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Development of 1,8-Naphthalimides as Clathrin Inhibitors

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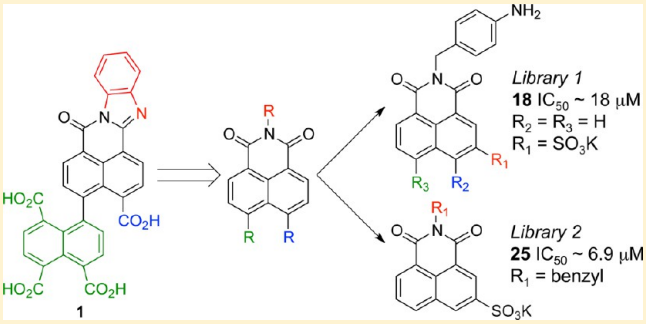
Supporting Information

ABSTRACT: We reported the first small molecule inhibitors of the interaction between the clathrin N-terminal domain (TD) and endocyctic accessory proteins (i.e., clathrin inhibition[1]). Initial screening of a ∼17 000 small molecule ChemBioNet library identified **1**. Screening of an existing in-house propriety library identified four substituted 1,8-napthalimides as ∼80−120 μM clathrin inhibitors. Focused library development gave 3-sulfo-*N*-(4-aminobenzyl)-1,8-naphthalimide, potassium salt (**18**, $IC_{50} \approx 18$ μM). A second library targeting the 4-aminobenzyl moiety was developed, and four analogues displayed comparable activity (**26**, **27**, **28**, **34** with IC_{50} values of 22, 16, 15, and 15 μM respectively) with a further four (**24**, **25**, **32**, **33**) more active than **18** with IC_{50} values of 10, 6.9, 12, and 10 μM, respectively. Docking studies rationalized the structure−activity relationship (SAR) with the biological data. 3-Sulfo-*N*-benzyl-1,8-naphthalimide, potassium salt (**25**) with an $IC_{50} \approx 6.9$ μM, is the most potent clathrin terminal domain−amphiphysin inhibitor reported to date.



INTRODUCTION

Endocytosis, the process whereby cells internalize membrane receptors, hormones, and nutrients, is pivotal to cellular wellbeing. Normal cells have numerous mechanisms to achieve accurate and efficient endocytosis; however, abnormal endocytosis underlies the pathology of diseases. Endocytosis uses several molecular mechanisms, classified as either clathrin-mediated endocytosis (CME) or as clathrin-independent endocytosis.[2,3] Cells are also capable of internalizing cargo by phagocytosis, macropinocytosis, caveolin-dependent, and clathrin- and caveolin-independent pathways.[4]

CME is the specific process of material uptake into a cell using clathrin-coated vesicles (CCV). CME is a complex, highly orchestrated process requiring the interaction of at least 30 proteins with roles at different stages of the process. CCV formation is initiated by FCHo and AP-2 proteins, which recruit clathrin triskelia to the membrane for coat assembly.[5,6] This is the first of five stages of the CCV cycle: (i) initiation, (ii) cargo selection, (iii) clathrin coat assembly, (iv) scission, and (v) clathrin uncoating, each requiring the synchronization of a wide range of protein−protein interactions to ensure successful cargo internalization.

Protein−protein interactions (PPIs) play a central role in CME. Current tools used to interfere with PPIs, such as monoclonal antibodies, dominant-negative proteins and peptides, suffer from a number of drawbacks that limit their utility.[7] Thus, there is an increasing need for small molecule modulators of PPIs, which could serve as tools for the acute study of physiological processes and potentially for the treatment of human diseases.[8] Gene fusions involving clathrin are found in a number of human cancers, such as an insertion in the CHC gene in DIGeorge syndrome/velocardiofacial syndrome,[9] a fusion of clathrin heavy chain and anaplastic lymphoma kinase (CHC-ALK) in non-Hodgkin's lymphomas (anaplastic large-cell lymphomas) and in inflammatory myofibroblastic tumors,[10] as well as the fusion of clathrin with the transcription factor gene TFE3 in renal adenocarcinomas.[11] Some core endocytic proteins such as clathrin also participate in nuclear signaling and transcriptional regulation, by modulating the activity of various nuclear factors. Many such proteins undergo nuclear translocation and affect gene expression directly (e.g., Hip1, arrestin, AP180/CALM, Dab1, epsin/Eps15, amphiphysin, endophilin).[12,13] Considerable evidence links elements of the CME pathway directly or indirectly to epilepsy in humans and animals. Furthermore, some CME genes cause epileptic-like seizures when genetically knocked-out in animals (e.g., amphiphysin or synaptojanin).[14]

