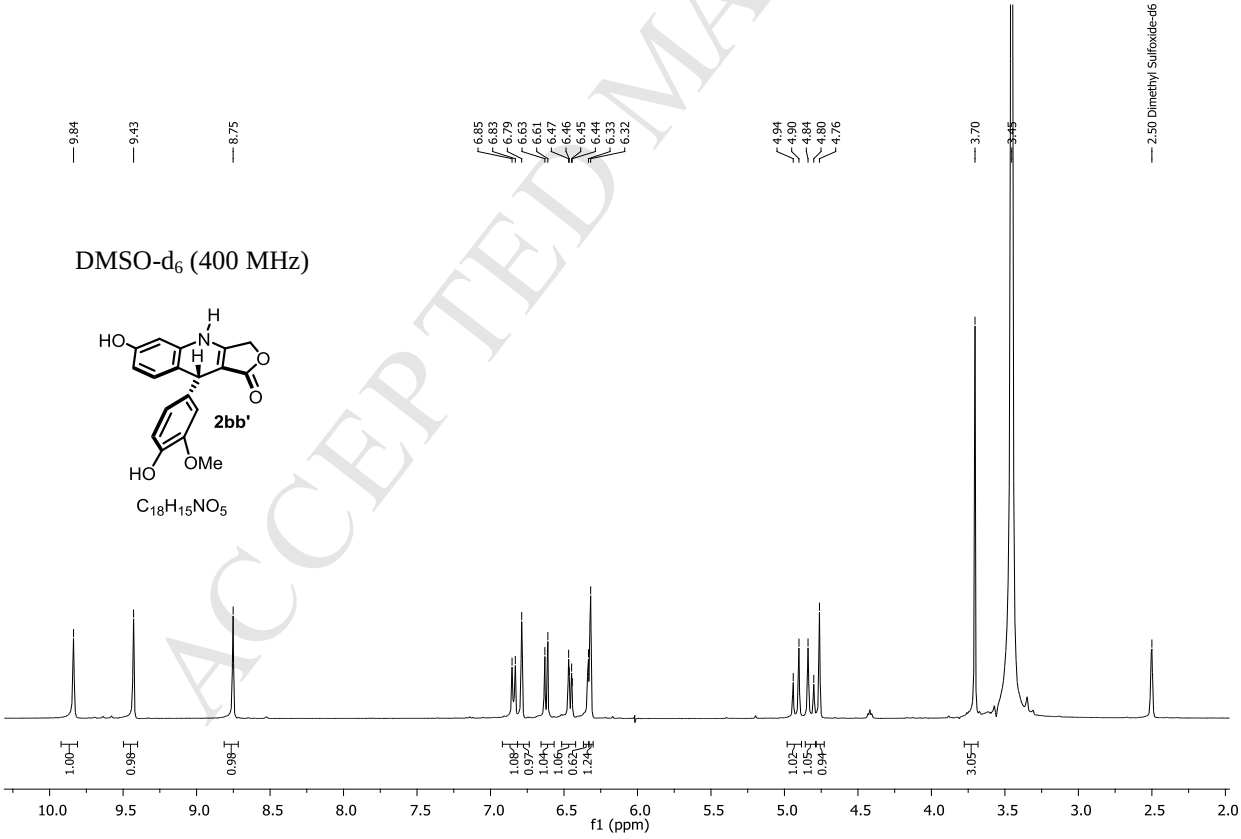
24% yield). White powder; $\mathbf{R_f}$ = 0.30 (hexanes/acetone 40 / 60); **m.p.** >260 ^{o}C; **IR** νmax (neat): 3357, 1728, 1621, 1610, 1485, 1109, 1035, 798 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 9.99 (bs, 1H), 6.95 (d, *J* = 8.2 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.72 (s, 1H), 6.65 (d, *J* = 7.9 Hz, 1H), 6.58 (d, *J* = 7.8 Hz, 2H), 5.93 (d, *J* = 3.4 Hz, 2H), 5.20 – 5.04 (m, 2H), 4.96 (d, *J* = 15.6 Hz, 1H), 4.87 (s, 1H), 4.84 (d, *J* = 15.6 Hz, 1H), 3.35 (s, 3H); **LC-MS (ESI)**: *m/z* 368.0 (M+H); **SMILES**: O=C(OC1)C2=C1NC3=CC(OCOC)=CC=C3C2C4=CC(OCO5)=C5C=C4.

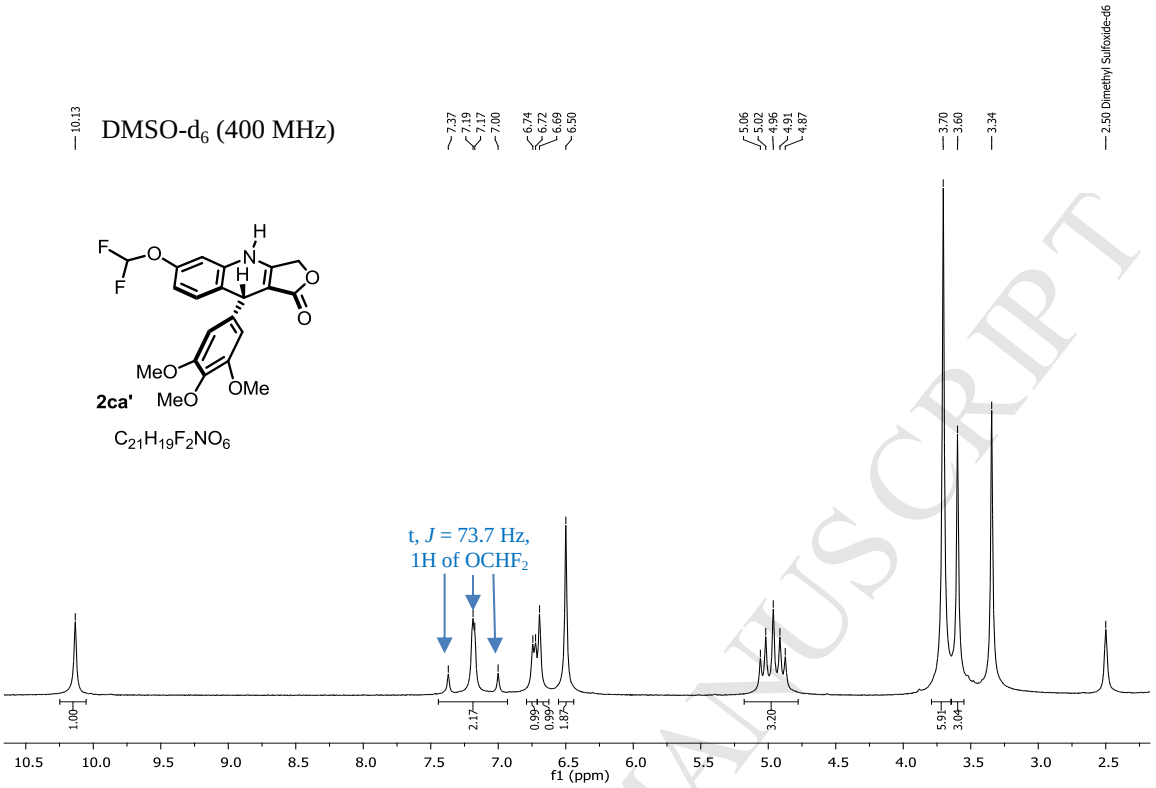
III. SELECTED ^{1}H, ^{13}C & LC-MS SPECTRA

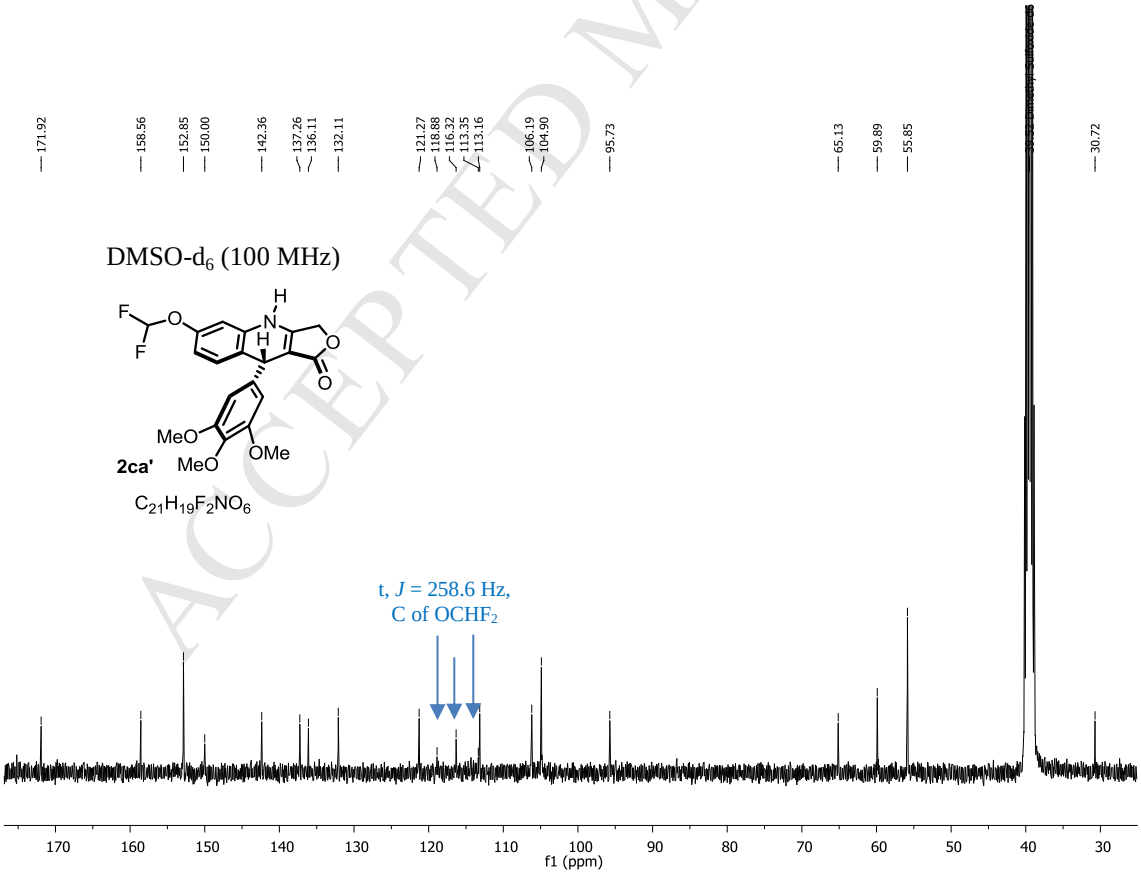


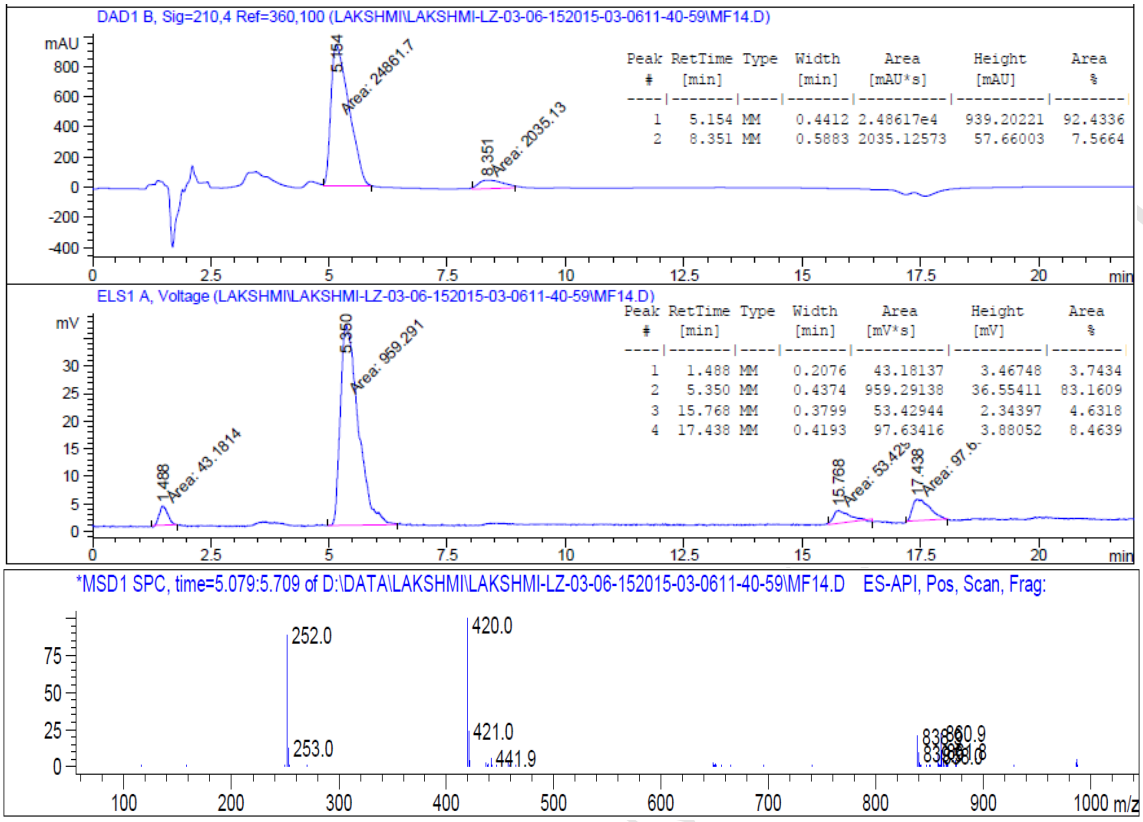




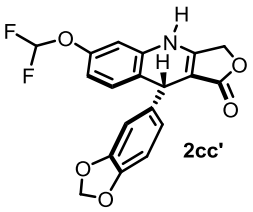




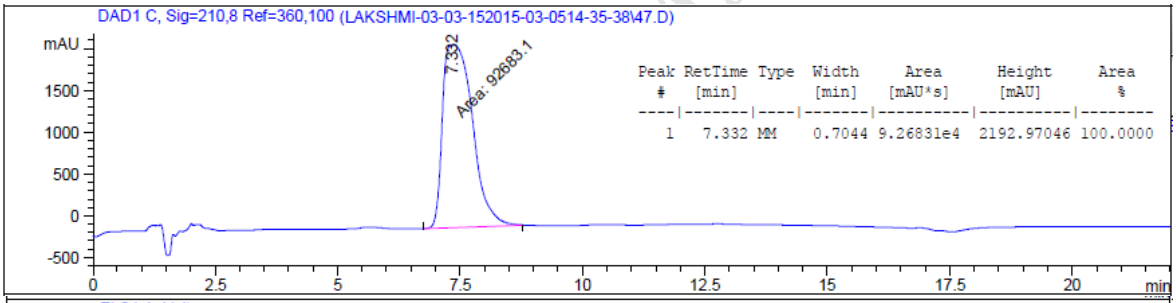


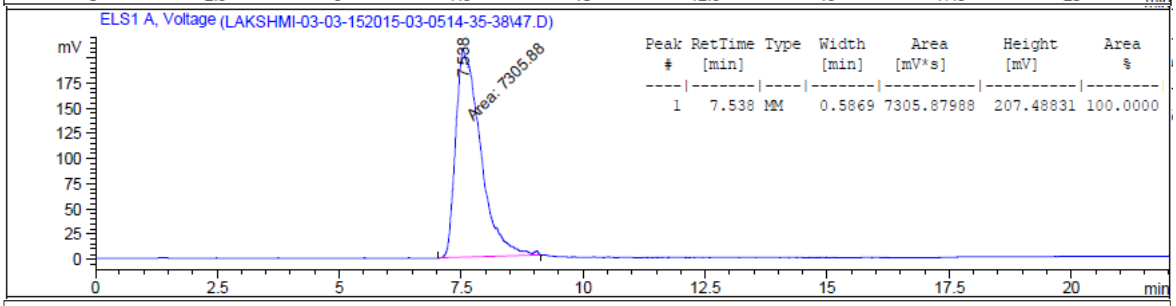


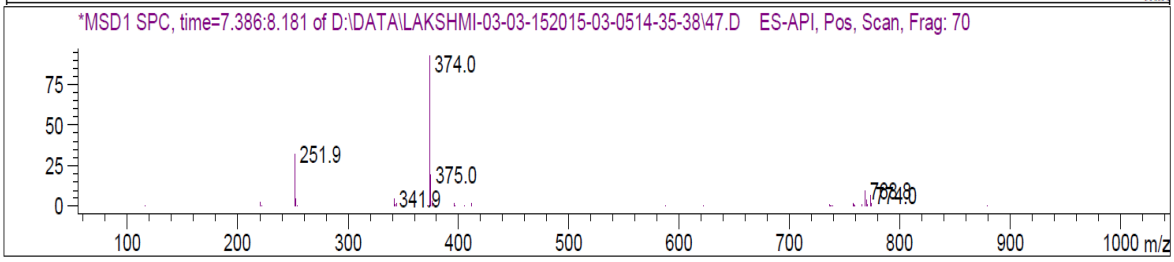
DMSO-d_6 (400 MHz)