We previously reported the first two small molecule clathrin terminal domain - amphiphysin inhibitors 3-sulfo-*N*-(4-aminobenzyl)-1,8-naphthalimide, potassium salt and (*Z*)-*N*-[5-(4-bromobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl]naphthalene-1-sulfonamide.[1] More recently chemical proteomics has identified bolinaquinone as a clathrin inhibitor.[15] Our clathrin

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inhibitor program commenced with library screening of ~17 000 small molecules from the ChemBioNet central open access technology platform for compounds that inhibit the clathrin N-terminal domain (TD)−amphiphysin interaction (which we herein call clathrin inhibitors).[1] From this we now report that we identified **1**, a binaphthalene tricarboxylic acid derivative, as one of the initial inhibitors of the clathrin TD−amphiphysin interaction at a fixed 100 μM concentration (Figure 1).[1] In this

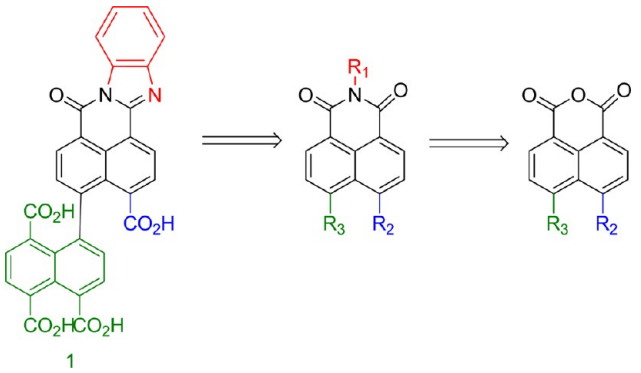


Figure 1. An ELISA-based high-throughput screening (HTS) approach identified (**1**) as an inhibitor of the clathrin TD−amphiphysin interaction. Scaffold simplification suggests a 1,8-naphthalic anhydride minimum pharmacophore.

report we describe how we used **1** as lead to develop a series of 3-sulfo-*N*-(4-aminobenzyl)-1,8-naphthalimide, potassium salt compound analogues, and describe the discovery of analogues that are more potent than this lead compound.

RESULTS

Lead **1** was a poor candidate for structure−activity relationship (SAR) development; its large size and complexity meant that it lacked synthetic tractability, and it also failed to meet development guidelines such as Lipinski's "rule of five". A fragmentation based approach was applied and allowed the development of a minimum pharmacophore based on **1** and is shown schematically in Figure 1.[16−18] The naphthalimide core was viewed as an attractive starting point as (a) it has well developed chemistry amenable to library optimization though the imide (R_1) position;[19,20] and has also been used in DNA targeting binders, anticancer and fluorescent cellular imaging agents;[21] (b) numerous substituted 1,8-naphthyl anhydrides are commercially available or synthetically accessible; and (c) we have developed libraries of naphthalimide based dynamin inhibitors (unpublished) and have developed expedient routes to the required analogues.[22]

Using our in-house dynamin inhibitor naphthalimide library as a start point for screening, we identified five differentially substituted naphthalimide displaying modest levels of potency (80−120 μM) as clathrin inhibitors (Table 1). Naphthalimides **2**−**4** were decorated with 3-SO_3K and 4-NH_2 moieties on the naphthalene core with a variety of *N*-alkyl substituents, while **5** and **6** possessed a single 3-NO_2 naphthalene substituent and an *N*-aromatic substituent. This suggested that there was considerable freedom in both the substitution pattern around the naphthalene ring as well as the imide substituent.