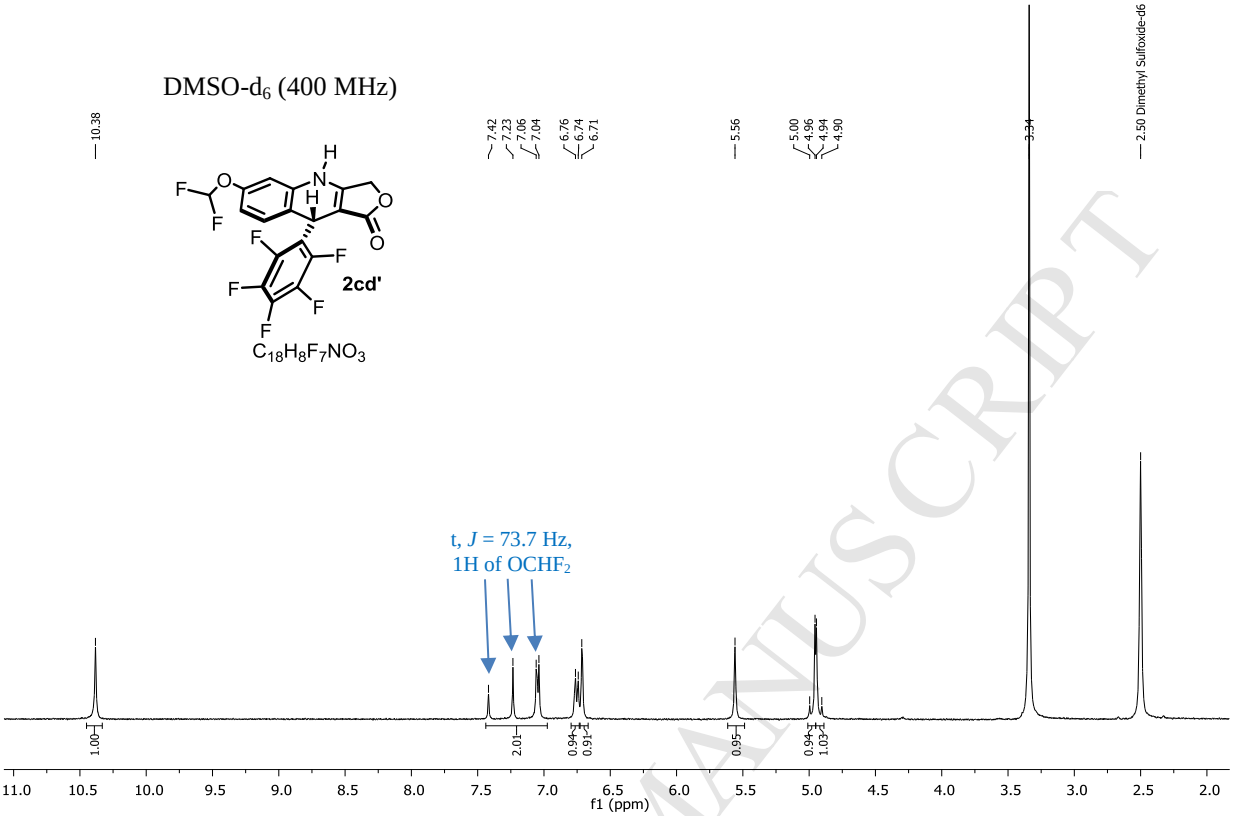


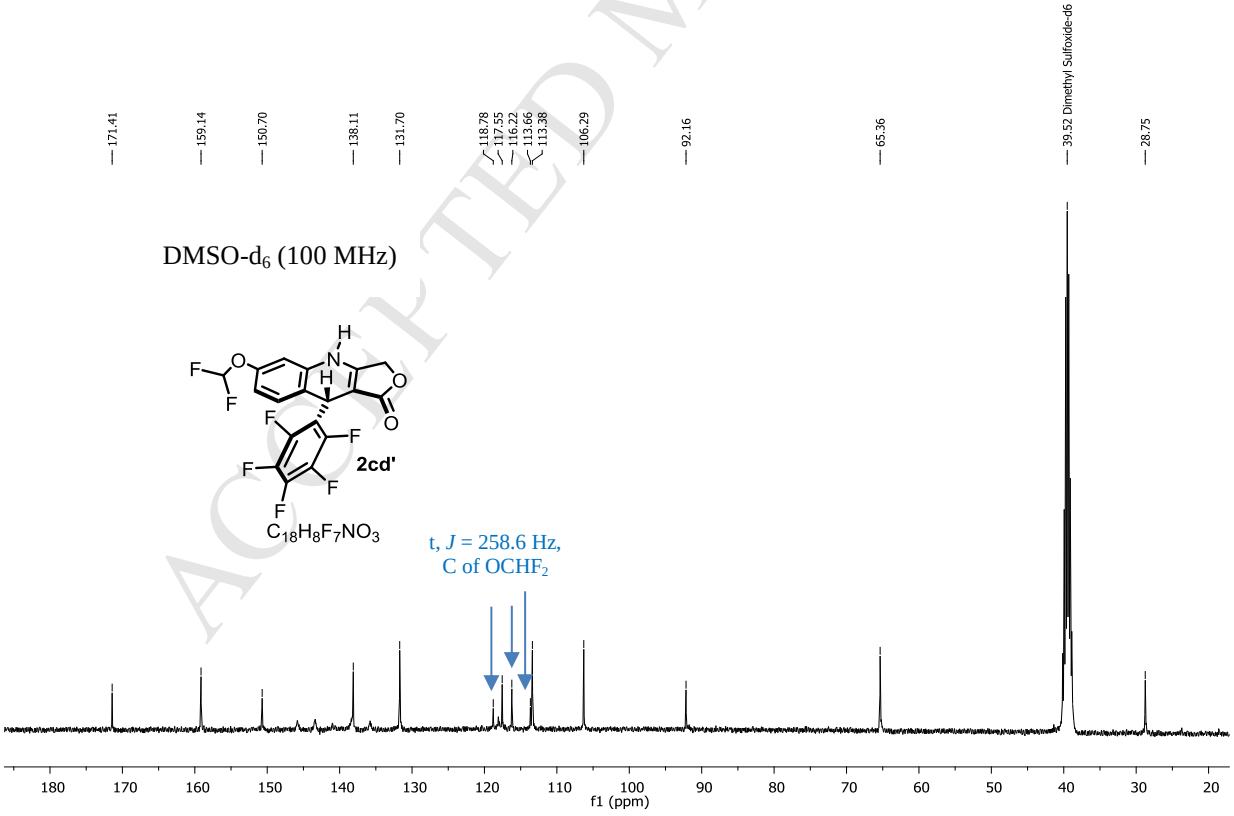
$C_{19}H_{13}F_2NO_5$

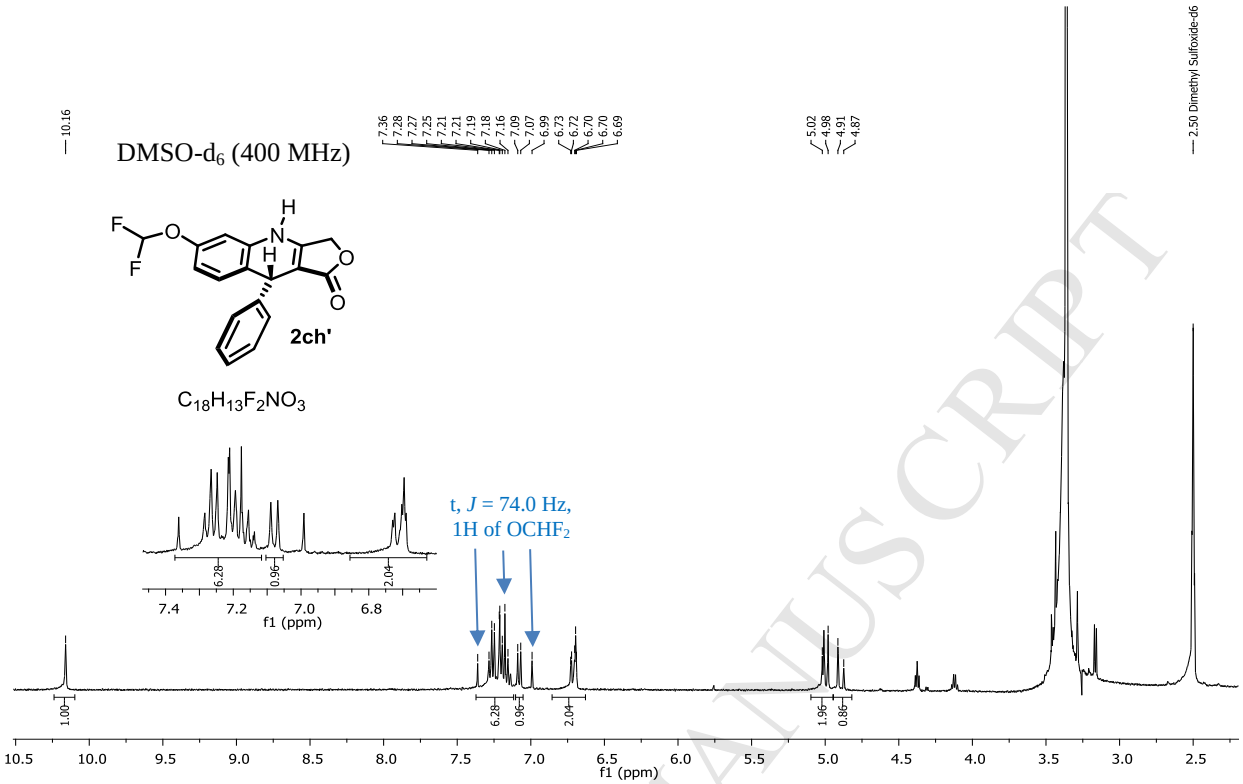




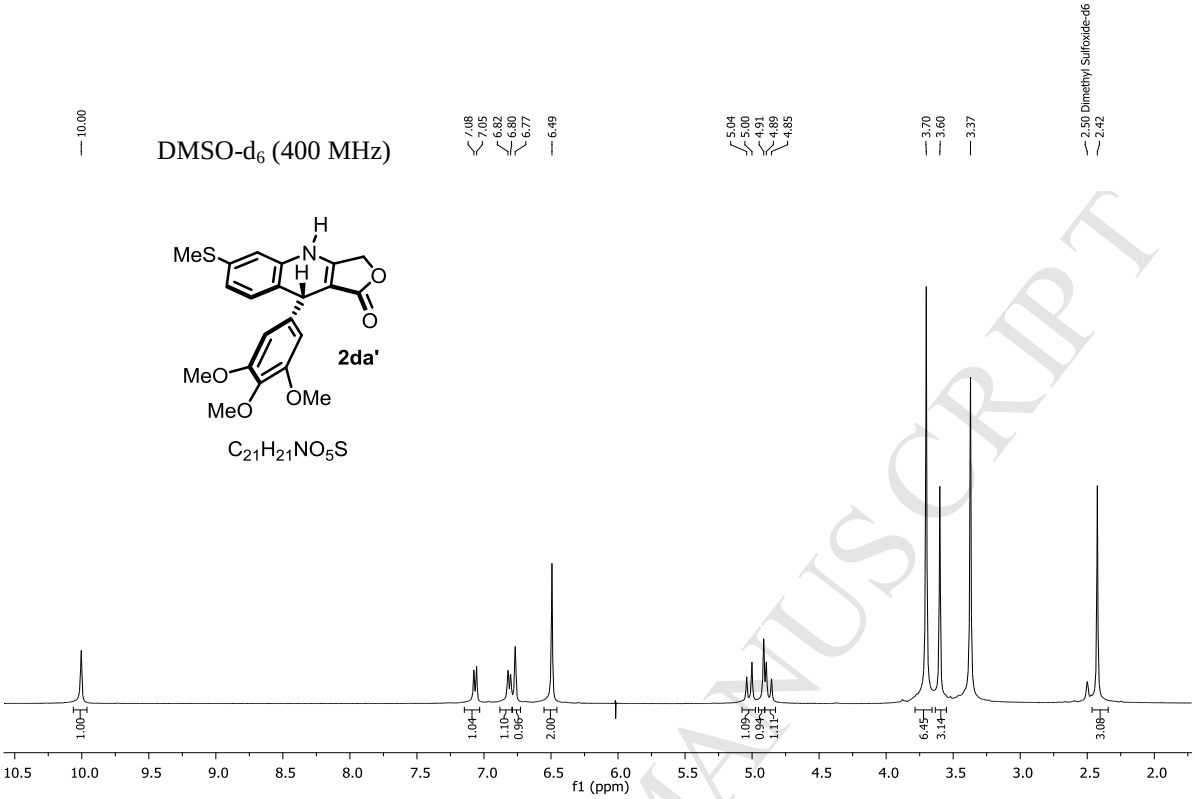




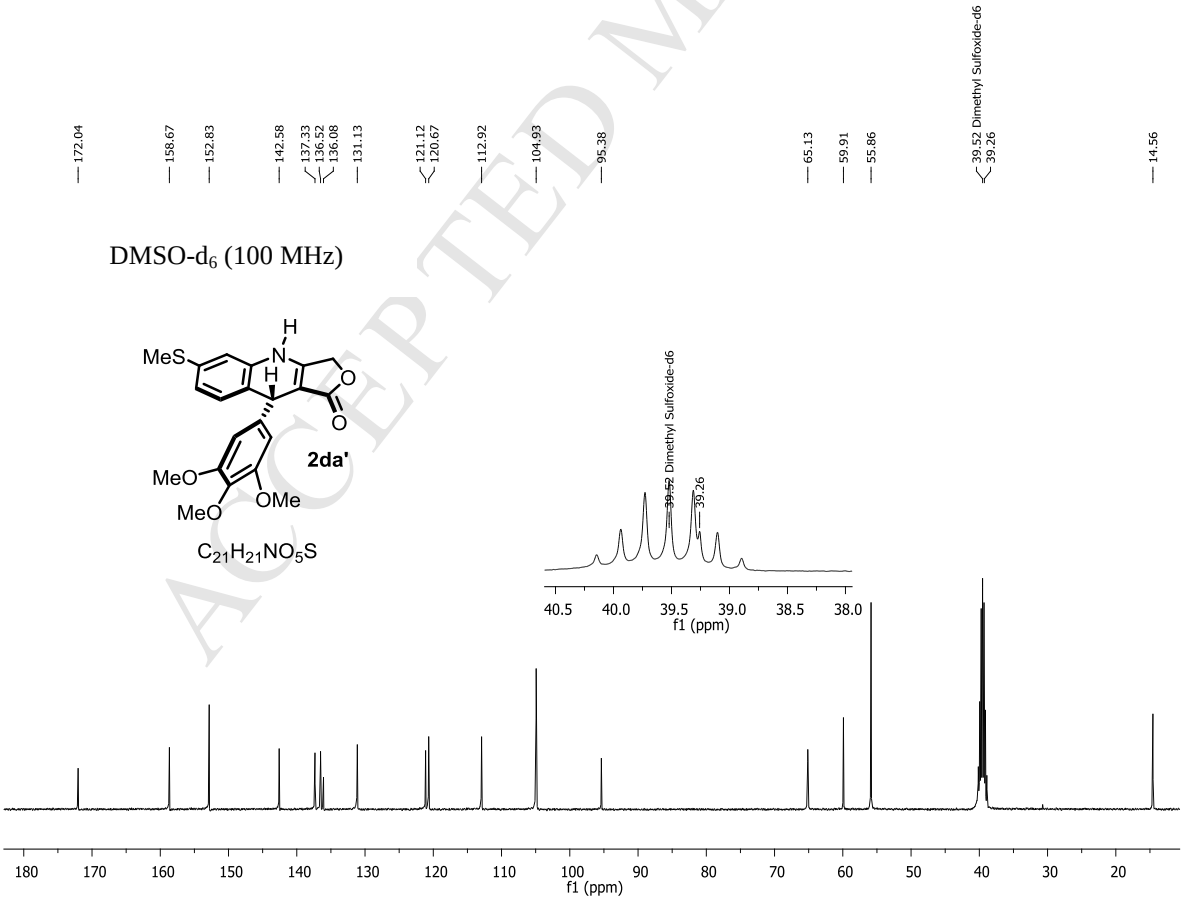


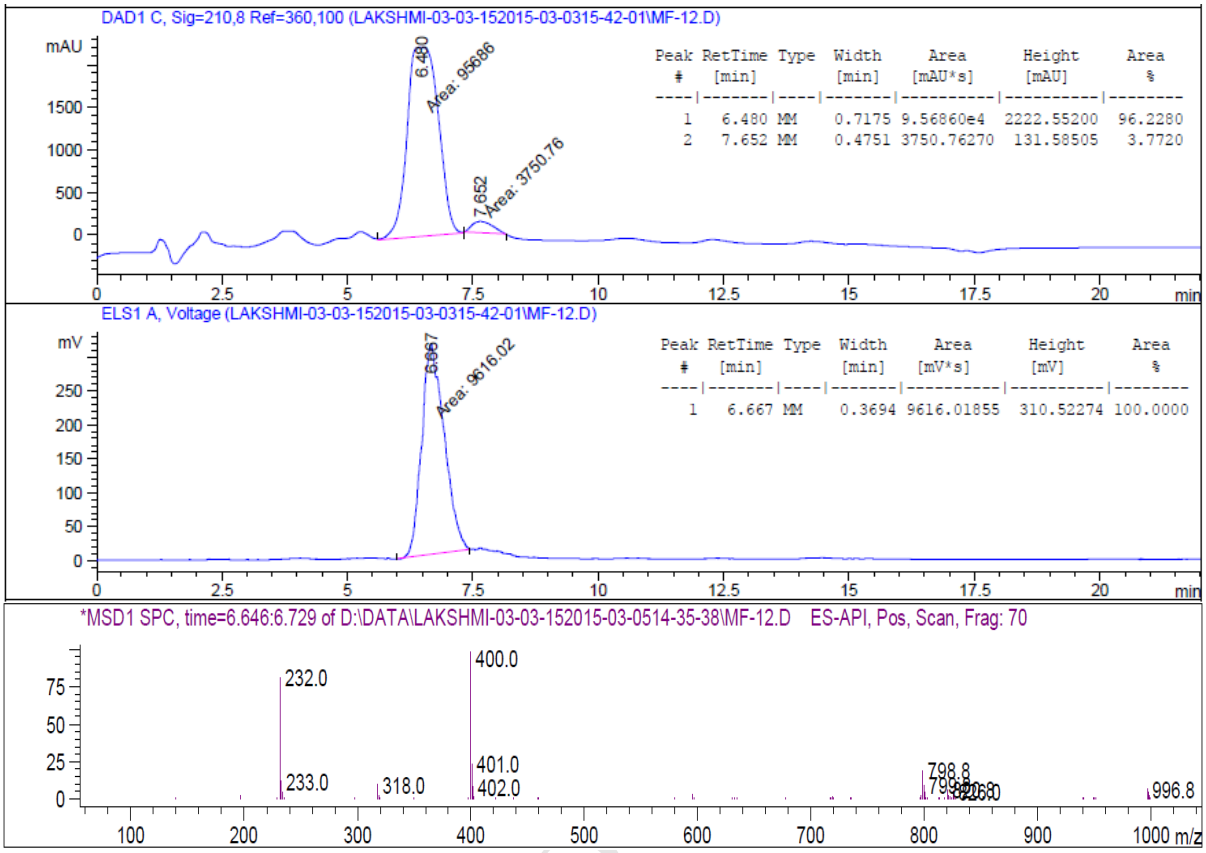


DMSO-d_6 (400 MHz)

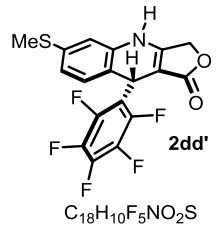


DMSO-d_6 (100 MHz)



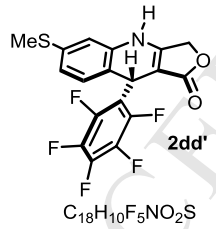

DAD1 C, Sig=210,8 Ref=360,100 (LAKSHMI-03-03-152015-03-0315-42-01\MF-12.D)
mAU
6.480
Area: 95686
7.652
Area: 3750.76
min
Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area %
1 | 6.480 | MM | 0.7175 | 9.56860e4 | 2222.55200 | 96.2280
2 | 7.652 | MM | 0.4751 | 3750.76270 | 131.58505 | 3.7720
ELS1 A, Voltage (LAKSHMI-03-03-152015-03-0315-42-01\MF-12.D)
mV
6.667
Area: 9616.02
min
Peak # | RetTime [min] | Type | Width [min] | Area [mV*s] | Height [mV] | Area %
1 | 6.667 | MM | 0.3694 | 9616.01855 | 310.52274 | 100.0000
*MSD1 SPC, time=6.646:6.729 of D:\DATA\LAKSHMI-03-03-152015-03-0514-35-38\MF-12.D ES-API, Pos, Scan, Frag: 70
232.0
400.0
233.0
318.0
401.0
402.0
798.8
996.8
m/z

DMSO-d_6 (400 MHz)

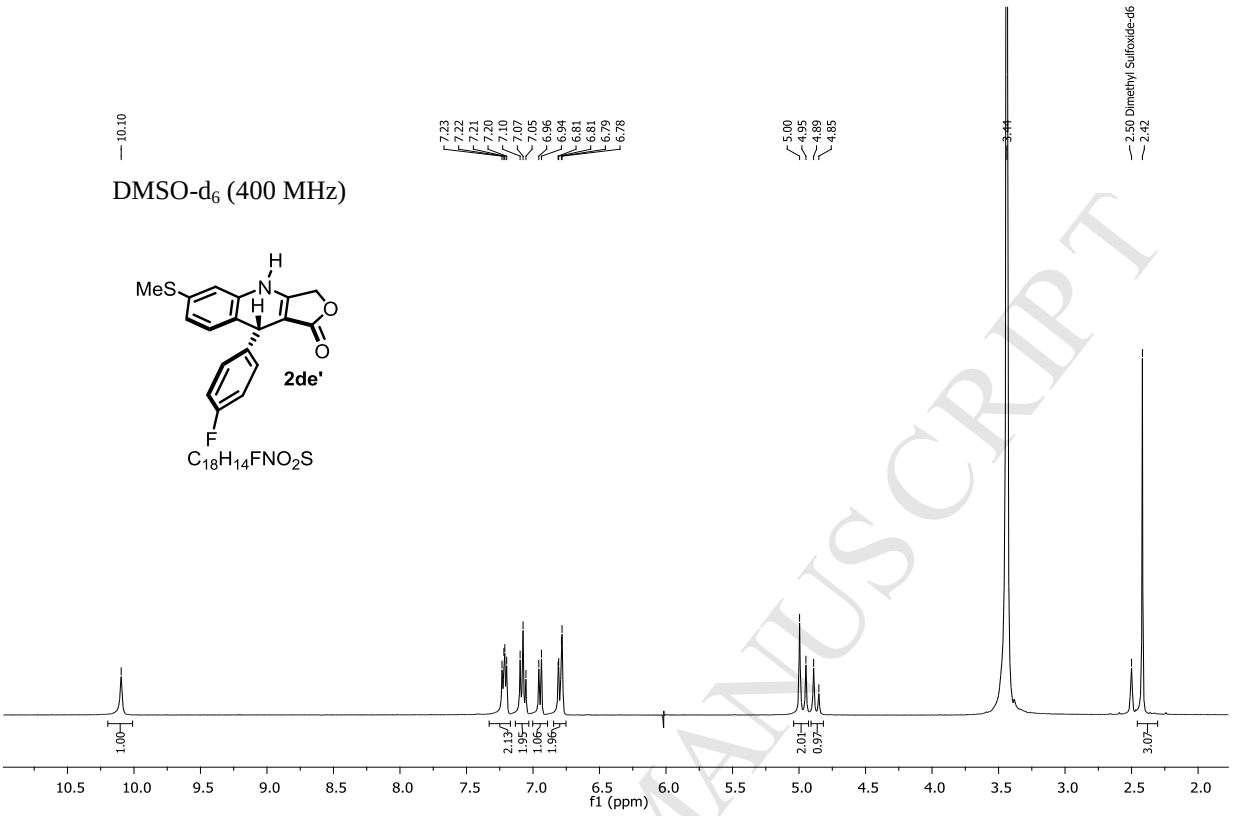


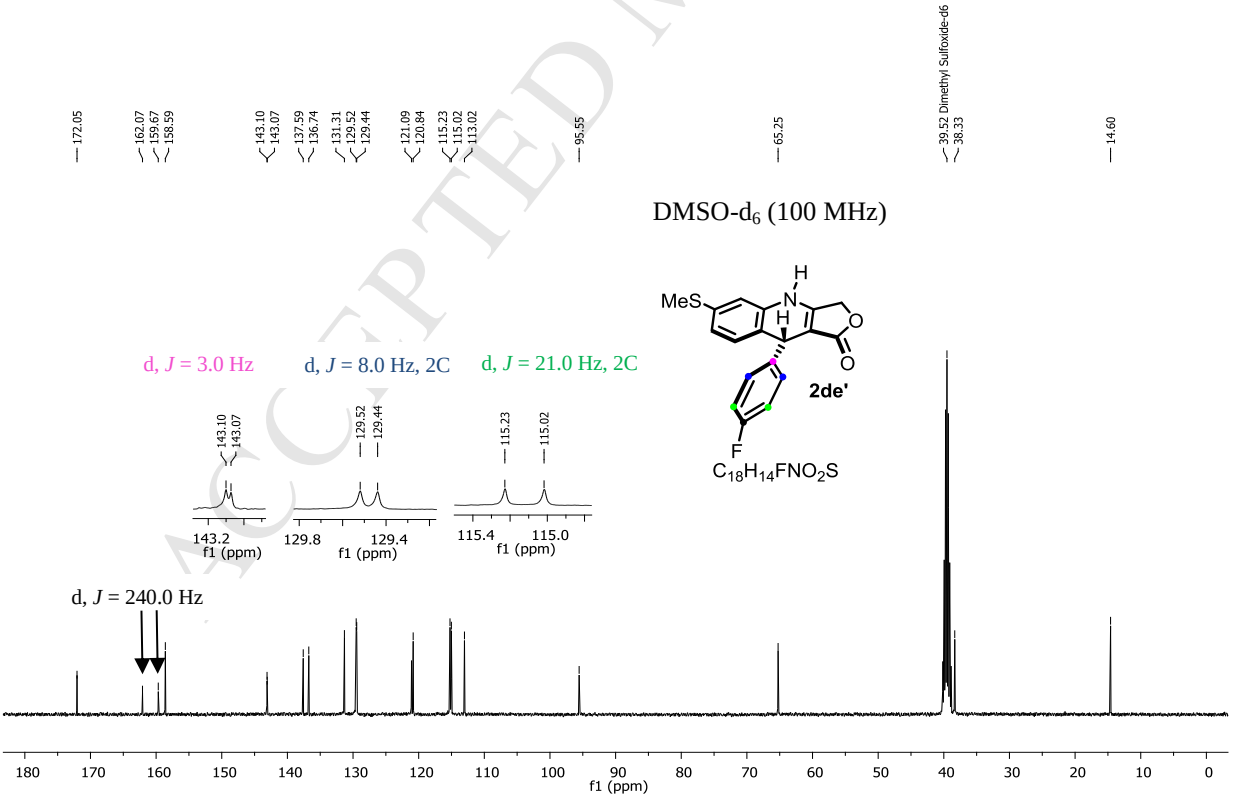
$C_{18}H_{10}F_5NO_2S$

DMSO-d_6 (100 MHz)

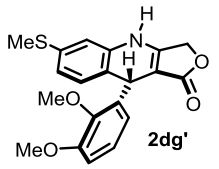


$C_{18}H_{10}F_5NO_2S$



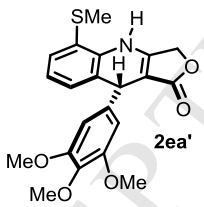


DMSO-d_6 (400 MHz)



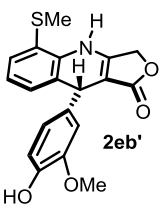
$C_{20}H_{19}NO_4S$

DMSO-d_6 (400 MHz)

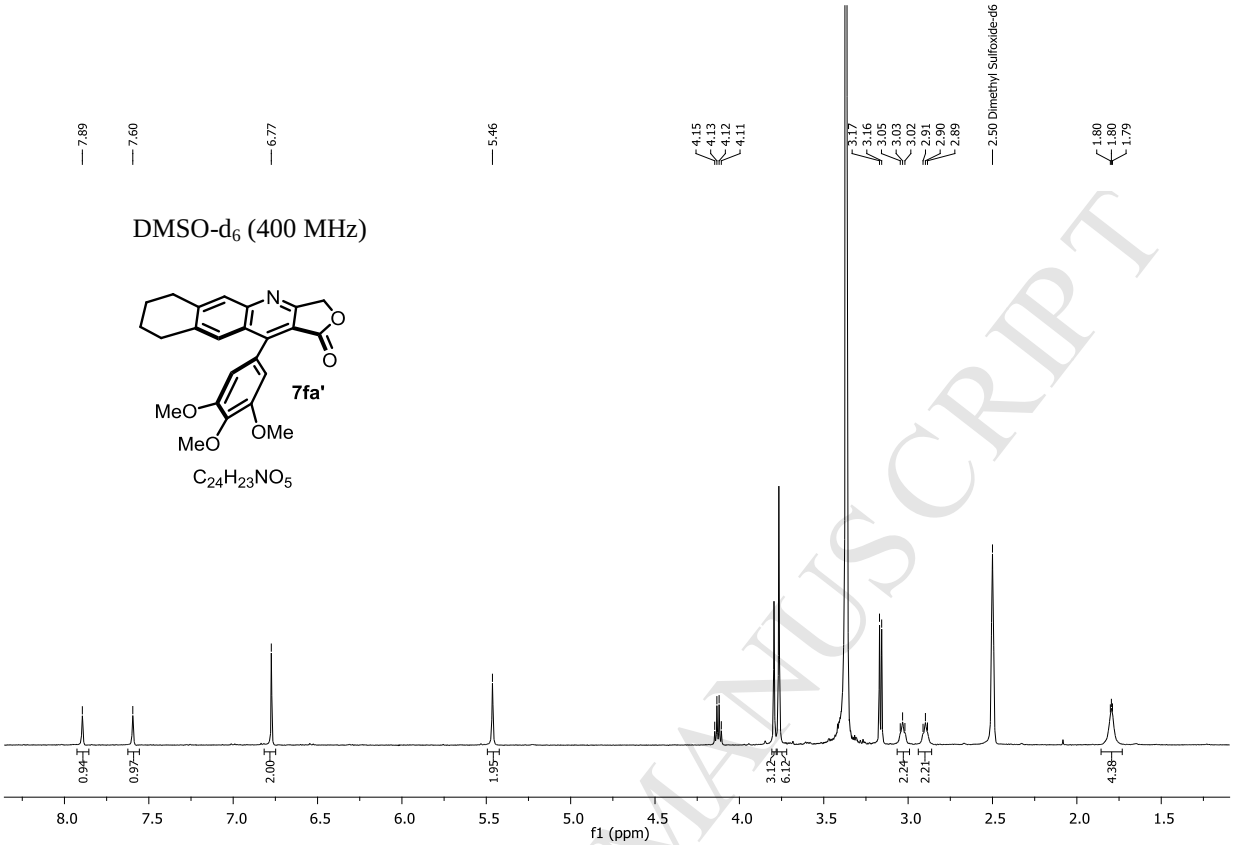


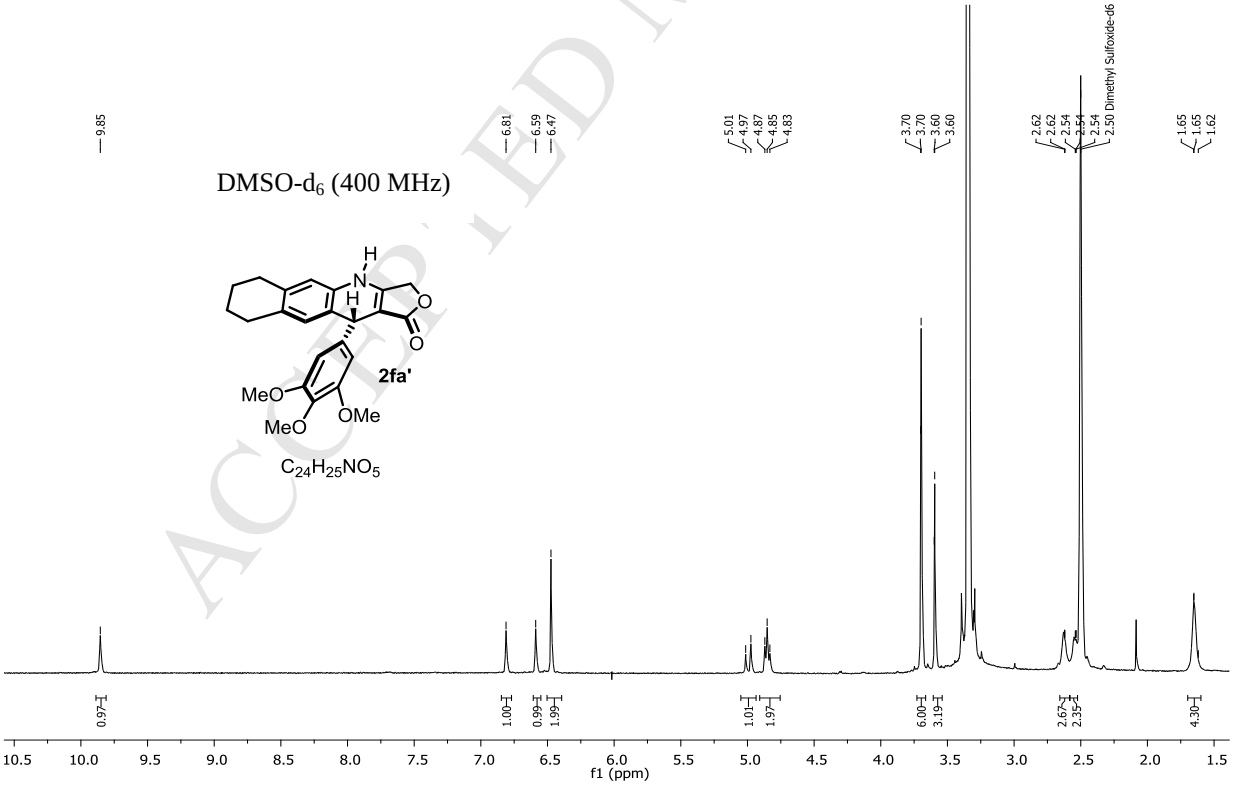
$C_{21}H_{21}NO_5S$

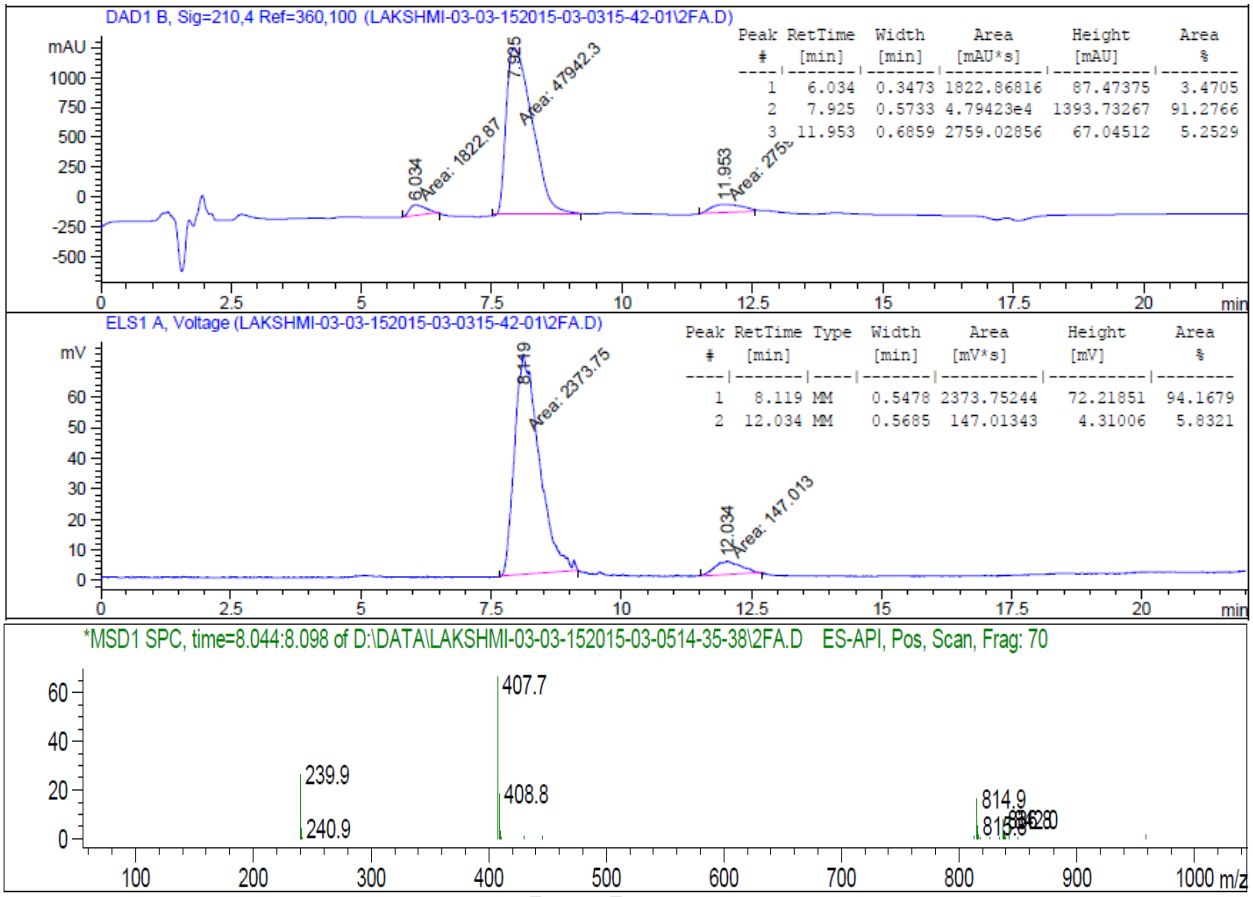
DMSO-d_6 (400 MHz)

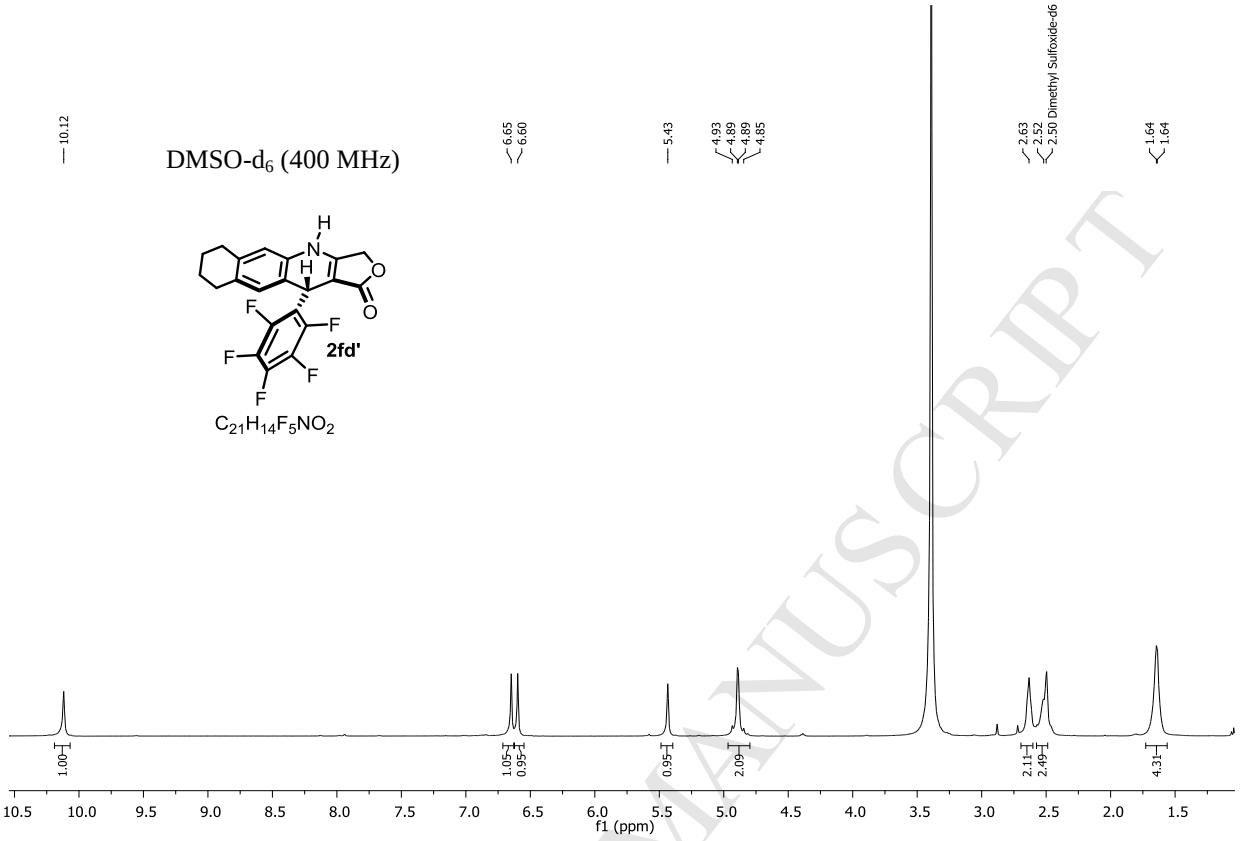


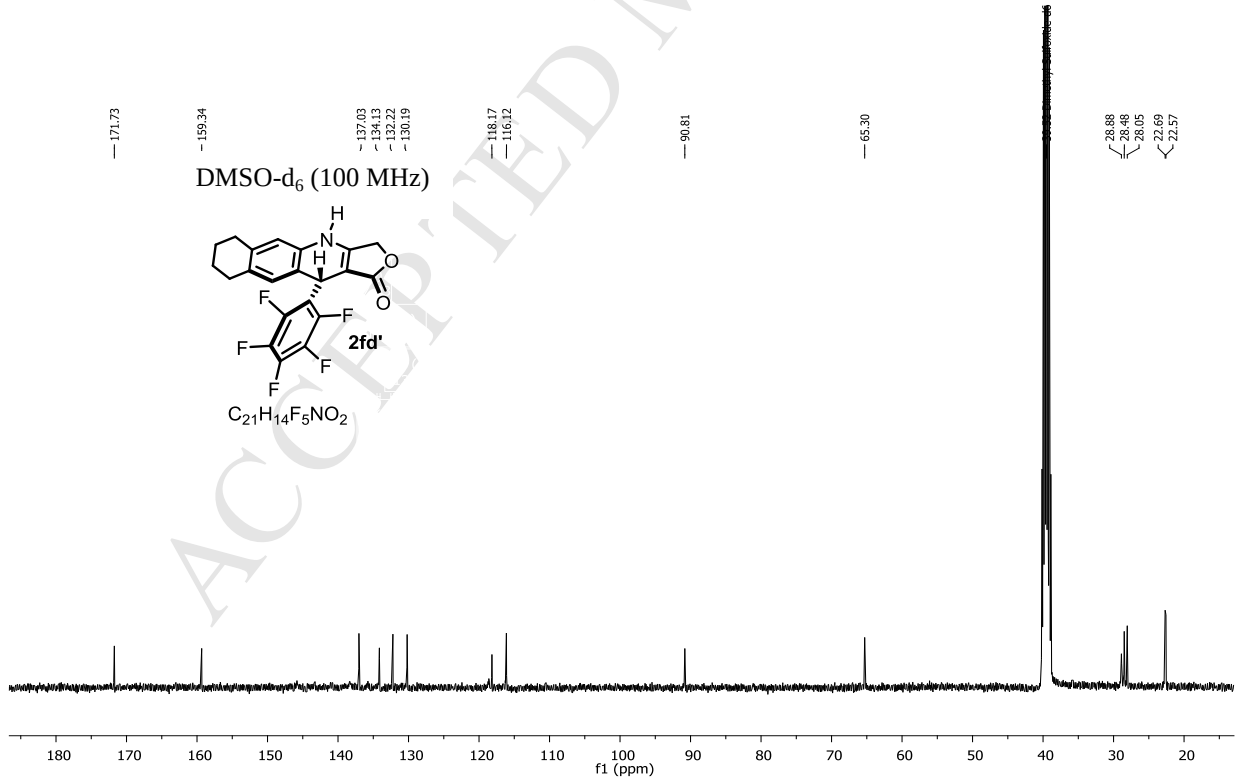
$C_{19}H_{17}NO_4S$



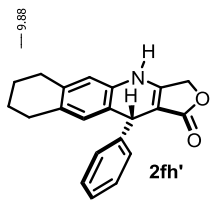



DAD1 B, Sig=210,4 Ref=360,100 (LAKSHMI-03-03-152015-03-0315-42-01\2FA.D)
mAU
Peak # | RetTime [min] | Width [min] | Area [mAU*s] | Height [mAU] | Area %
1 | 6.034 | 0.3473 | 1822.86816 | 87.47375 | 3.4705
2 | 7.925 | 0.5733 | 4.79423e4 | 1393.73267 | 91.2766
3 | 11.953 | 0.6859 | 2759.02856 | 67.04512 | 5.2529
6.034
Area: 1822.87
7.925
Area: 47942.3
11.953
min
ELS1 A, Voltage (LAKSHMI-03-03-152015-03-0315-42-01\2FA.D)
mV
Peak # | RetTime [min] | Type | Width [min] | Area [mV*s] | Height [mV] | Area %
1 | 8.119 | MM | 0.5478 | 2373.75244 | 72.21851 | 94.1679
2 | 12.034 | MM | 0.5685 | 147.01343 | 4.31006 | 5.8321
8.119
Area: 2373.75
12.034
Area: 147.013
min
*MSD1 SPC, time=8.044:8.098 of D:\DATA\LAKSHMI-03-03-152015-03-0514-35-38\2FA.D ES-API, Pos, Scan, Frag: 70
407.7
239.9
240.9
408.8
814.9
m/z



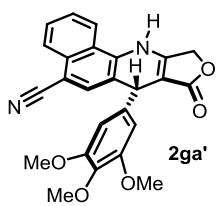


DMSO-d_6 (400 MHz)

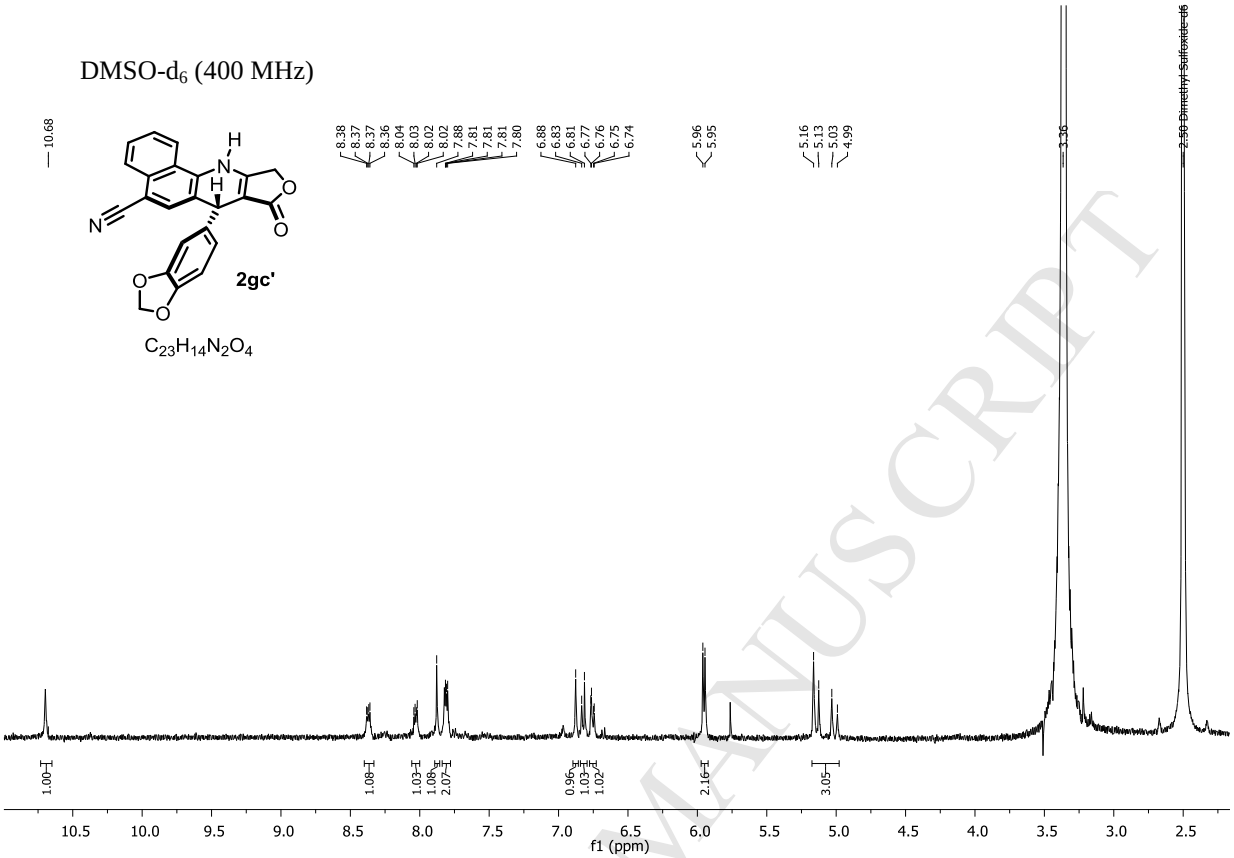


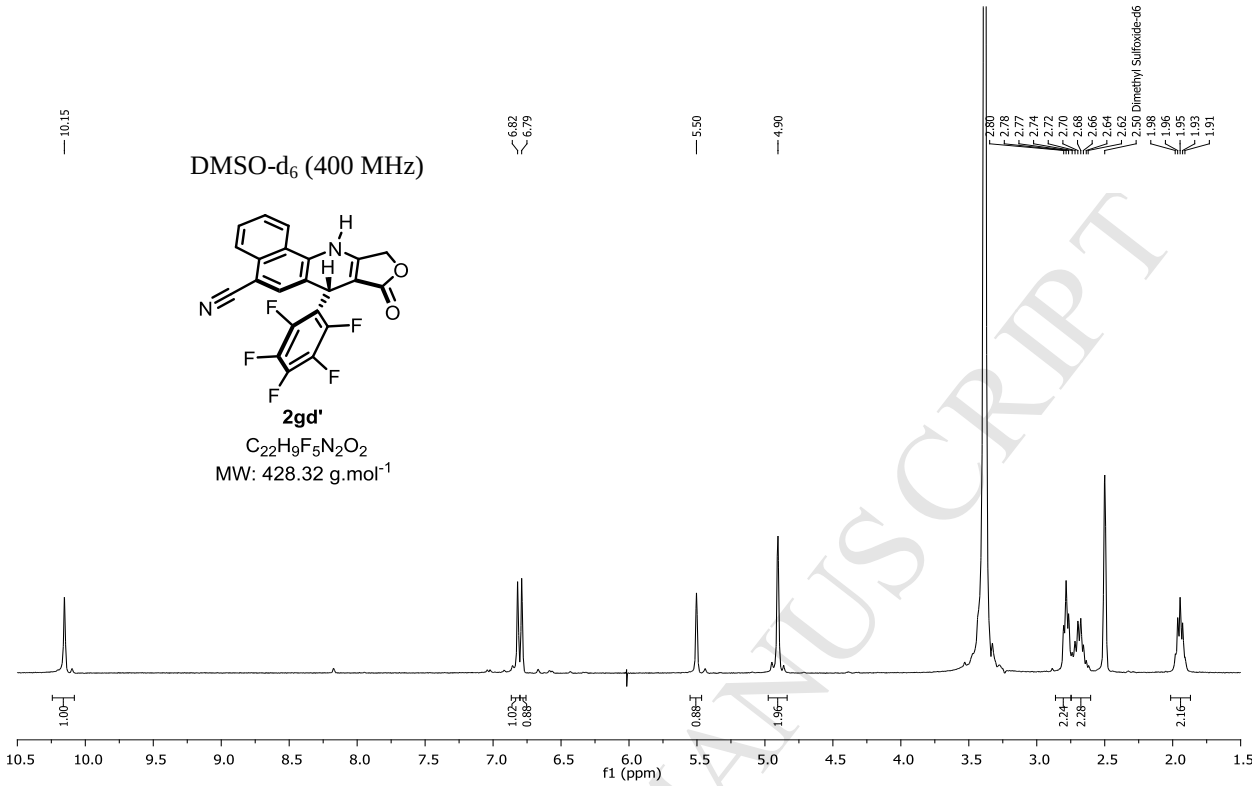
$C_{21}H_{19}NO_2$

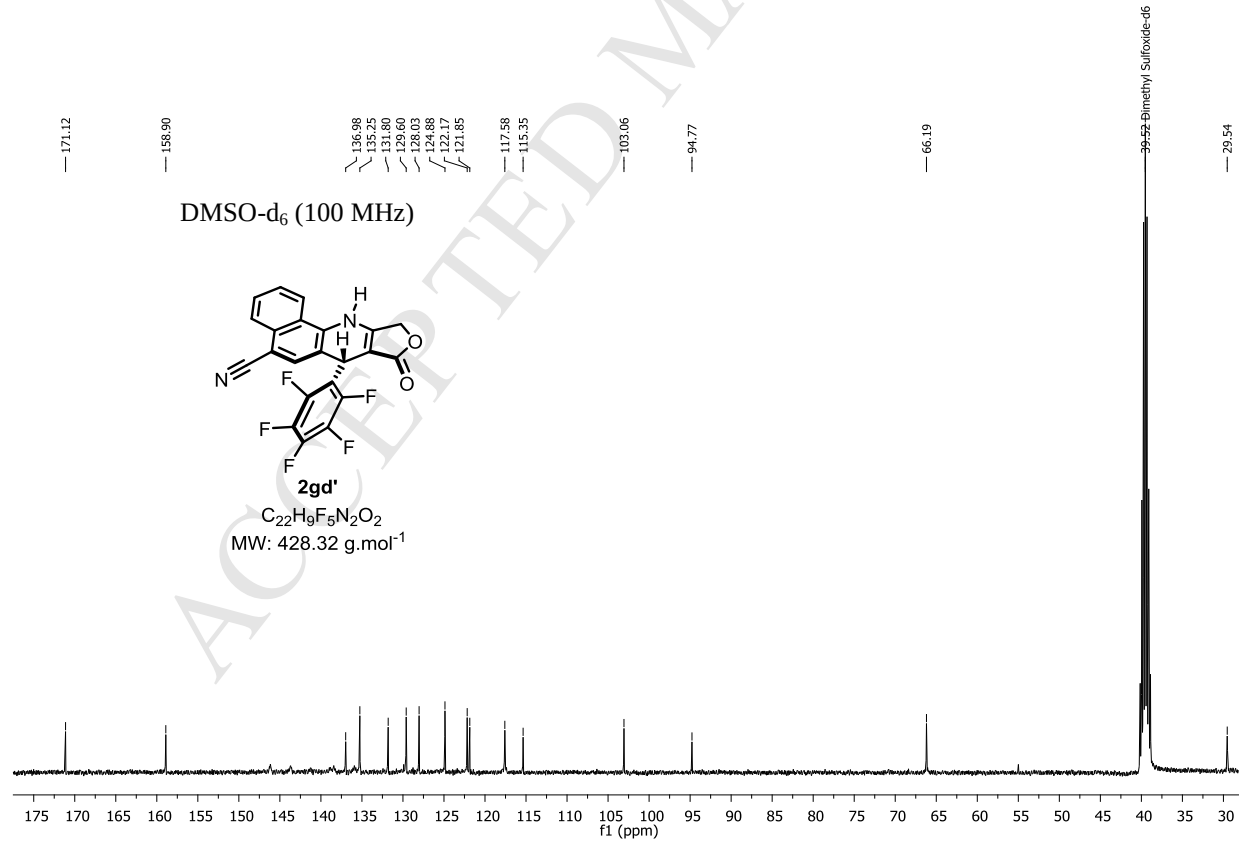
DMSO-d_6 (400 MHz)