For the development of a clathrin inhibitor, we viewed inhibition of dynamin GTPase as an undesirable off-target effect. All compounds synthesized were screened against dynamin I GTPases as previously described.[22] Of the leads identified in Table 1 only compound **2**, bearing a *N*-4-aminobenzyl moiety was devoid of dynamin activity (data not shown). Therefore **2** was chosen as the initial *N*-substituent lead for our subsequent efforts in the development of clathrin inhibitor SAR of this class of compounds.

Our initial investigations explored of the optimal naphthalene core substitution through the synthesis and biological screening of a library of 13 compounds from commercially available and/or easily accessible 1,8-napthalene anhydrides (Table 2). These Library 1 analogues were accessed via a simple anhydride-amine condensation reaction (Scheme 1). In a typical synthesis,

Table 1. Initial Screening of the 1,8-Naphthalimide Core As a Lead Candidate for Clathrin Inhibition

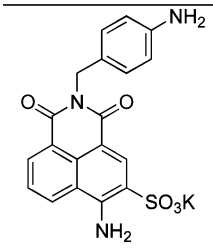
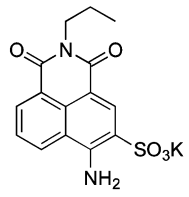
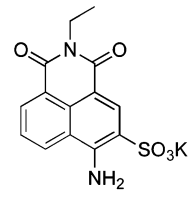
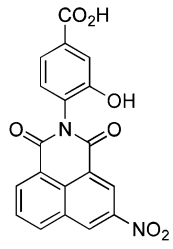
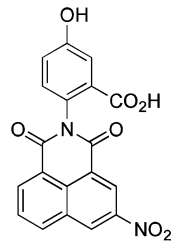
Structure	IC_{50} (μM)	Structure	IC_{50} (μM)	Structure	IC_{50} (μM)
 **2**	~100	 **3**	~120	 **4**	~100
 **5**	~80	 **6**	~100		

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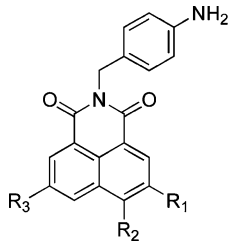
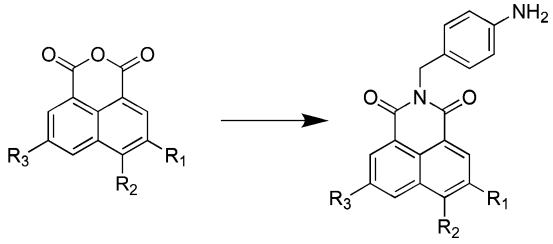
Table 2. Inhibition of the Clathrin TD−Amphiphysin Interaction by *N*-(4-Aminobenzyl)-1,8-naphthyl Analogues 7−19 (Library 1)



compound	R_1	R_2	R_3	clathrin IC_{50} (μM)
7	H	H	H	*a*
8	H	NO_2	H	*a*
9	H	Br	H	*a*
10	H	NH_2	H	*a*
11	H	Cl	H	*a*
12	H	SO_3K	H	*a*
13	NO_2	H	H	*a*
14	OH	H	H	*a*
15	Br	H	H	*a*
16	NH_2	H	H	*a*
17	OCH_3	H	H	*a*
18	SO_3Na	H	H	18
19	NO_2	H	NO_2	*a*

[a]Not active at 100 μM compound concentration.