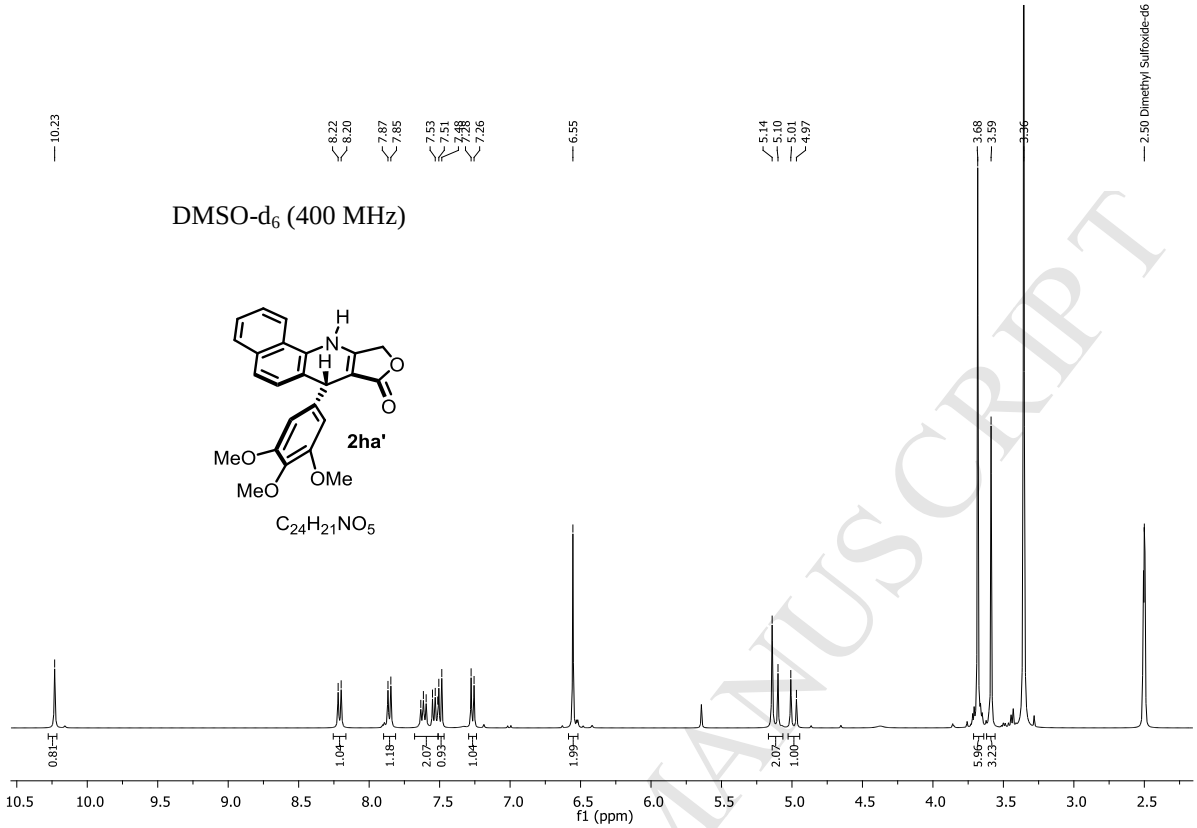


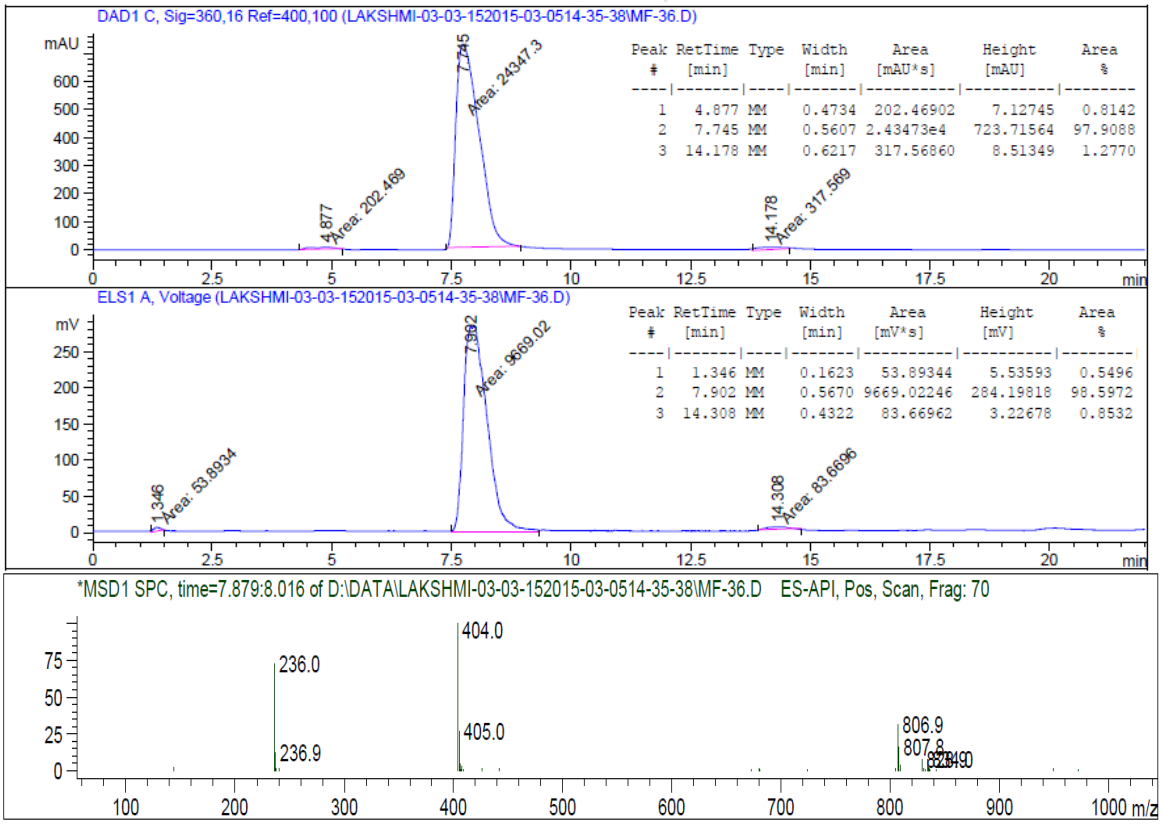
$C_{25}H_{20}N_2O_5$





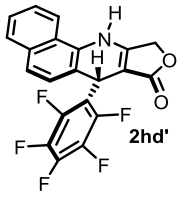



DMSO-d6 (400 MHz)
2ha'
MeO
MeO
OMe
C24H21NO5
f1 (ppm)


DAD1 C, Sig=360,16 Ref=400,100 (LAKSHMI-03-03-152015-03-0514-35-38\MF-36.D)
Peak # RetTime [min] Type Width [min] Area [mAU*s] Height [mAU] Area %
1 4.877 MM 0.4734 202.46902 7.12745 0.8142
2 7.745 MM 0.5607 2.43473e4 723.71564 97.9088
3 14.178 MM 0.6217 317.56860 8.51349 1.2770
Area: 202.469
Area: 24347.3
Area: 317.569
ELS1 A, Voltage (LAKSHMI-03-03-152015-03-0514-35-38\MF-36.D)
Peak # RetTime [min] Type Width [min] Area [mV*s] Height [mV] Area %
1 1.346 MM 0.1623 53.89344 5.53593 0.5496
2 7.902 MM 0.5670 9669.02246 284.19818 98.5972
3 14.308 MM 0.4322 83.66962 3.22678 0.8532
Area: 53.8934
Area: 9669.02
Area: 83.6696

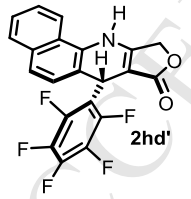
*MSD1 SPC, time=7.879:8.016 of D:\DATA\LAKSHMI-03-03-152015-03-0514-35-38\MF-36.D ES-API, Pos, Scan, Frag: 70
404.0
236.0
405.0
236.9
806.9
807.8

DMSO-d_6 (400 MHz)

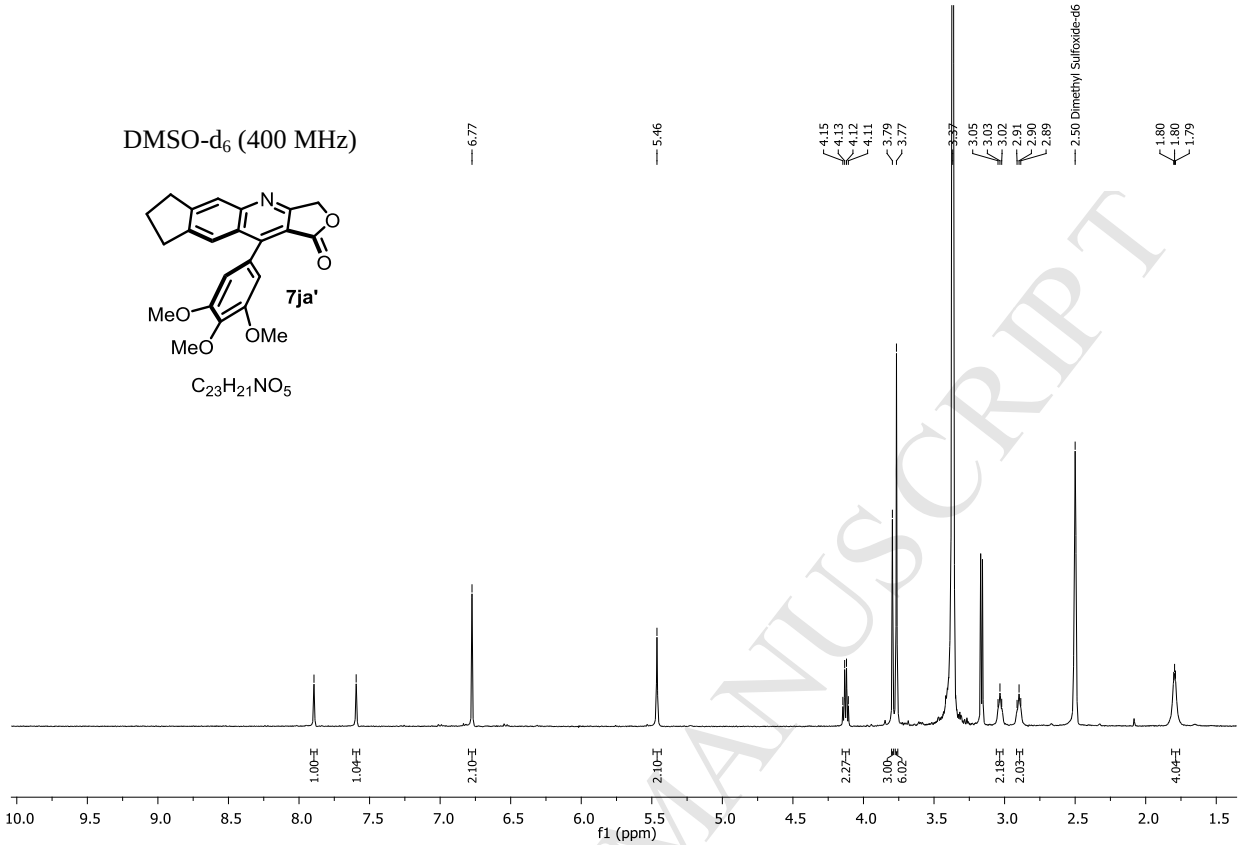


$C_{21}H_{10}F_5NO_2$

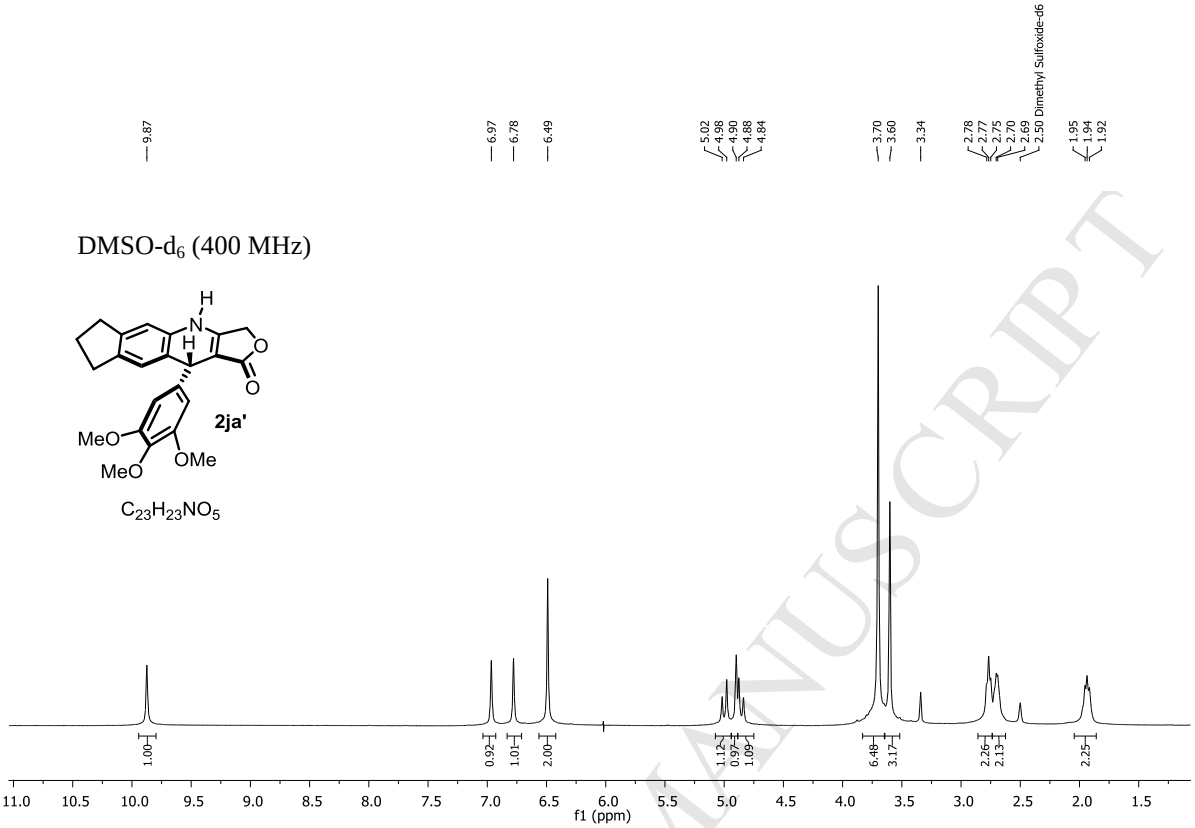
DMSO-d_6 (100 MHz)



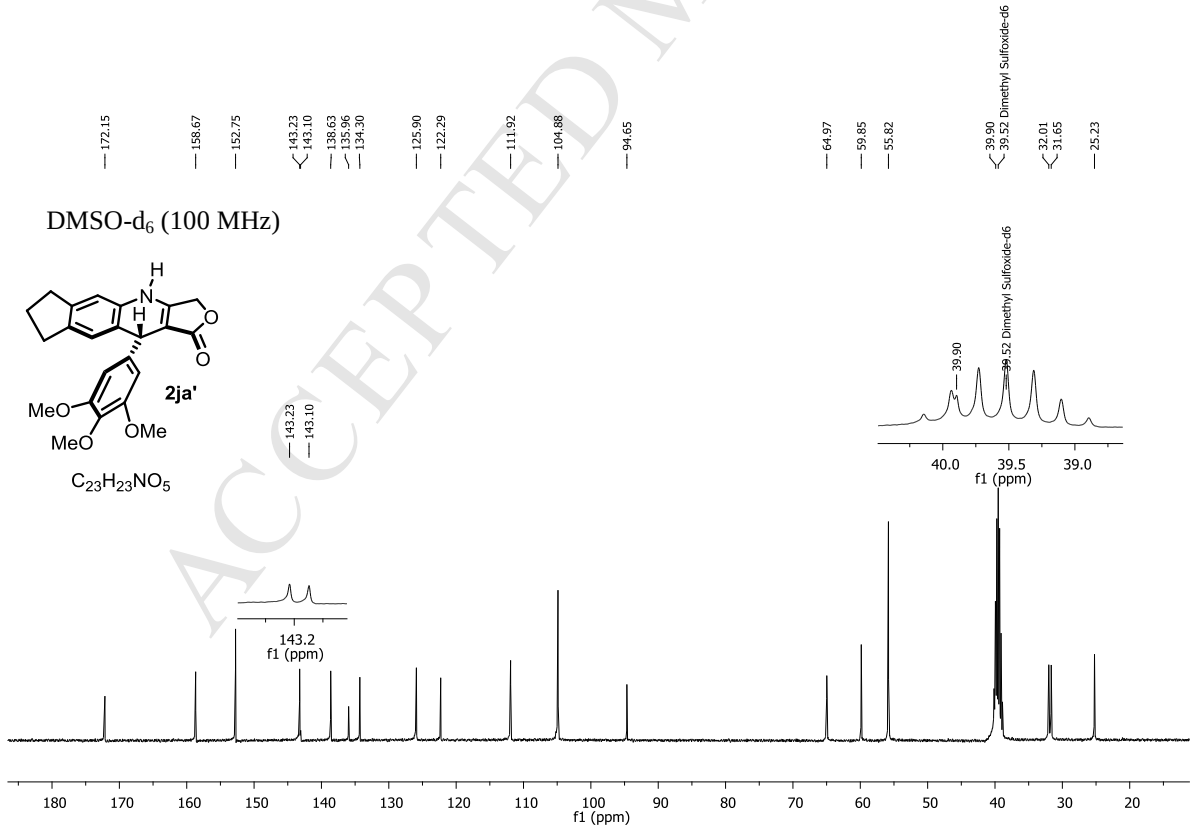
$C_{21}H_{10}F_5NO_2$

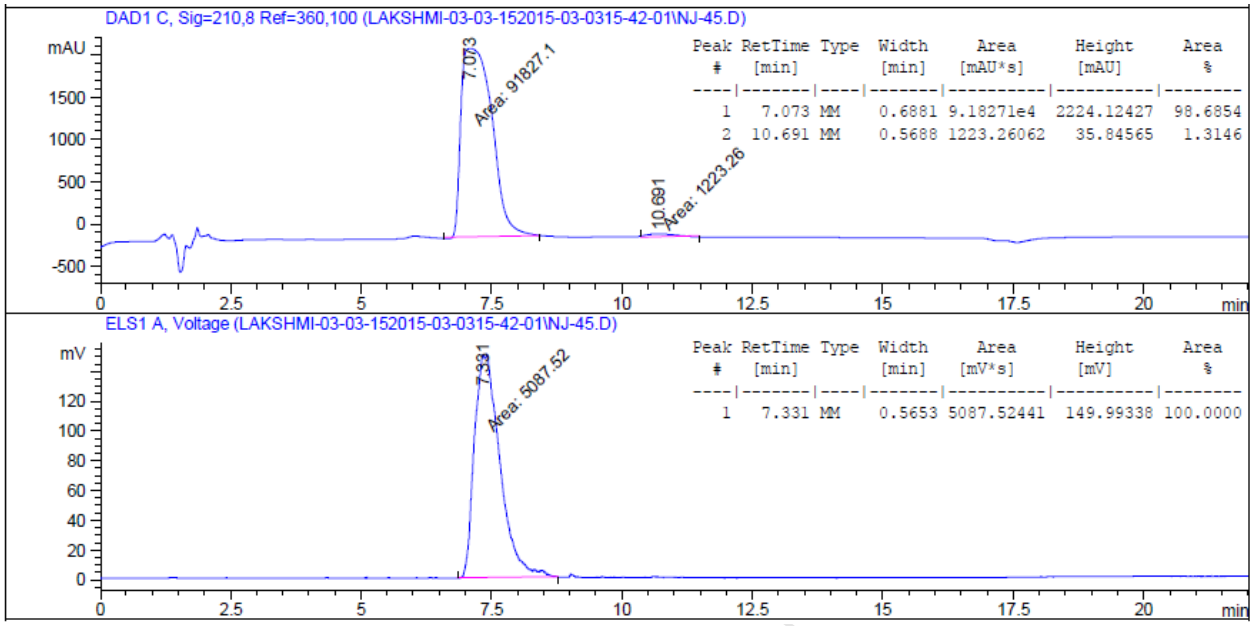


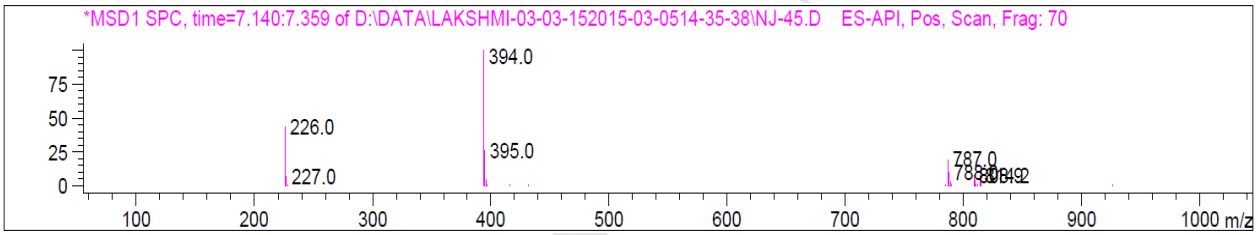
DMSO-d_6 (400 MHz)

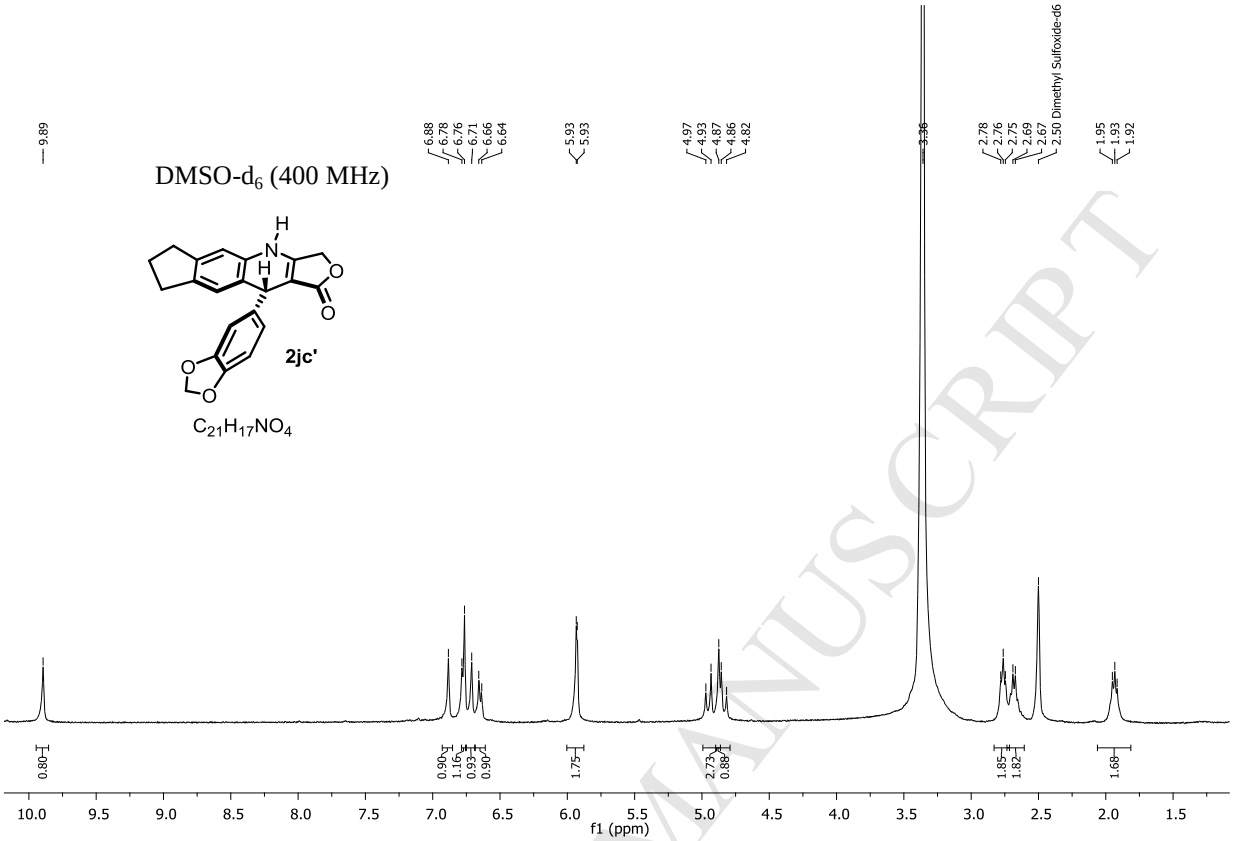


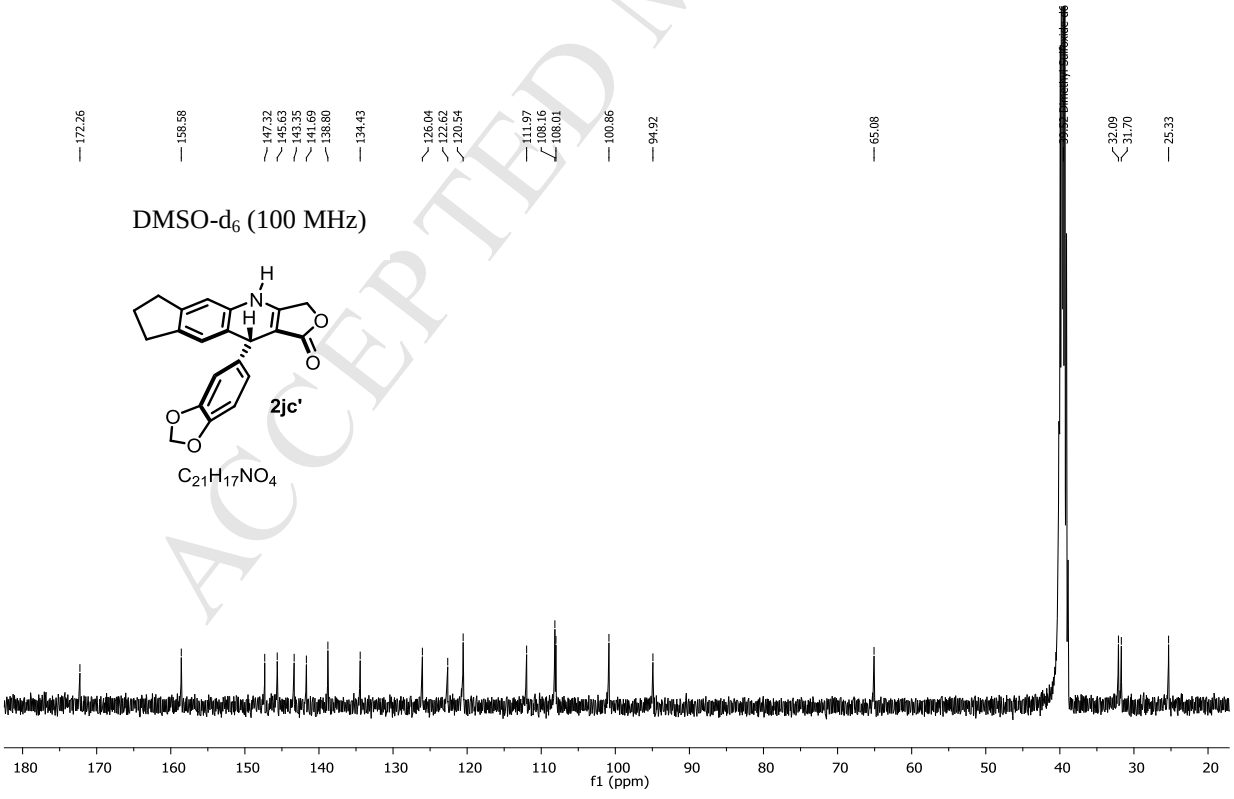
DMSO-d_6 (100 MHz)

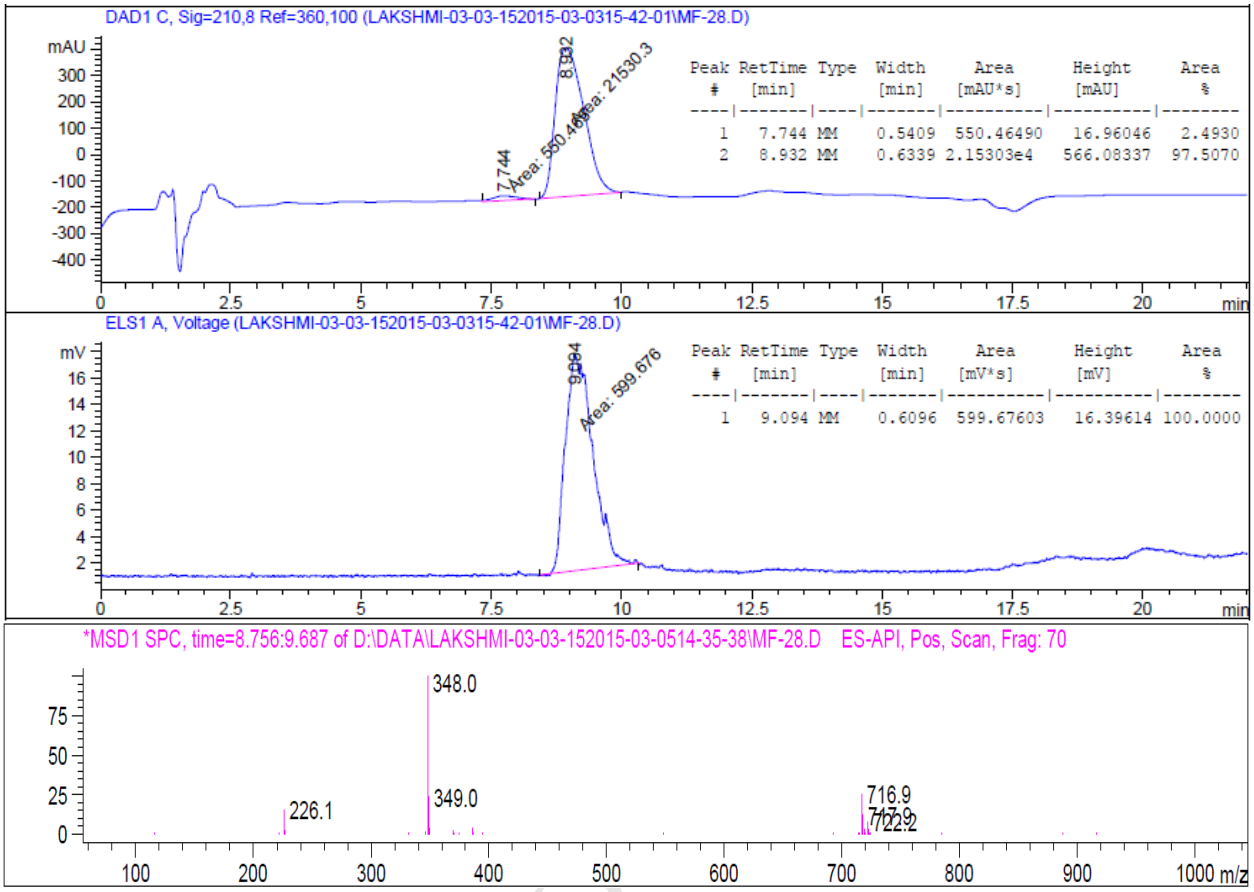



DAD1 C, Sig=210,8 Ref=360,100 (LAKSHMI-03-03-152015-03-0315-42-01\NJ-45.D)
mAU
7.073
Area: 91827.1
10.691
Area: 1223.26
Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area %
1 | 7.073 | MM | 0.6881 | 9.18271e4 | 2224.12427 | 98.6854
2 | 10.691 | MM | 0.5688 | 1223.26062 | 35.84565 | 1.3146
min
ELS1 A, Voltage (LAKSHMI-03-03-152015-03-0315-42-01\NJ-45.D)
mV
7.331
Area: 5087.52
Peak # | RetTime [min] | Type | Width [min] | Area [mV*s] | Height [mV] | Area %
1 | 7.331 | MM | 0.5653 | 5087.52441 | 149.99338 | 100.0000
min

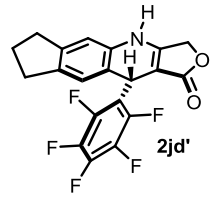

*MSD1 SPC, time=7.140:7.359 of D:\DATA\LAKSHMI-03-03-152015-03-0514-35-38\NJ-45.D ES-API, Pos, Scan, Frag: 70
394.0
226.0
395.0
227.0
787.0
m/z





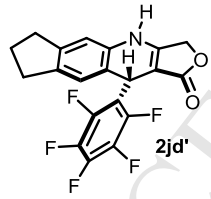

DAD1 C, Sig=210,8 Ref=360,100 (LAKSHMI-03-03-152015-03-0315-42-01\MF-28.D)
mAU
Peak RetTime Type Width Area Height Area
[min] [min] [mAU*s] [mAU] %
1 7.744 MM 0.5409 550.46490 16.96046 2.4930
2 8.932 MM 0.6339 2.15303e4 566.08337 97.5070
7.744
Area: 550.46
8.932
Area: 21530.3
min
ELS1 A, Voltage (LAKSHMI-03-03-152015-03-0315-42-01\MF-28.D)
mV
Peak RetTime Type Width Area Height Area
[min] [min] [mV*s] [mV] %
1 9.094 MM 0.6096 599.67603 16.39614 100.0000
9.094
Area: 599.676
min
*MSD1 SPC, time=8.756:9.687 of D:\DATA\LAKSHMI-03-03-152015-03-0514-35-38\MF-28.D ES-API, Pos, Scan, Frag: 70
348.0
349.0
226.1
716.9
722.2
m/z

DMSO-d_6 (400 MHz)

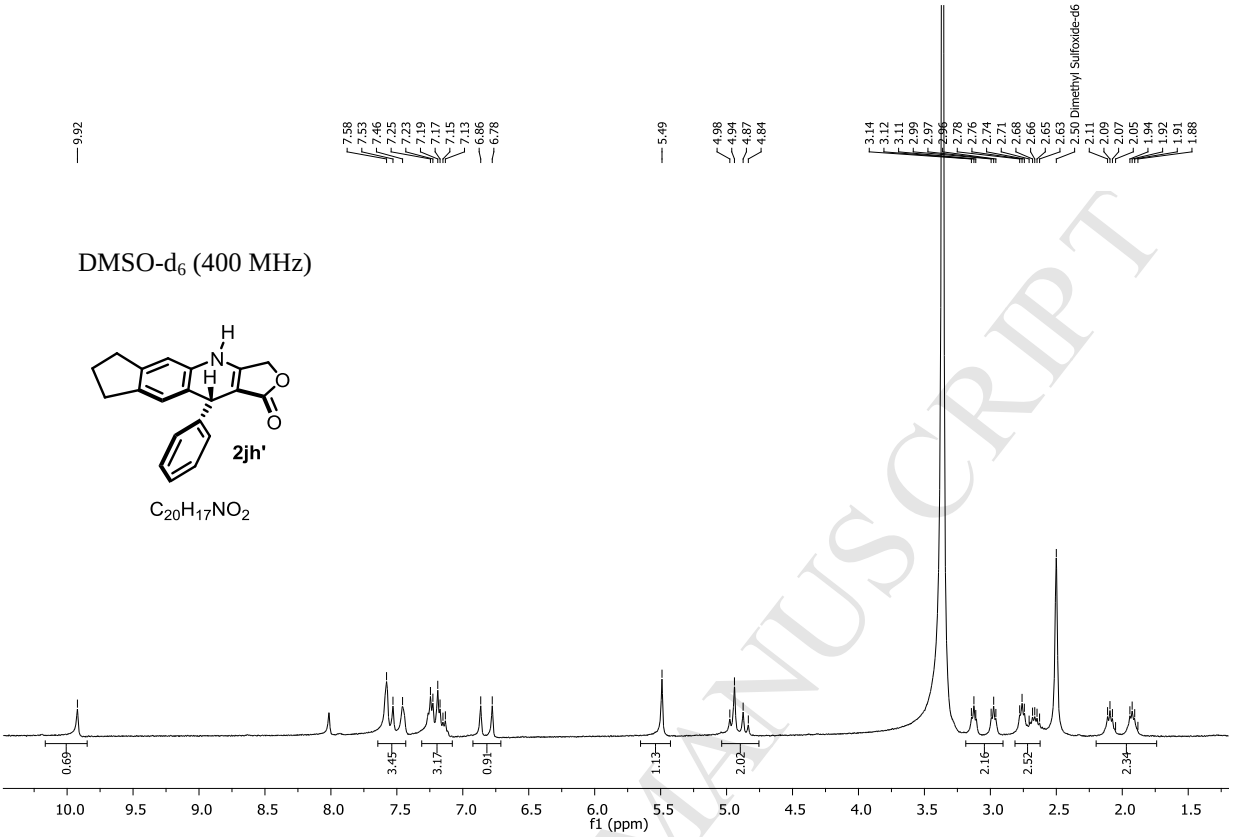


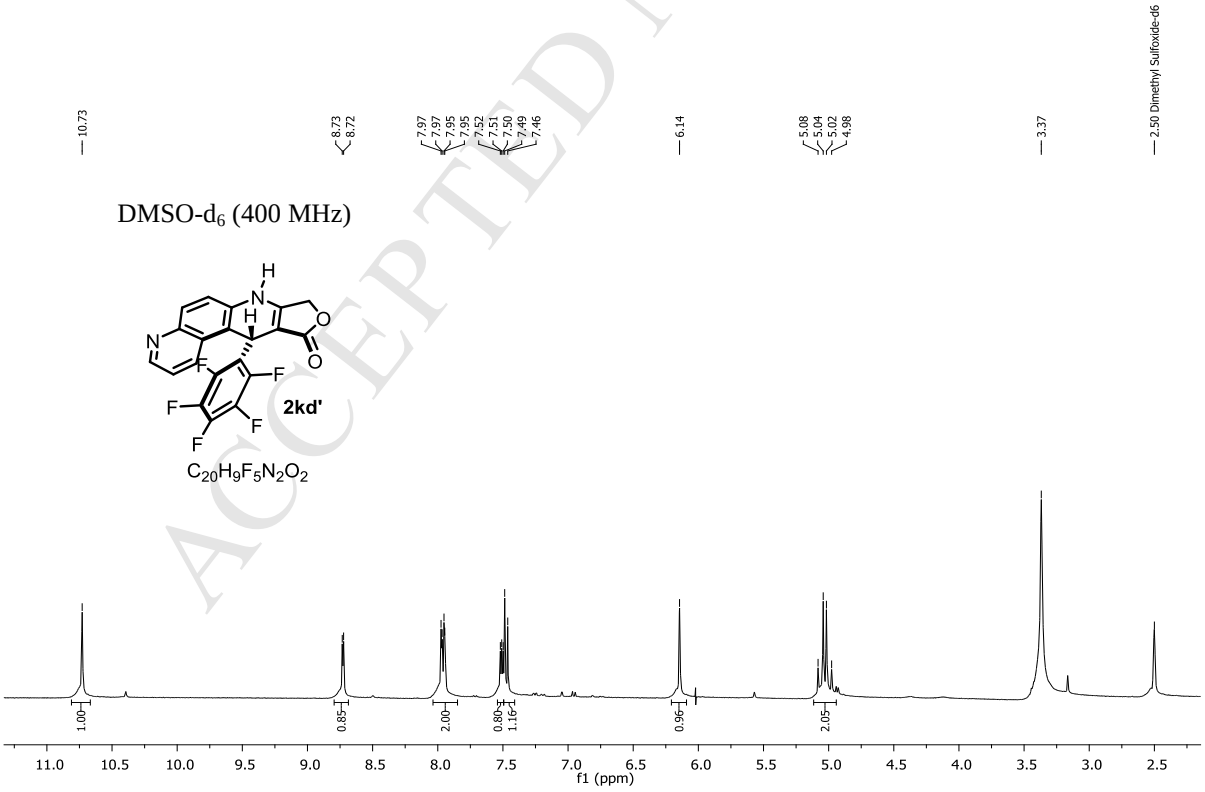
$C_{20}H_{12}F_5NO_2$

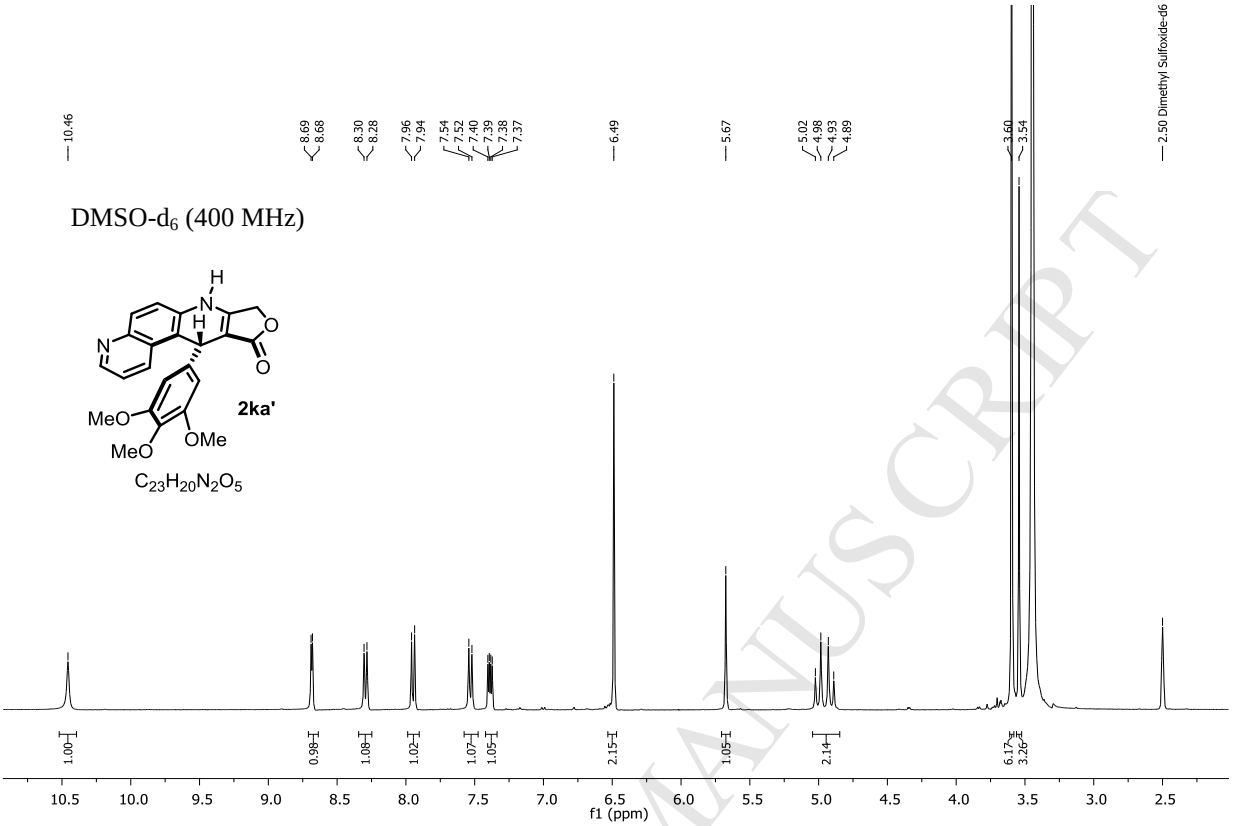
DMSO-d_6 (100 MHz)

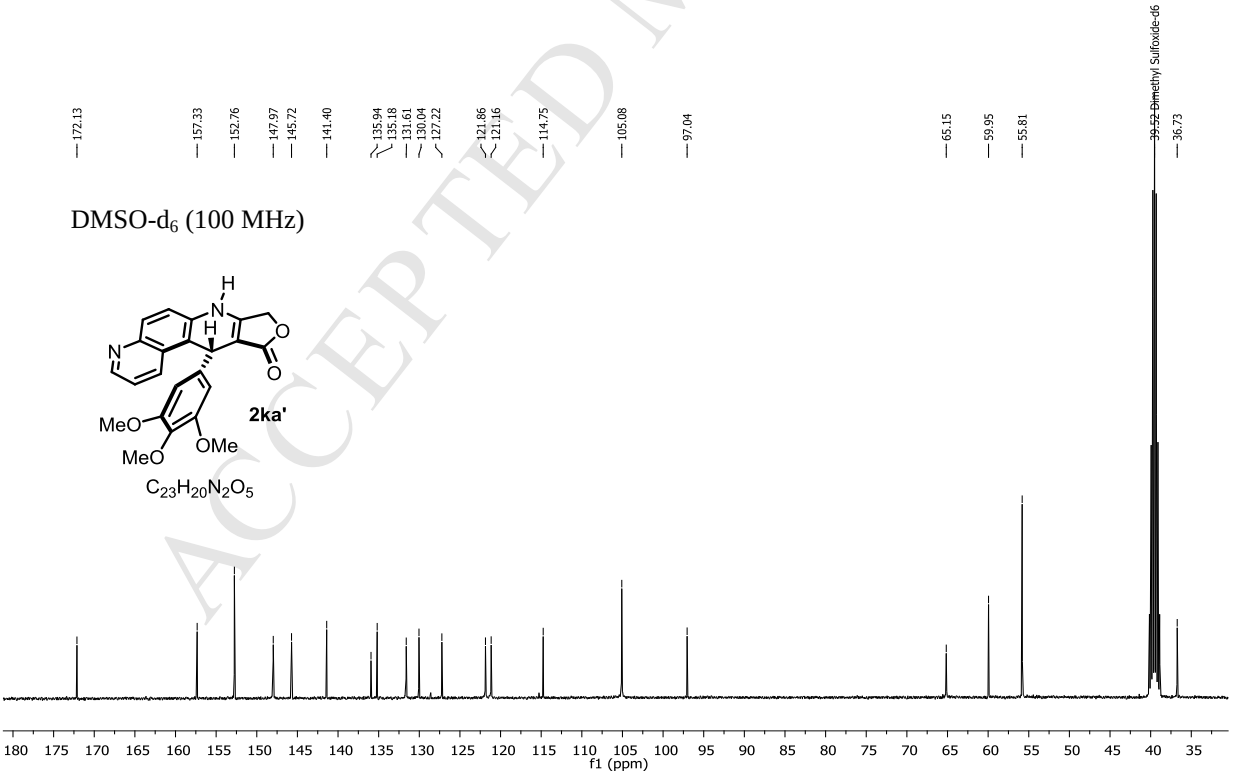


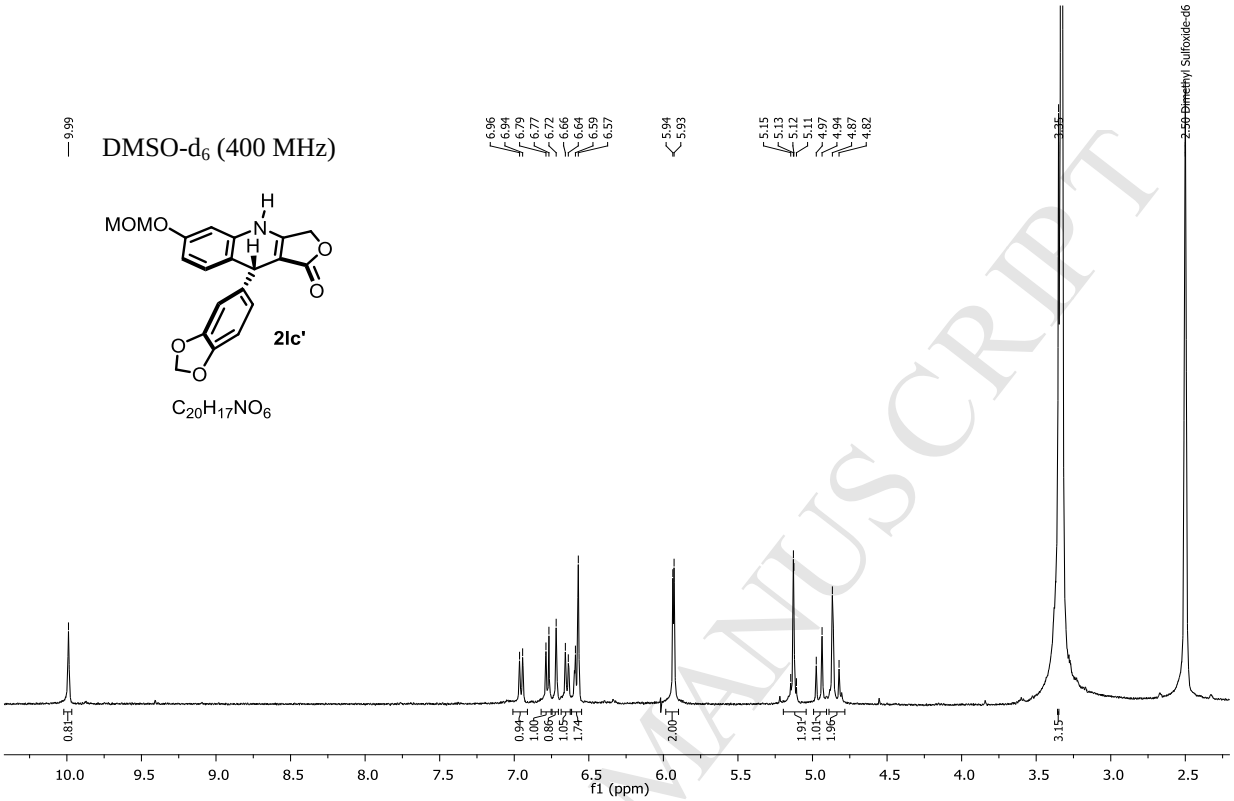
$C_{20}H_{12}F_5NO_2$





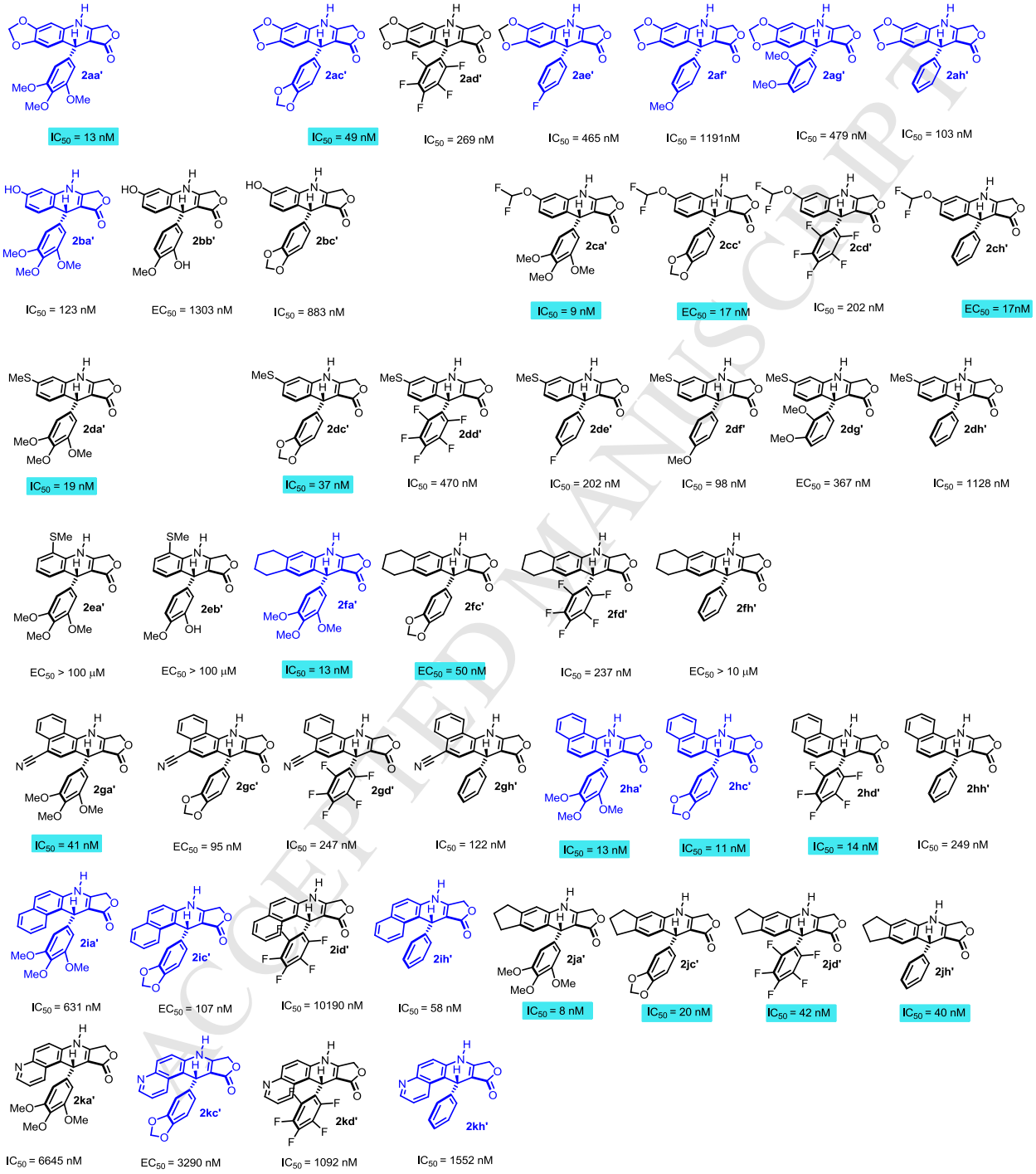






IV. BIOLOGICAL EVALUATION

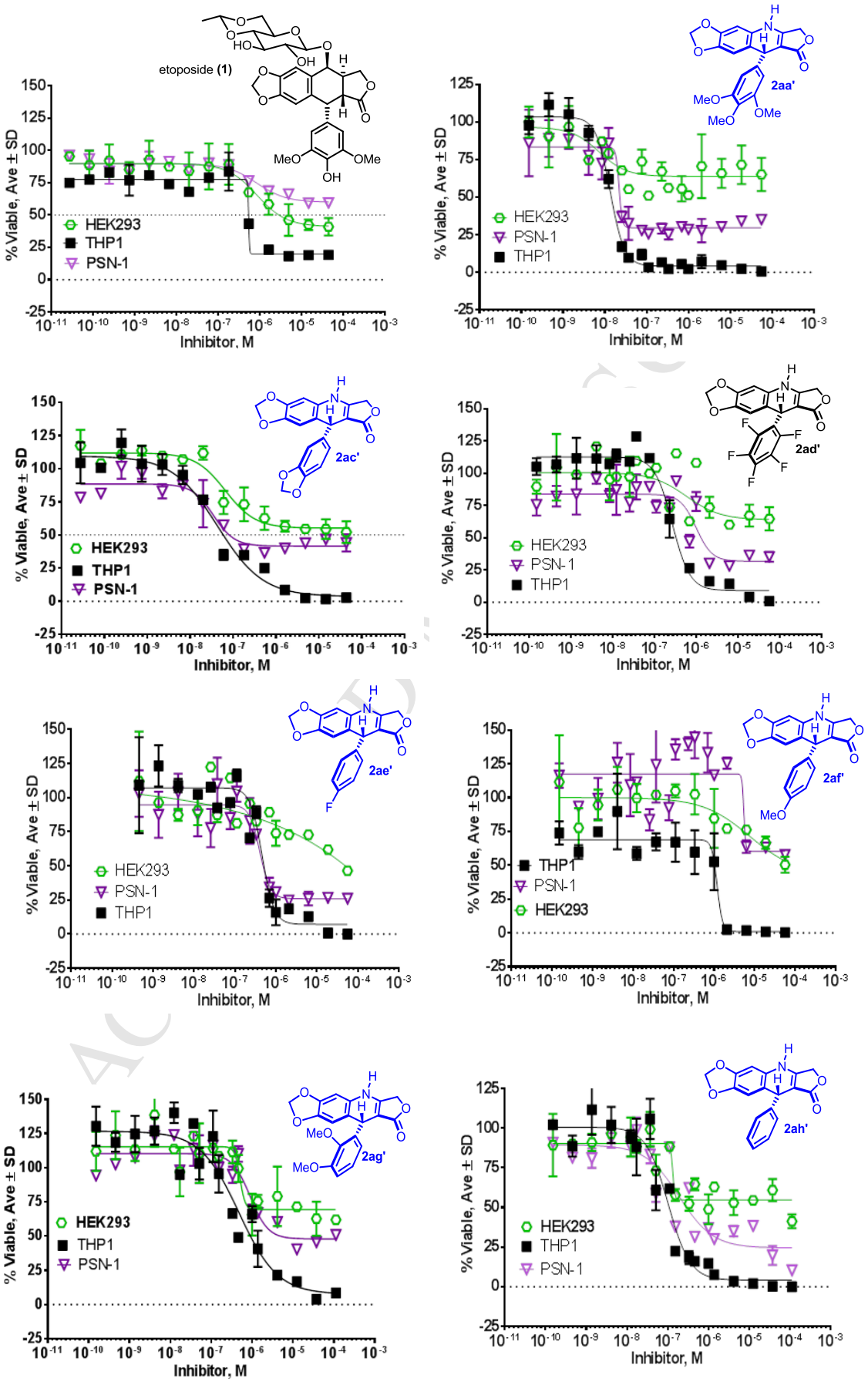
Scheme SI-2. Biological evaluation of a library of 4-aza-2,3didehydropodophyllotoxins (APT) **2** [a,b]

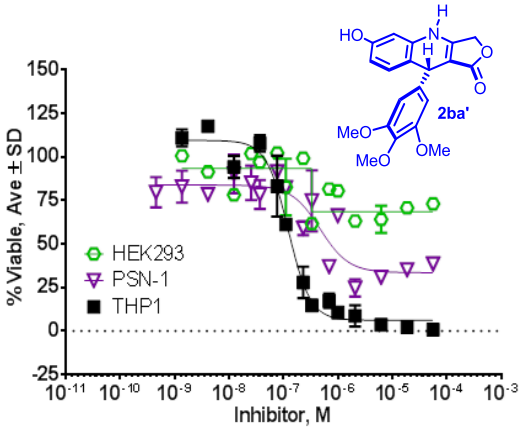


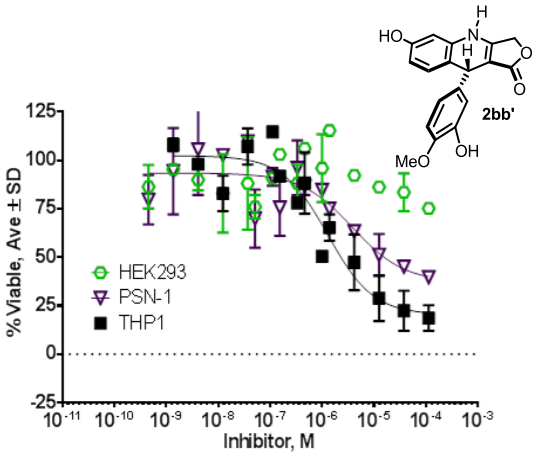
[a]Note. Compounds presented in blue have been synthesized previously and compounds in black are reported for the first time in our library. [b] The reported IC_{50} or EC_{50} values are against the human THP-1 leukemia cell line.

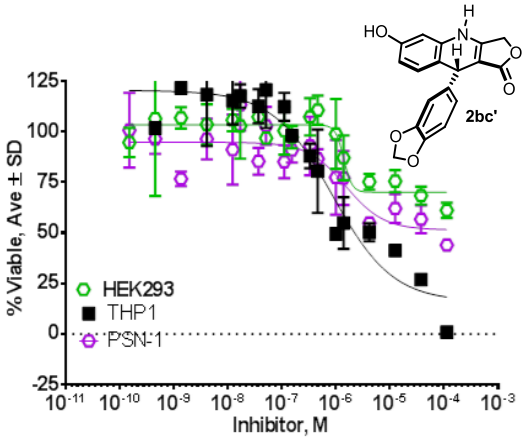
CellTiter-Glo® cell viability assays. Test compounds were solubilized in 100% DMSO and added to polypropylene 384 well plates (Greiner cat# 781280). 1,250 of THP-1, PSN-1, or HEK293 cells were plated in 384-well plates in 8 µl of serum-free media (RPMI-1640 for THP-1 and PSN-1, EMEM for HEK293). Test compounds and etoposide (pharmacological assay control) were prepared as 10-point, 1:3 serial dilutions starting at 10 mM, then added to the cells using the pin tool mounted on Biomek NXP. Plates were incubated for 72 h at 37°C, 5% CO_2 and 95% RH. After incubation, 8 µL of CellTiter-Glo® (Promega cat#: G7570) were added to each well, and incubated for 15 min at room temperature. Luminescence was recorded using a Biotek Synergy H4 multimode microplate reader. Viability was expressed as a percentage relative to wells containing media only (0%) and wells containing cells treated with 1% DMSO only (100%). Three parameters were calculated on a per-plate basis: (a) the signal-to-background ratio (S/B); (b) the coefficient for variation [CV; CV = (standard deviation/mean) x 100)] for all compound test wells; and (c) the Z'-factor. The IC_{50} value of the pharmacological control (etoposide, LC Laboratories # E-4488) was also calculated to ascertain the assay robustness. Each dose response curve was examined and curve type was determined according to Inglese et al.. In cases where a complete response was observed (viability < 20%, both asymptotes present) the IC_{50} value derived from GraphPad software was used. In cases of partial response (viability 20-50%, both asymptotes present) the lowest concentration that induced > 50% viability loss was assigned as EC_{50} value. In cases where the highest efficacy observed was > 50% viability, IC_{50} value was assigned as " > highest concentration tested" (*e.g.*, > 100 µM).

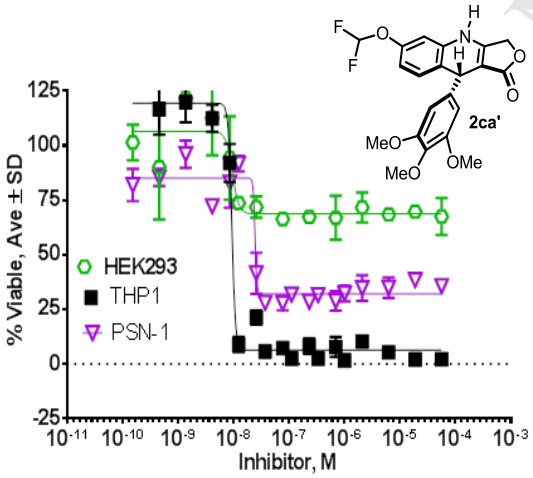
Figure SI-1. Biological activity ploys presenting IC_{50} and EC_{50} for all APT **2** synthesized

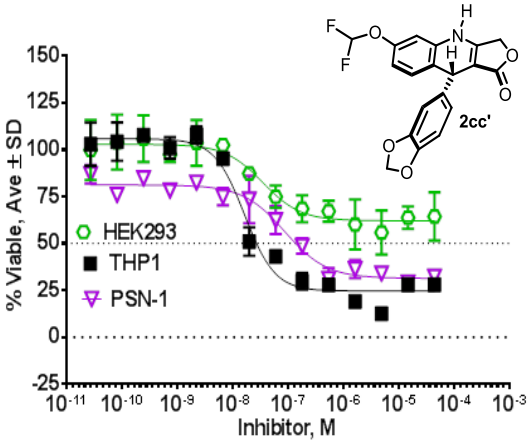


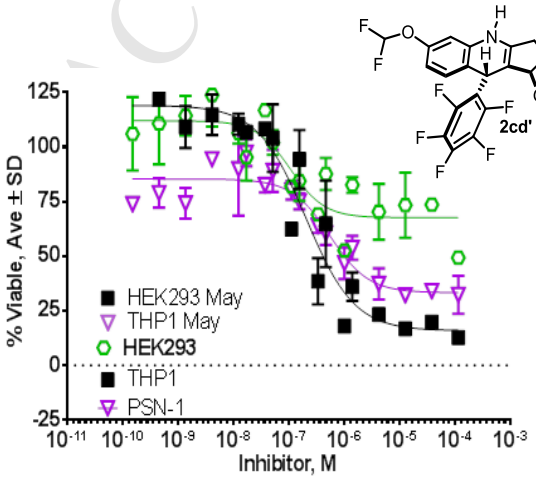

2ba'
% Viable, Ave ± SD
Inhibitor, M
HEK293
PSN-1
THP1

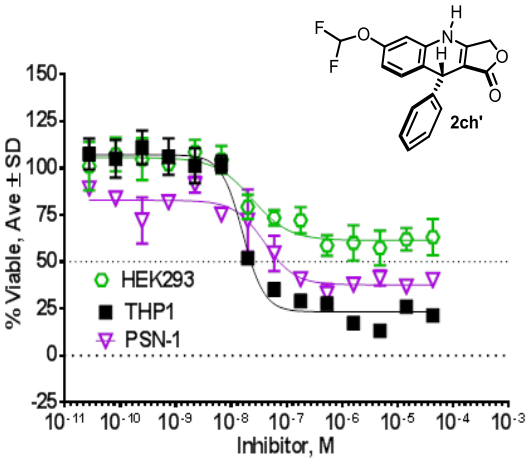

2bb'
% Viable, Ave ± SD
Inhibitor, M
HEK293
PSN-1
THP1

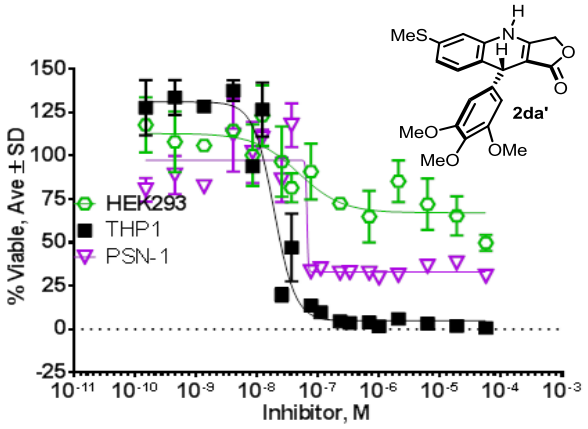

2bc'
% Viable, Ave ± SD
Inhibitor, M
HEK293
THP1
PSN-1

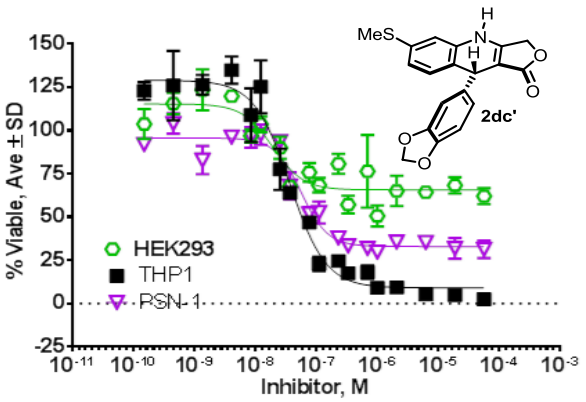

2ca'
% Viable, Ave ± SD
Inhibitor, M
HEK293
THP1
PSN-1

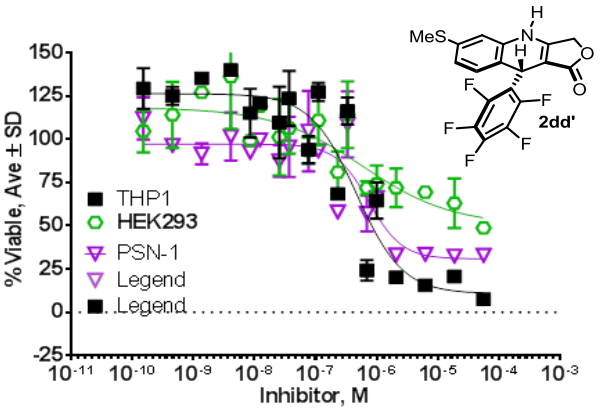

2cc'
% Viable, Ave ± SD
Inhibitor, M
HEK293
THP1
PSN-1

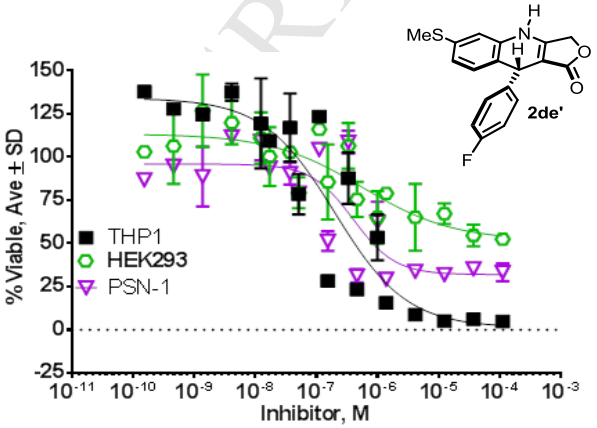

2cd'
% Viable, Ave ± SD
Inhibitor, M
HEK293 May
THP1 May
HEK293
THP1
PSN-1

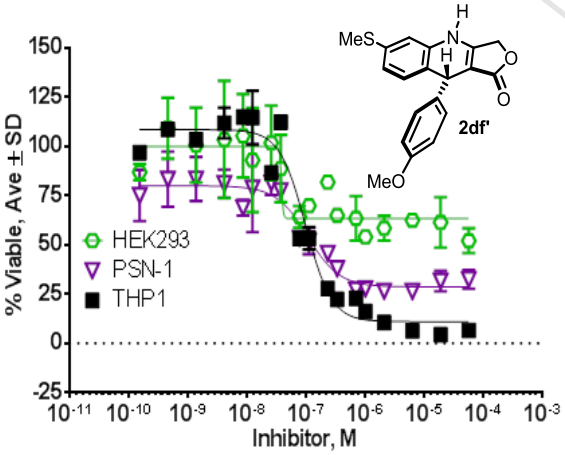

2ch'
% Viable, Ave ± SD
Inhibitor, M
HEK293
THP1
PSN-1

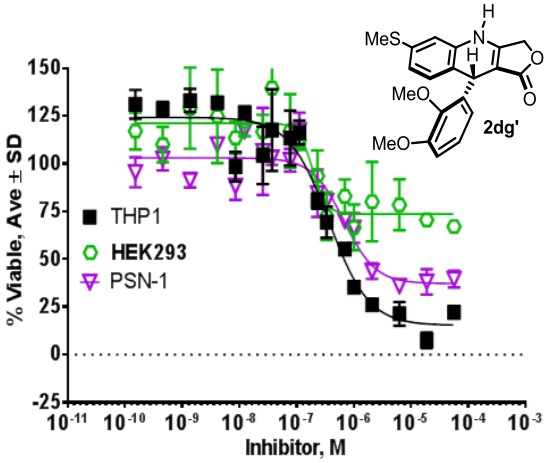

% Viable, Ave ± SD
Inhibitor, M
HEK293
THP1
PSN-1
2da'

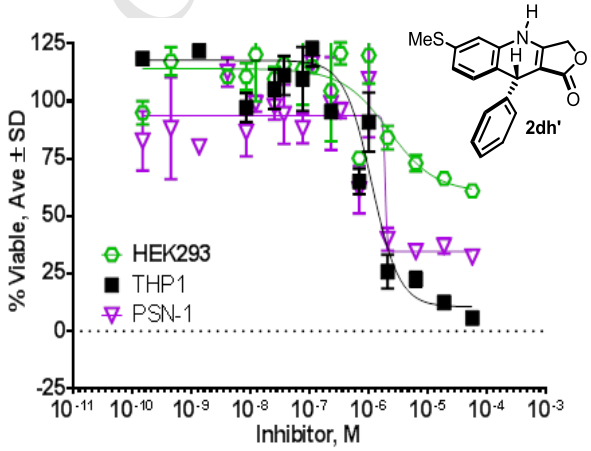

% Viable, Ave ± SD
Inhibitor, M
HEK293
THP1
PSN-1
2dc'

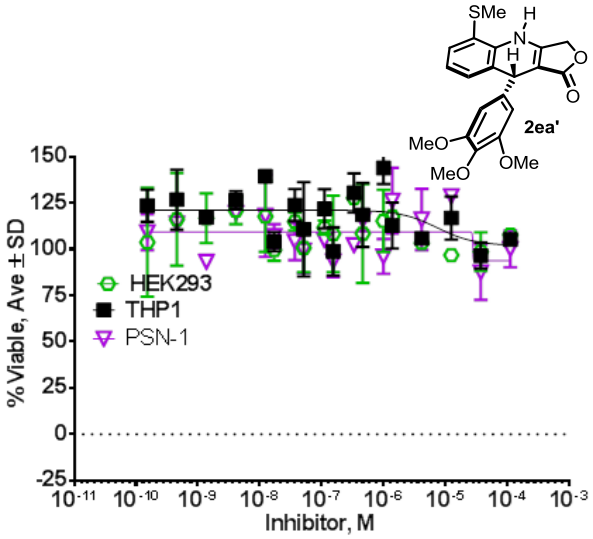

% Viable, Ave ± SD
Inhibitor, M
THP1
HEK293
PSN-1
Legend
Legend
2dd'

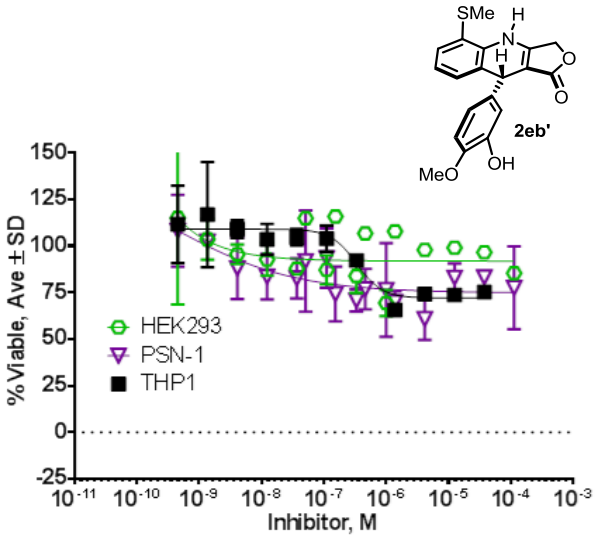

% Viable, Ave ± SD
Inhibitor, M
THP1
HEK293
PSN-1
2de'

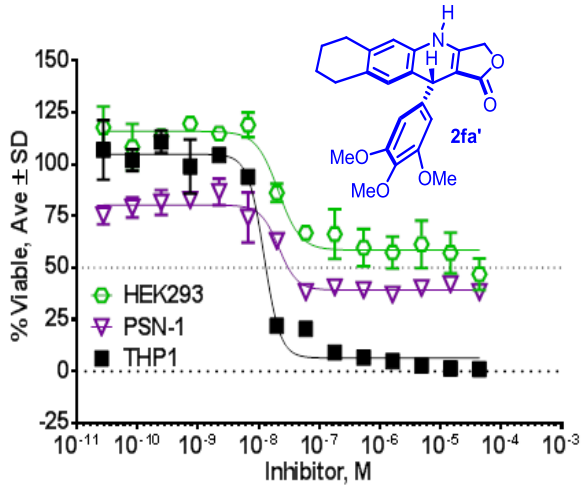

% Viable, Ave ± SD
Inhibitor, M
HEK293
PSN-1
THP1
2df'

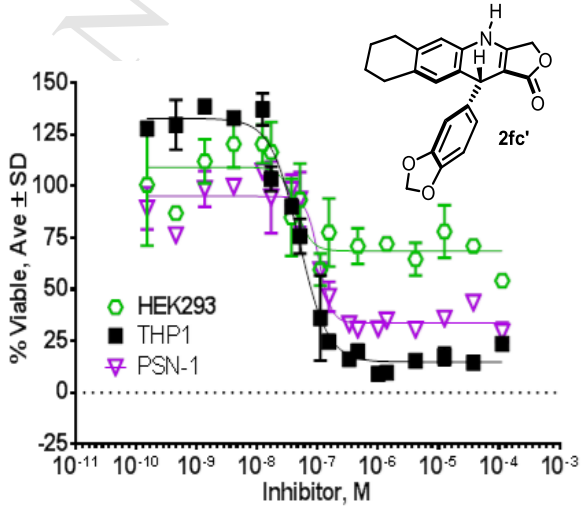

% Viable, Ave ± SD
Inhibitor, M
THP1
HEK293
PSN-1
2dg'

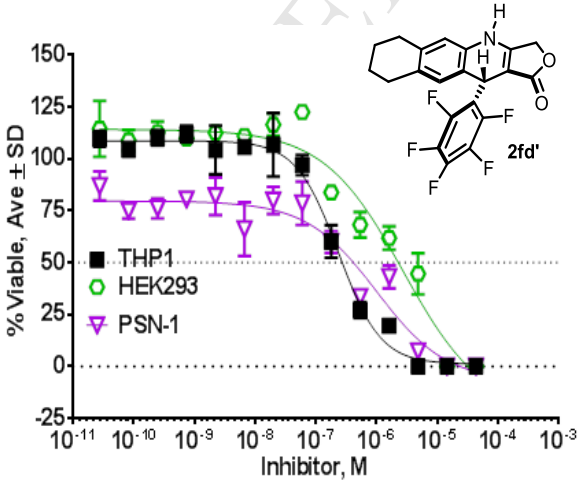

% Viable, Ave ± SD
Inhibitor, M
HEK293
THP1
PSN-1
2dh'

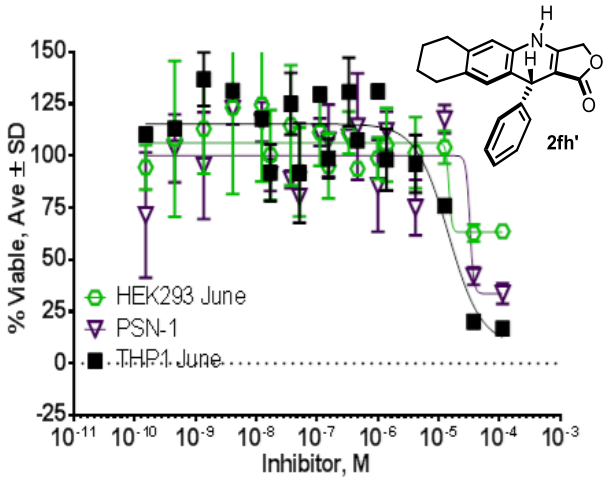

SMe
H
N
H
O
O
2ea'
MeO
MeO
OMe
% Viable, Ave ± SD
HEK293
THP1
PSN-1
Inhibitor, M

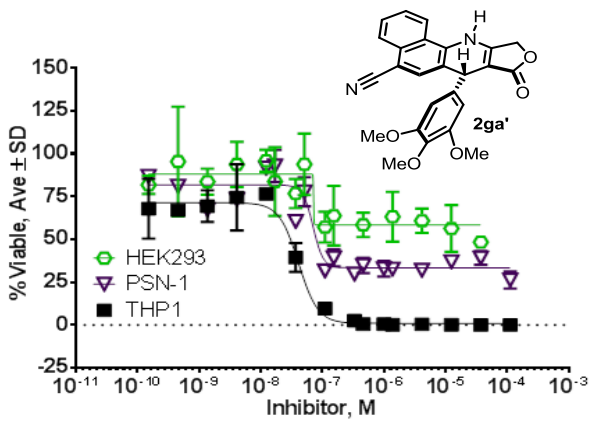

SMe
H
N
H
O
O
2eb'
MeO
OH
% Viable, Ave ± SD
HEK293
PSN-1
THP1
Inhibitor, M

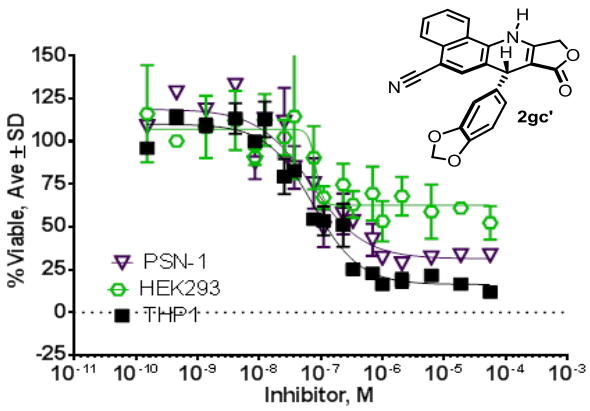

H
N
H
O
O
2fa'
MeO
MeO
OMe
% Viable, Ave ± SD
HEK293
PSN-1
THP1
Inhibitor, M

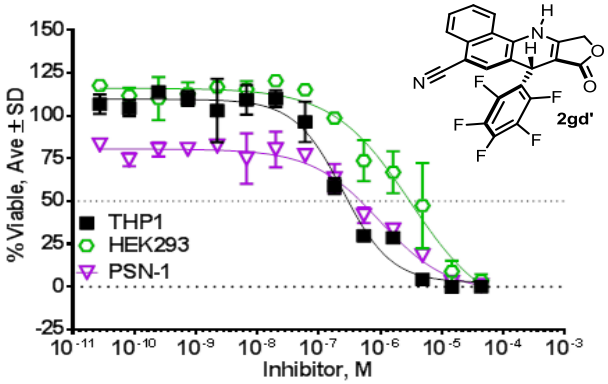

H
N
H
O
O
2fc'
O
O
% Viable, Ave ± SD
HEK293
THP1
PSN-1
Inhibitor, M

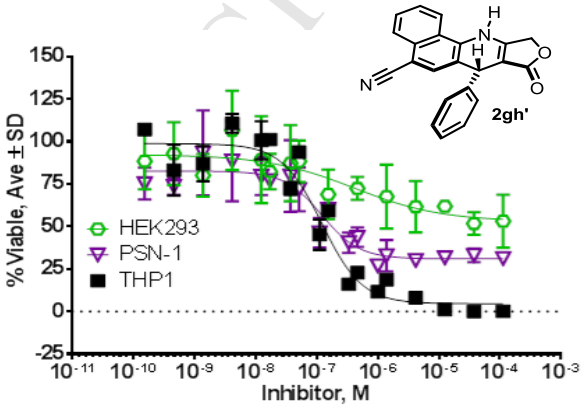

H
N
H
O
O
F
F
F
F
F
2fd'
% Viable, Ave ± SD
THP1
HEK293
PSN-1
Inhibitor, M

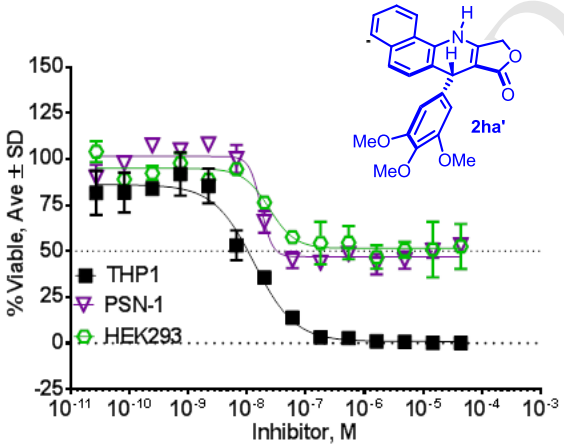

H
N
H
O
O
2fh'
% Viable, Ave ± SD
HEK293 June
PSN-1
THP1 June
Inhibitor, M

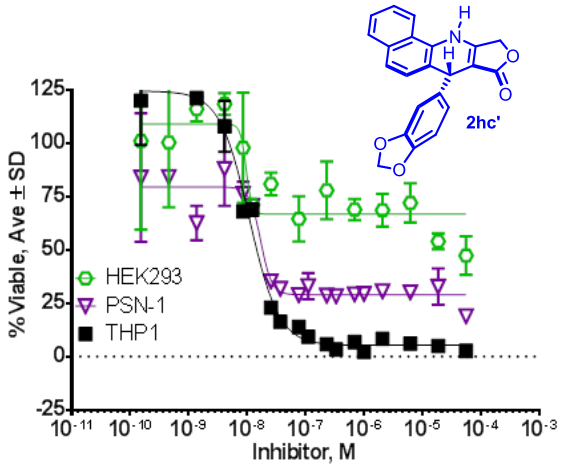

2ga'
MeO
MeO
OMe
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M

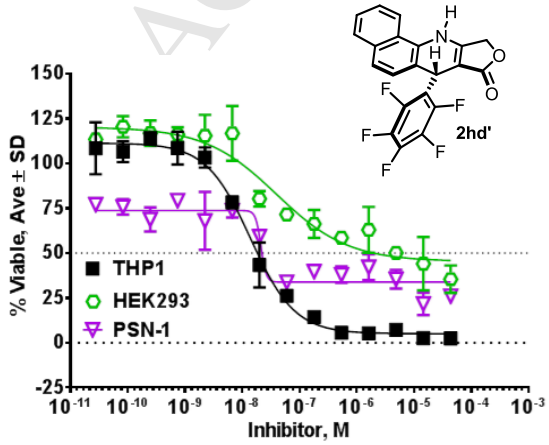

2gc'
PSN-1
HEK293
THP1
% Viable, Ave ± SD
Inhibitor, M

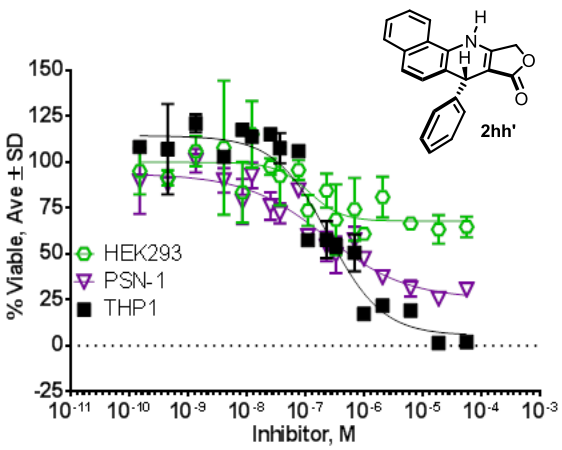

2gd'
THP1
HEK293
PSN-1
% Viable, Ave ± SD
Inhibitor, M

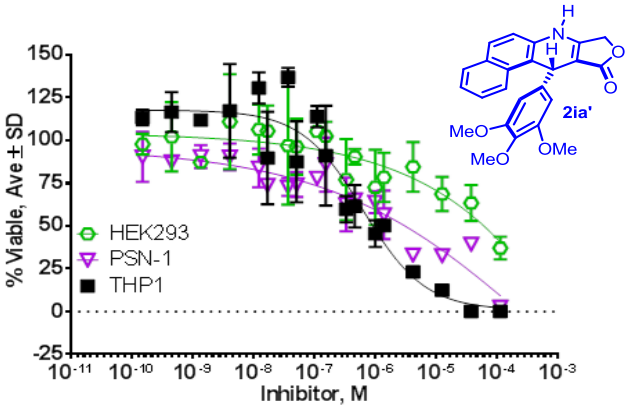

2gh'
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M

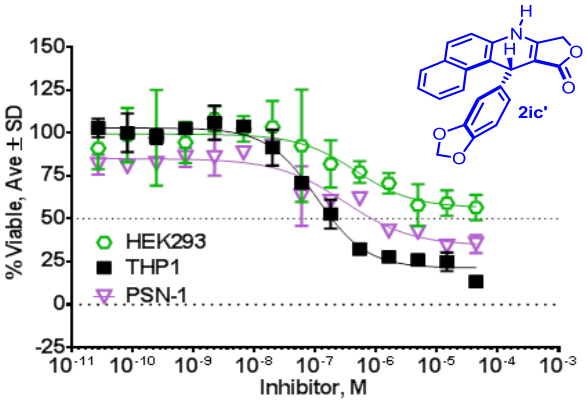

2ha'
MeO
MeO
OMe
THP1
PSN-1
HEK293
% Viable, Ave ± SD
Inhibitor, M

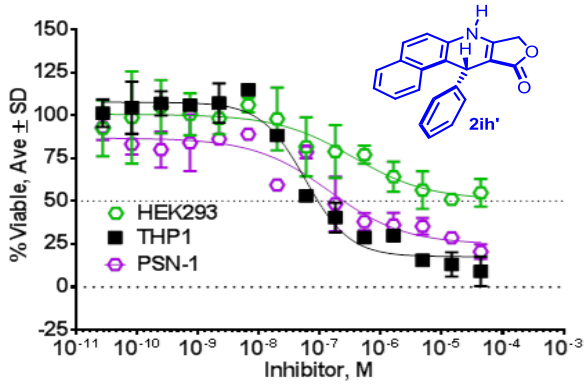

2hc'
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M

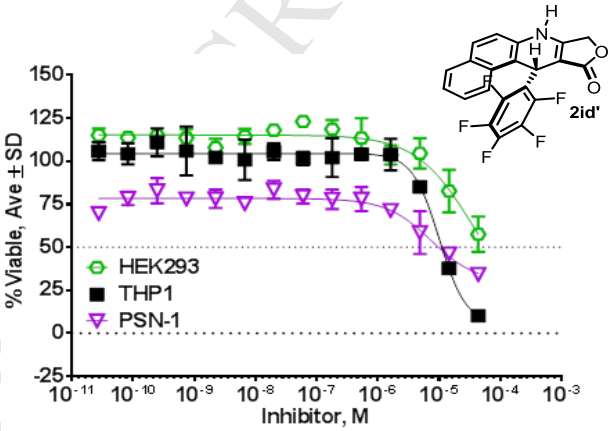

2hd'
THP1
HEK293
PSN-1
% Viable, Ave ± SD
Inhibitor, M

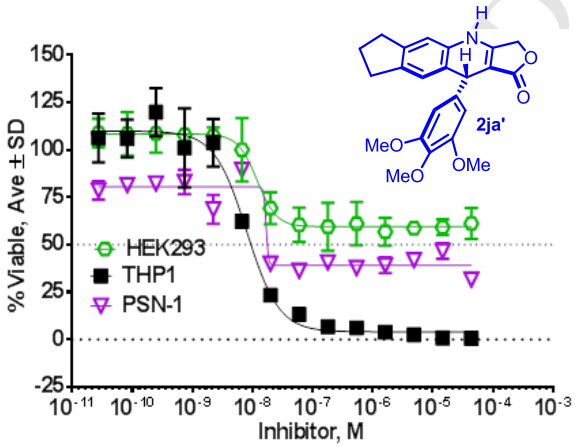

2hh'
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M

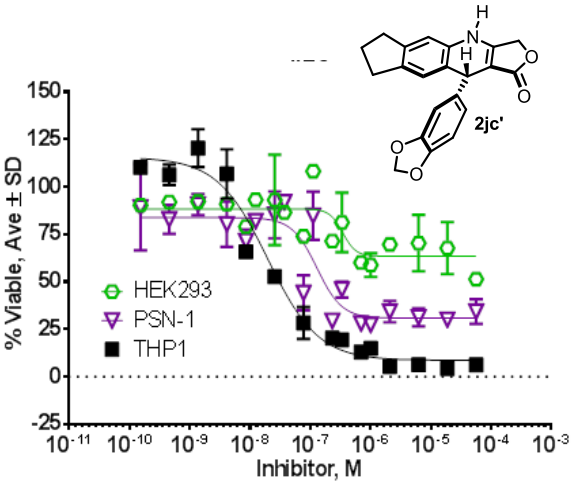

2ia'
MeO
MeO
OMe
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M

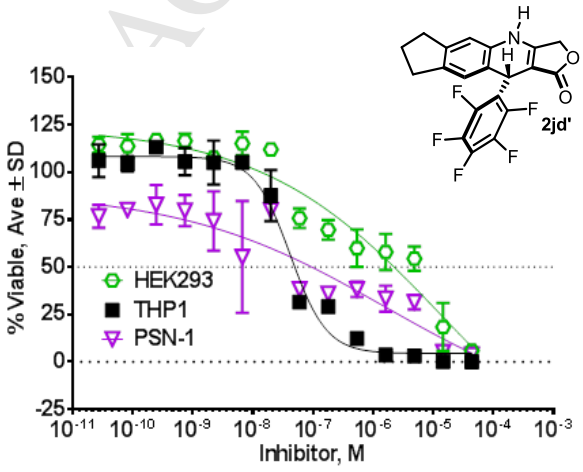

2ic'
HEK293
THP1
PSN-1
% Viable, Ave ± SD
Inhibitor, M

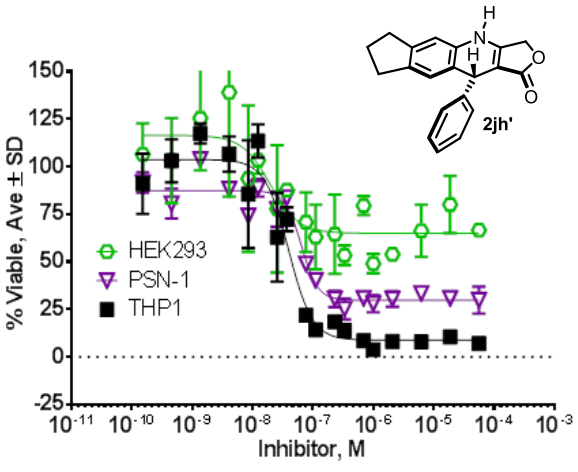

2ih'
HEK293
THP1
PSN-1
% Viable, Ave ± SD
Inhibitor, M

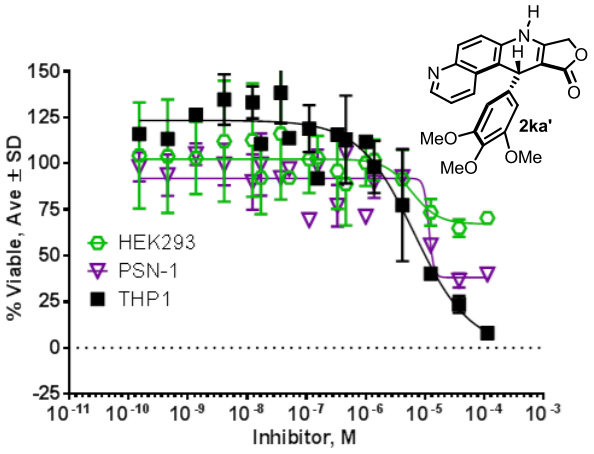

2id'
HEK293
THP1
PSN-1
% Viable, Ave ± SD
Inhibitor, M

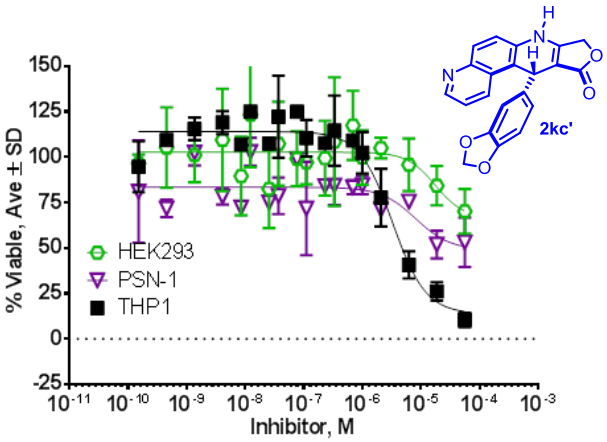

2ja'
MeO
MeO
OMe
HEK293
THP1
PSN-1
% Viable, Ave ± SD
Inhibitor, M

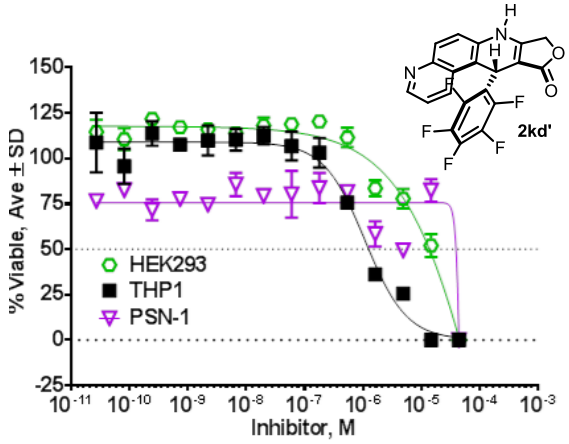

2jc'
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M


2jd'
HEK293
THP1
PSN-1
% Viable, Ave ± SD
Inhibitor, M


2jh'
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M







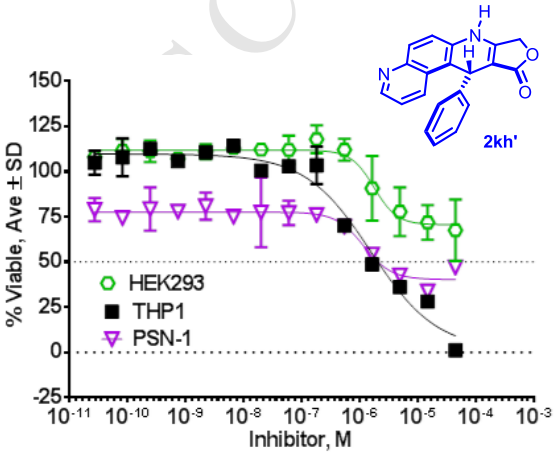
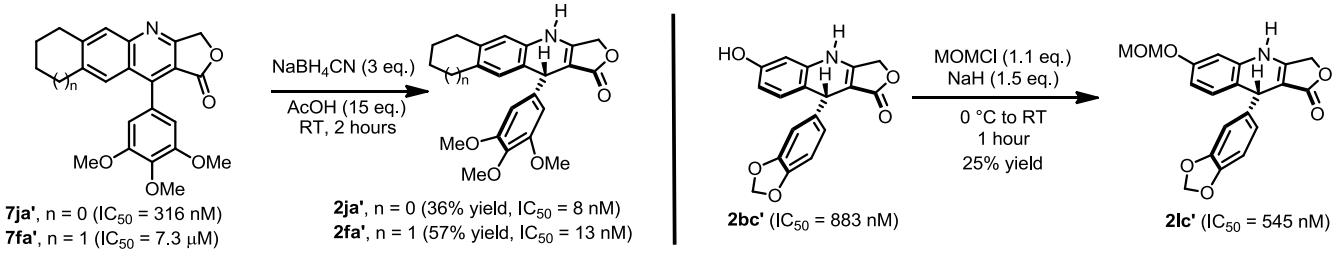


Table SI-1. Biological evaluation of the APT library. In cases where maximal response was greater than 20% EC_{50} value is reported instead of IC_{50}.

Entry	Compound	THP1 (Leukemia) IC_{50} M (EC_{50} M)	PSN1 (Pancreatic cancer) EC_{50} M (IC_{50} M)	HEK, (non-malignant kidney) EC_{50} M (IC_{50} M)
1	**etoposide**	(540 ± 42 x 10^{-9})	>50 X 10^{-6}	4645± 419 x 10^{-9}
2	**2aa'**	13.5 ± 1.41 x 10^{-9}	22.0 ± 1.91 x 10^{-9}	> 100 X 10^{-6}
3	**2ac'**	49 ± 3 x 10^{-9}	180 ± 13 x 10^{-9}	>50 X 10^{-6}
4	**2ad'**	269 ± 24 x 10^{-9}	1002 ± 91 x 10^{-9}	> 100 X 10^{-6}
5	**2ae'**	465 ± 42 x 10^{-9}	405 ± 39 x 10^{-9}	> 100 X 10^{-6}
6	**2af'**	1191 ± 89 x 10^{-9}	> 100 X 10^{-6}	> 100 X 10^{-6}
7	**2ag'**	479± 49 x 10^{-9}	> 10.0 X 10^{-6}	> 100 X 10^{-6}
8	**2ah'**	103±9.2 x 10^{-9}	191 ± 16 x 10^{-9}	> 100 X 10^{-6}
9	**2ba'**	123 ± 12 x 10^{-9}	473 ± 42 x 10^{-9}	> 100 X 10^{-6}
10	**2bb'**	(1303 ± 120 x 10^{-9})	(3891 ± 420 x 10^{-9})	(> 100 X 10^{-6})
11	**2bc'**	(883 ± 7.2 x 10^{-9})	(100 ± 153 X 10^{-6})	(> 100 X 10^{-6})
12	**2ca'**	9.3 ± 0.81 x 10^{-9}	24.3 ± 2.41 x 10^{-9}	> 100 X 10^{-6}
13	**2cc'**	(17 ± 2 x 10^{-9})	(88 ± 6 x 10^{-9})	(>50 X 10^{-6})
14	**2cd'**	(202 ± 9.0 x 10^{-9})	(549 ± 30 x 10^{-9})	(> 100 X 10^{-6})
15	**2ch'**	(17 ± 2 x 10^{-9})	(40 ± 3 x 10^{-9})	(>50 X 10^{-6})
16	**2da'**	19.4 ± 1.9 x 10^{-9}	66.3 ± 6.9 x 10^{-9}	> 100 X 10^{-6}
17	**2dc'**	37 ± 3.0 x 10^{-9}	56.7 ± 5.41 x 10^{-9}	> 100 X 10^{-6}
18	**2dd'**	470 ± 39 x 10^{-9}	745 ± 69 x 10^{-9}	> 100 X 10^{-6}
19	**2de'**	202± 19 x 10^{-9}	402 ± 28 x 10^{-9}	> 100 X 10^{-6}
20	**2df'**	98 ± 6.3 x 10^{-9}	106 ± 10 x 10^{-9}	> 100 X 10^{-6}
21	**2dg'**	(367± 39 x 10^{-9})	(670 ± 58 x 10^{-9})	(> 100 X 10^{-6})
22	**2dh'**	1128 ± 81 x 10^{-9}	1902 ± 171 x 10^{-9}	> 100 X 10^{-6}
23	**2ea'**	(> 100 X 10^{-6})	(> 100 X 10^{-6})	(> 100 X 10^{-6})
24	**2eb'**	(> 100 X 10^{-6})	(> 100 X 10^{-6})	(> 100 X 10^{-6})
25	**2fa'**	13 ± 1.1 x 10^{-9}	61 ± 6 x 10^{-9}	>50 X 10^{-6}
26	**2fc'**	(50 ± 3.0 x 10^{-9})	(106 ± 11 x 10^{-9})	(> 100 X 10^{-6})
27	**2fd'**	237 ± 22 x 10^{-9}	1004 ± 100 x 10^{-9}	2742± 219 x 10^{-9}
28	**2fh'**	(> 10 X 10^{-6})	(> 10 X 10^{-6})	(> 100 X 10^{-6})
29	**2ga'**	41 ± 3 x 10^{-9}	71 ± 4.1 x 10^{-9}	> 100 X 10^{-6}
30	**2gc'**	(95 ± 6 x 10^{-9})	(213 ± 20.1 x 10^{-9})	(> 100 X 10^{-6})
31	**2gd'**	247 ± 22 x 10^{-9}	968± 93 x 10^{-9}	3063 ± 219 x 10^{-9}
32	**2gh'**	122 ± 11 x 10^{-9}	121 ± 10 x 10^{-9}	> 100 X 10^{-6}

Entry	Compound	THP1 (Leukemia) IC_{50} M (EC_{50} M)	PSN1 (Pancreatic cancer) EC_{50} M (IC_{50} M)	HEK, (non-malignant kidney) EC_{50} M (IC_{50} M)
33	**2ha'**	13 ± 2 x 10^{-9}	60 ± 5.1 X 10^{-9}	>50 X 10^{-6}
34	**2hc'**	10.9 ± 1.41 x 10^{-9}	17.1 ± 2.0 x 10^{-9}	> 100 X 10^{-6}
35	**2hd'**	14 ± 2 x 10^{-9}	60 ± 5 x 10^{-9}	>50 X 10^{-6}
36	**2hh'**	249 ± 22 x 10^{-9}	222 ± 19 x 10^{-9}	> 100 X 10^{-6}
37	**2ia'**	631± 59 x 10^{-9}	4547± 419 x 10^{-9}	> 100 X 10^{-6}
38	**2ic'**	(107 ± 10 x 10^{-9})	(1620 ± 159 x 10^{-9})	(>50 X 10^{-6})
39	**2id'**	10.2 ± 1.2 x 10^{-6}	>50 X 10^{-6}	>50 X 10^{-6}
40	**2ih'**	58 ± 5.2 x 10^{-9}	144 ± 9 x 10^{-9}	>50 X 10^{-6}
41	**2ja'**	8 ± 0.7 x 10^{-9}	20 ± 2 x 10^{-9}	>50 X 10^{-6}
42	**2jc'**	19.6 ± 1.41 x 10^{-9}	129 ± 10 x 10^{-9}	> 100 X 10^{-6}
43	**2jd'**	42± 3 x 10^{-9}	65 ± 10 x 10^{-9}	14 ± 1.2 x 10^{-6}
44	**2jh'**	39.7 ±3.5 x 10^{-9}	64.6 ± 7.1 x 10^{-9}	> 100 X 10^{-6}
45	**2ka'**	6645± 619 x 10^{-9}	> 10 X 10^{-6}	> 100 X 10^{-6}
46	**2kc'**	(3290 ± 320 x 10^{-9})	(> 100 X 10^{-6})	(> 100 X 10^{-6})
47	**2kd'**	1092 ± 100 x 10^{-9}	>50 X 10^{-6}	>50 X 10^{-6}
48	**2kh'**	1552 ± 120 x 10^{-9}	1159 ± 120 x 10^{-9}	>50 X 10^{-6}

Scheme SI-3. Modulations of the APTs pharmacophores



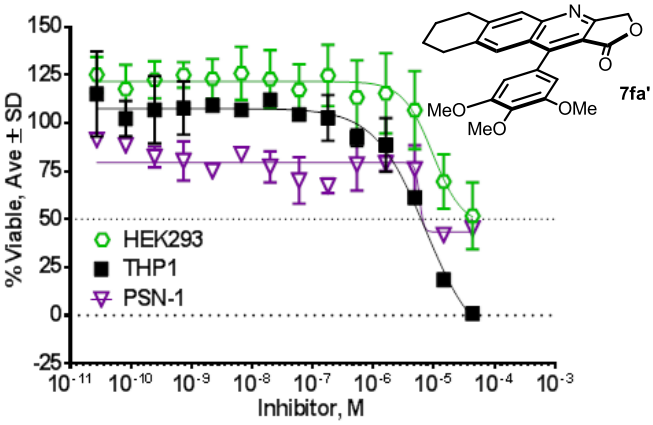
7ja', n = 0 (IC_{50} = 316 nM)
7fa', n = 1 (IC_{50} = 7.3 μM)

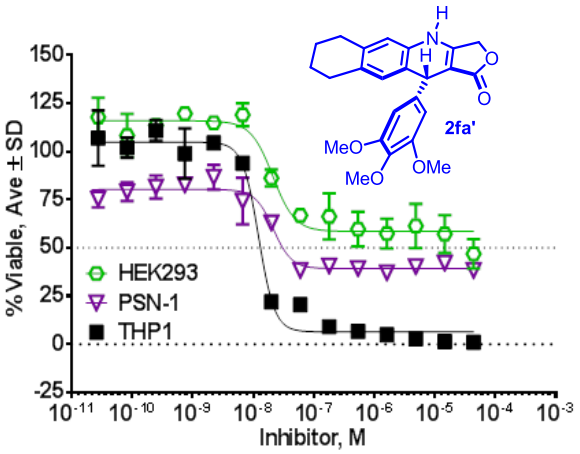
2ja', n = 0 (36% yield, IC_{50} = 8 nM)
2fa', n = 1 (57% yield, IC_{50} = 13 nM)

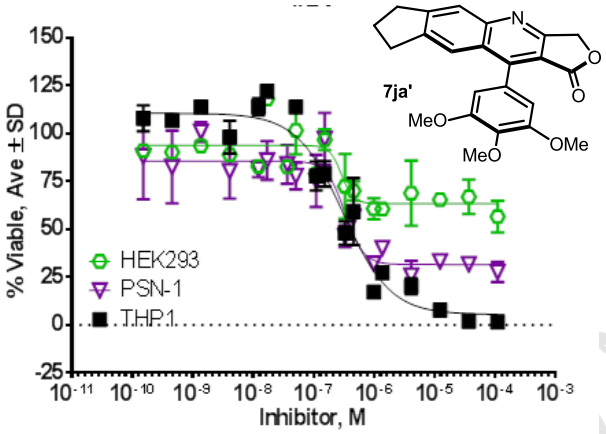
2bc' (IC_{50} = 883 nM)

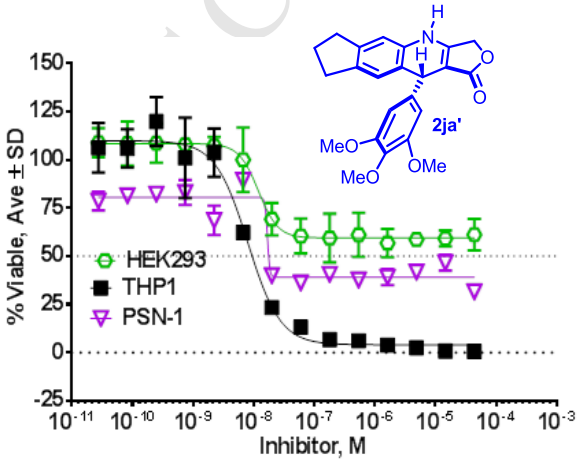
2lc' (IC_{50} = 545 nM)

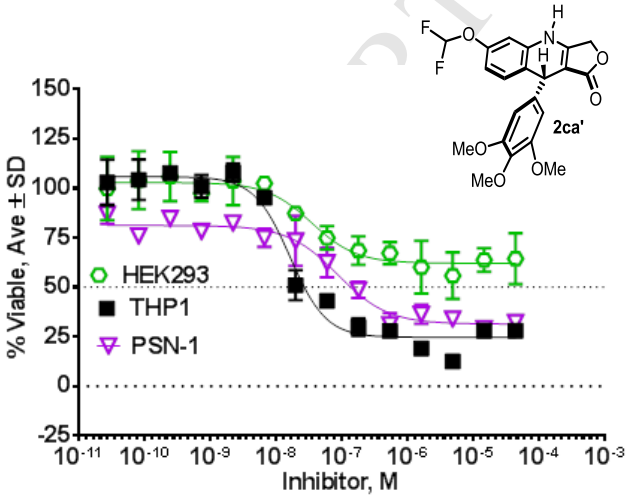
Entry	Compound	THP1 (Leukemia) IC_{50} M (EC_{50} M)	PSN1 (Pancreatic cancer) EC_{50} M (IC_{50} M)	HEK, (non-malignant kidney) EC_{50} M (IC_{50} M)
1	**7ja'**	316 ± 32 x 10^{-9}	335 ± 32 x 10^{-9}	> 100 X 10^{-6}
2	**2ja'**	8 ± 0.7 x 10^{-9}	60 ± 6 x 10^{-9}	>50 X 10^{-6}
3	**7fa'**	7332 ± 720 x 10^{-9}	>50 X 10^{-6}	>50 X 10^{-6}
4	**2fa'**	13 ± 1.1 x 10^{-9}	61 ± 6 x 10^{-9}	>50 X 10^{-6}
5	**2bc'**	883 ± 7.2 x 10^{-9}	100 ± 153 X 10^{-6}	> 100 X 10^{-6}
6	**2lc'**	545 ± 52 x 10^{-9}	>50 X 10^{-6}	>50 X 10^{-6}


7fa'
MeO
MeO
OMe
HEK293
THP1
PSN-1
% Viable, Ave ± SD
Inhibitor, M


2fa'
MeO
MeO
OMe
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M


7ja'
MeO
MeO
OMe
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M


2ja'
MeO
MeO
OMe
HEK293
THP1
PSN-1
% Viable, Ave ± SD
Inhibitor, M


2ca'
MeO
MeO
OMe
HEK293
THP1
PSN-1
% Viable, Ave ± SD
Inhibitor, M

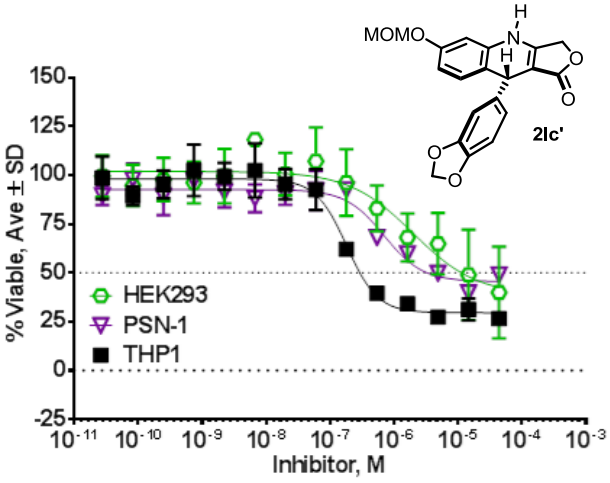
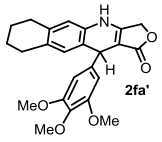
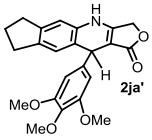
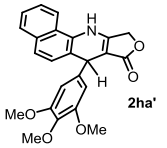
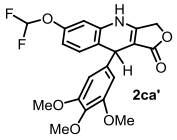
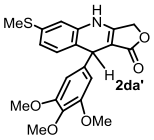
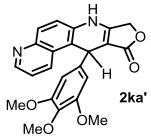
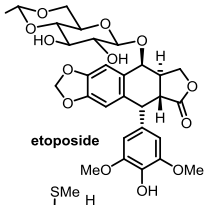
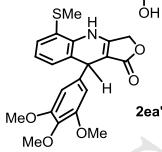
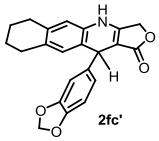
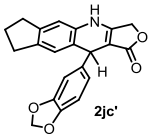
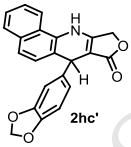
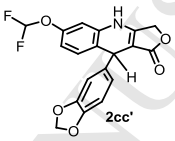
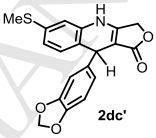
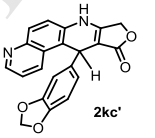
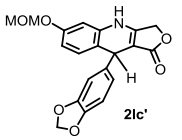
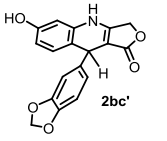

MOMO
2lc'
HEK293
PSN-1
THP1
% Viable, Ave ± SD
Inhibitor, M

Table SI-2. Selected physicochemical properties for several APT molecules

Entry	compound	THP-1 cell lines IC_{50} (nM)	cLogP	TPSA (Å^2)	H-bond donor & acceptor count
1	 **2fa'**	13 ± 1.1	3.7	66.02	6
2	 **2ja'**	8 ± 0.7	3.26	66.02	6
3	 **2ha'**	13 ± 2	3.24	66.02	6
4	 **2ca'**	9.3 ± 0.81	3.02	75.25	7
5	 **2da'**	19.4 ± 1.9	2.88	66.02	6
6	 **2ka'**	6645 ± 619	2.41	78.91	7
7	 **etoposide**	540 ± 42	1.16	160.83	15
8	 **2ea'**	>100	2.88	66.02	6
9	 **2fc'**	50 ± 3.0	3.8	56.79	5
10	 **2jc'**	19.6 ± 1.41	3.35	56.79	5
11	 **2hc'**	10.9 ± 1.41	3.33	56.79	5
12	 **2cc'**	17 ± 2	3.11	66.02	6
13	 **2dc'**	37 ± 3.0	2.97	56.79	5
14	 **2kc'**	3290 ± 320	2.5	69.68	6
15	 **2lc'**	545 ± 52	2.25	75.25	7
16	 **2bc'**	883 ± 7.2	2.04	77.02	7

Calculated physicochemical properties.

Lipophilicity is an important physicochemical property that plays a major role in identifying the suitable drug candidates because it encrypts how a compound or drug transportation is affected by inter- and intramolecular forces. Etoposide **1** exhibits poor physicochemical properties for a drug, likely causing its modest solubility in water (cLogP =1.16) and absorption. In fact, **1** disobeys three of the five Lipinski rules[SI-2] with a high molecular weight (MW of 588.6 DA >500

[SI-2] Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **2012**, *64*, 4-17.

DA) presents 12 hydrogen-bond acceptor sites (HBA>10) and has a topological polar surface area of 160.83 Å^2 (>140 Å^2).

Our approach to the APT-library diversity was to maintain a low toxicity against non-malignant cells and improve the compounds Absorption, Distribution, Metabolism and Excretion properties (ADME) in comparison to the reference drug etoposide **1**. In the present library, all the synthetic analogues have very similar molecular weights (MW< 450 Da) and number of rotatable bonds (RtB). For this purpose, specific physicochemical parameters have been analyzed: cLogP values (cLogP < 3-5)[SI-3] to enhance absorption and cell permeation, topological polar surface area values (61-75 Å^2< TPSA in Å^2<140 Å^2)[SI-4] to maintain a good availability and transport of the molecules and finally a direct correlation was proposed with the numbers of hydrogen-bond acceptor and donors sites (HBA+HBD < 12) to potentially increase the number of available binding sites and enhance chemical interactions with the cellular biological target. Partition coefficient values (cLogP), the total number of HBA/HBD sites at physiological pH and the TPSA were calculated for all APT molecules from the library, using the Plexus discovery software from Plexus Suite which is a web-based software package that integrates the ChemAxon's structure-based property calculations application.[SI-5] A meaningful selected data set of these properties is presented in **Table-SI2**. All the calculated physicochemical values for these compounds (cLogP < 3.7; 56 Å^2< TPSA <79 Å^2; HBA+HBD<7) were in acceptable range according to the literature which does not afford a satisfactory explanation to interpret the low IC_{50} values for several compounds such as **2ka'**, **2kc'**, **2ia'**, **2ic'**or **2ea'**. Although the calculated physicochemical values could not be directly correlated to the biological activity and IC_{50} values (against leukemia), a trend can be observed. For example, in the series of compounds **2bc'**, **2lc'**, **2dc'**, **2cc'**, **2hc'** and **2jc'**, the lipophilicity increased substantially (cLogP = 2.0, 2.2, 3.0, 3.1, 3.3 and 3.35) which also correspond to an increase in cell permeation and in biological activity (corresponding IC_{50} values of 883, 545, 37, 17, 11 and 20 nM). The increase in lipophilicity resulting from the ether appendages (*e.g.* **2bc'** vs **2lc'** and **2cc'**) at C7 may well favor a better cell permeation without affecting the binding properties of the small-molecules. Therefore we could conclude that lead compounds such as **2ca'** and **2cc'** may present an interesting balance between a relatively high lipophilicity to facilitate cell permeation (cLogP ~ 3.0) while presenting a relatively large TPSA (TPSA ~ 70 Å^2) to maintain an important binding affinity to the cellular target. Also, as shown by recent studies, compounds with cLogP values< 3 are likely to present less toxicity.[SI-3]

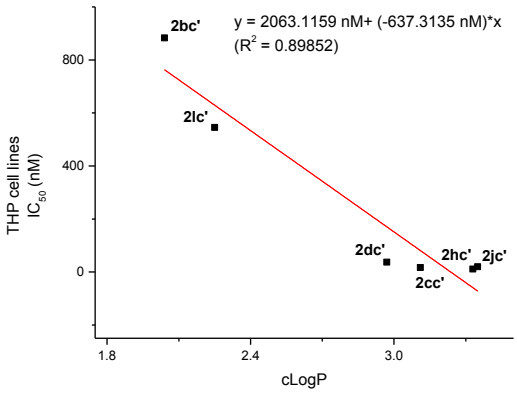


Figure SI-2. Relationship between IC_{50} and clogP values for a series of ATP molecules

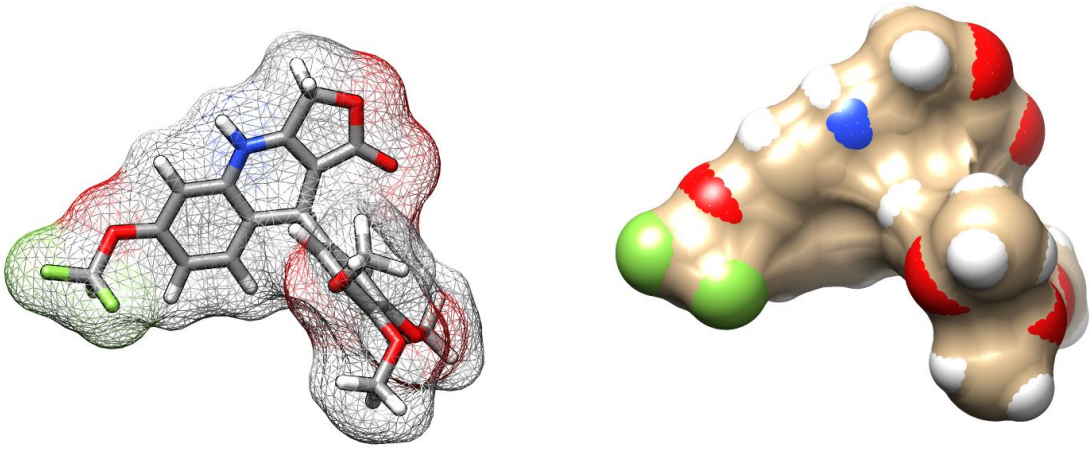
[SI-3] Abad-Zapatero, C. Ligand efficiency indices for effective drug discovery. *Expert Opin. Drug Discov.* **2007**, *2*, 469-488.

[SI-4] Palm, K.; Luthman, K.; Ungell, A.-L.; Strandlund, G.; Artursson, P. Correlation of drug absorption with molecular surface properties. *J. Pharm. Sci.* **1996**, *85*, 32-39.

[SI-5] https://www.chemaxon.com/products/calculator-plugins/

Overall, we are delighted that most APT-molecules from the synthetic library have a much better "drugability" profile than the parent and reference drug **1** which likely translated in molecules such as **2ca'**, **2cc'**, **2da'**, **2fa'**, **2ha'**, **2ja'** and **2jc'** to be highly potent and selective against leukemia. Based on the aforementioned physicochemical properties, the APT molecules synthesized in this study demonstrated high solubility, a favorable cell permeability and an important level of biological validation, therefore these small-molecules should have the appropriate structural and electronic features (high TPSA and count of HBA+HBD) to induce a high binding affinity to the biological target. Our preliminary data showed that compounds bearing a 3,4,5-trimethoxy substitution pattern on the **E-ring** are slightly more potent against the leukemia THP-1 cells than compounds having a 3,4-dioxolane (**Table SI-2**, entry 1 vs 9, entry 2 vs 10, entry 4 vs 12, entry 5 vs 13). Positional substitution on the **B-ring** is also key to the biological activity and the best pharmacophore positioned at C6, C7 and C8 are presented in **Table SI-2**. From our preliminary data, it appears that functionalization on the A/B-rings has a drastic influence on the biological activity with **2ca'** and **2da'** being two of the most active compounds tested to date (R = $OCHF_2$ or SCH_3 at position C7).The degree of interaction or affinity of the APT molecules and the biological target leading to a potent activity on leukemia THP-1 cells, can be related mostly to the stereoelectronic pattern of substitution on the B-ring.

Figure SI-2. Visualization of topological polar surface area (TPSA) of the lead compound **2ca'**



SMILES	Compound_ID	IC_{50}
O=C(OC1)C2=C1NC3=CC(OCO4)=C4C=C3C2C5=CC(OCO6)=C6C=C5	**2ac'**	49 nM
O=C(OC1)C2=C1NC3=CC(OCO4)=C4C=C3C2C5=C(F)C(F)=C(F)C(F)=C5F	**2ad'**	269 nM
O=C(OC1)C2=C1NC3=CC(OCO4)=C4C=C3C2C5=CC=C(OC)C=C5	**2af'**	1191 nM
O=C(OC1)C2=C1NC3=CC(O)=CC=C3C2C4=CC=C(OC)C(O)=C4	**2bb'**	1303 nM
O=C(OC1)C2=C1NC(C=C(OC(F)F)C=C3)=C3C2C4=CC(OCO5)=C5C=C4	**2cc'**	17 nM
FC(F)OC1=CC=C(C(C2=C(F)C(F)=C(F)C(F)=C2F)C(C(OC3)=O)=C3N4)C4=C1	**2cd'**	202 nM
FC(F)OC(C=C1)=CC2=C1C(C(C(OC3)=O)=C3N2)C4=CC=CC=C4	**2ch'**	17 nM
O=C(OC1)C2=C1NC3=CC(SC)=CC=C3C2C4=CC(OC)=C(OC)C(OC)=C4	**2da'**	19 nM
CSC1=CC=C(C(C2=C(F)C(F)=C(F)C(F)=C2F)C(C(OC3)=O)=C3N4)C4=C1	**2dd'**	470 nM
CSC1=CC=C(C(C2=CC=C(F)C=C2)C(C(OC3)=O)=C3N4)C4=C1	**2de'**	202 nM
CSC1=CC=C(C(C2=C(OC)C(OC)=CC=C2)C(C(OC3)=O)=C3N4)C4=C1	**2dg'**	367 nM
O=C(OC1)C2=C1NC3=C(SC)C=CC=C3C2C4=CC(OC)=C(OC)C(OC)=C4	**2ea'**	>100 µM
O=C(OC1)C2=C1NC3=C(SC)C=CC=C3C2C4=CC=C(OC)C(O)=C4	**2eb'**	>100 µM
O=C1C2=C(C3=CC(OC)=C(OC)C(OC)=C3)C4=CC5=C(CCCC5)C=C4N=C2CO1	**7fa'**	7.3 µM
O=C(OC1)C2=C1NC3=CC(CCCC4)=C4C=C3C2C5=CC(OC)=C(OC)C(OC)=C5	**2fa'**	13 nM
O=C(OC1)C2=C1NC3=CC(CCCC4)=C4C=C3C2C5=C(F)C(F)=C(F)C(F)=C5F	**2fd'**	237 nM
O=C(OC1)C2=C1NC3=CC(CCCC4)=C4C=C3C2C5=CC=CC=C5	**2fh'**	>50 µM
N#CC1=C2C(C=CC=C2)=C3C(C(C4=CC(OC)=C(OC)C(OC)=C4)C(C(OC5)=O)=C5N3)=C1	**2ga'**	41 nM
N#CC1=C2C(C=CC=C2)=C3C(C(C4=CC(OCO5)=C5C=C4)C(C(OC6)=O)=C6N3)=C1	**2gc'**	95 nM
O=C(OC1)C2=C1NC3=C(C=CC=C4)C4=C(C#N)C=C3C2C5=C(F)C(F)=C(F)C(F)=C5F	**2gd'**	247 nM
O=C1OCC2=C1[C@H](C3=CC(OC)=C(OC)C(OC)=C3)C(C=CC4=C5C=CC=C4)=C5N2	**2ha'**	13 nM
O=C(OC1)C2=C1NC3=C(C=CC=C4)C4=CC=C3C2C5=C(F)C(F)=C(F)C(F)=C5F	**2hd'**	17 nM
O=C1C2=C(C3=CC(OC)=C(OC)C(OC)=C3)C4=CC5=C(CCC5)C=C4N=C2CO1	**2ja'**	8 nM
O=C(OC1)C2=C1NC3=CC(CCC4)=C4C=C3C2C5=CC(OC)=C(OC)C(OC)=C5	**7ja'**	316 nM
O=C(OC1)C2=C1NC(C=C(CCC3)C3=C4)=C4C2C5=CC=C6C(OCO6)=C5	**2jc'**	20 nM
FC(C(F)=C(F)C(F)=C1F)=C1C2C3=CC4=C(CCC4)C=C3NC5=C2C(OC5)=O	**2jd'**	42 nM
O=C(OC1)C2=C1NC(C=C(CCC3)C3=C4)=C4C2C5=CC=CC=C5	**2jh'**	40 nM
O=C(OC1)C2=C1NC(C=CC3=C4C=CC=N3)=C4C2C5=CC(OC)=C(OC)C(OC)=C5	**2ka'**	6645 nM
O=C(OC1)C2=C1NC3=CC=C(N=CC=C4)C4=C3C2C5=C(F)C(F)=C(F)C(F)=C5F	**2kd'**	1092 nM
O=C(OC1)C2=C1NC3=CC(OCOC)=CC=C3C2C4=CC(OCO5)=C5C=C4	**2lc'**	545 nM