Scheme 1. Synthesis of Library 1 Analogues[a]



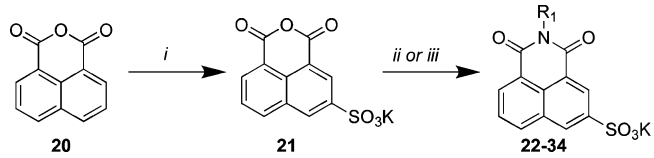
[a]Reagents and conditions: 4-aminobenzylamine (3 equivalents), TEA (cat), ethanol, reflux 18 h.

treatment of the appropriate anhydride with 3 equiv of 4-aminobenzylamine and a catalytic amount of triethylamine (TEA) at 100 °C for 18 h afforded the desired compounds in modest (52%, **19**) to excellent (95%, **7**) yields. Alternatively the same transformation could be accomplished in the room temperature ionic liquid, [BMIM][NO_3] at 140 °C for 20 min.[23] All products precipitated cleanly from the reaction mixture with the exception of **19**, which was purified by column chromatography.

From the data presented in Table 2, the correct orientation of the naphthyl substituents was crucial for clathrin inhibition. With the exception of compound **18**, no compound bearing a single substituent in the 3- or 4-position returned any activity. The single disubstituted nitro analogue (**19**) was also inactive; however, this added functionality gave rise to off-target effects against dynamin that will be described in a separate report. The active compound **18** retained the C3-SO_3H but lost the C4-NH_2 moiety of the lead, **2**.[1] These subtle modifications impart a 5-fold potency enhancement and increase the synthetic accessibility of subsequent analogues. Interestingly the C4-SO_3H isomer **12** was inactive.

Our focus turned to the role of the *N*-substituent in the naphthalimide based analogues on clathrin inhibition. Retaining the C3-SO_3K moiety, Library 2 was constructed with variations in substituent type and pattern on the aromatic imide ring (Scheme 2).

Scheme 2. Generic Synthesis of *N*-Substituted 3-SO_3K 1,8-Naphthylimides 22−34[a]



[a]Reagents and conditions: (i) (a) fuming sulfuric acid (30% SO_3), 90 °C, 90 min; (b) saturated $KCl_{(aq)}$, 4 °C, 18 h; (ii) RNH_2, ethanol, reflux 18 h; (iii) primary amine (0.1−1.5 equiv), 1 M lithium acetate, 130 °C, 18−72 h.

The parent anhydride, 3-sulfo-1,8-naphthalic anhydride potassium salt (**21**), was generated by treatment of 1,8-napthalic anhydride (**20**) with fuming sulfuric acid (H_2SO_4/30% SO_3) at 90 °C for 30 min, followed by precipitation as the potassium sulfonate salt. Termination of the reaction after 30 min prevents the formation of the bis-SO_3K analogue. Treatment of the 3-sulfo-1,8-naphthalic anhydride potassium salt with 3 equiv of substituted benzylamines in ethanol and TEA at 100 °C for 18 h yielded the desired products. Of the Library 2 analogues, only the four nitrogen containing benzylamines (**22−24**, and **29**) afforded the desired product in good yields (77−86%), without the need for further purification. The other analogues resulted in the incomplete conversion to the desired naphthalimide. Variations to the temperature, time, base concentration, and molar equivalents of amine failed to resolve the issue, and purification attempts were unsuccessful. Consequently, an alternative synthetic method was sought. Conducting the amine-anhydride condensation in aqueous media (1 M lithium acetate, pH 5)[20] with variations in the ratio of amine to anhydride allowed access to all Library 2 analogues, although in some instances a 10-fold excess anhydride **21** was required. The outcomes of Library 2 screening for clathrin and dynamin I inhibition are presented in Table 3. In general, modifications to **18**'s *p*-aminobenzyl moiety were better tolerated than modifications to the naphthalene moiety. Of the 13 compounds within Library 2, three showed only modest levels of clathrin inhibition (~100 μM), while the remaining analogues exhibited levels of potency that were comparable to, or better than, **18**. Among the compounds in this Library 2, only **31** showed moderate dynamin inhibition (defined as an IC_{50} <100 μM) but was not a clathrin inhibitor. Importantly, among the clathrin active compounds **24**, **30**, **32**, dynamin inhibition was weak (IC_{50}> 100 μM) or absent (up to 300 μM compound) with analogues **25−29** and **34** (Table 3).

Removal of the benzylmethylene linker gave the phenyl analogue (**22**) which was clathrin inactive most likely due to installation of unfavorable interactions presumably a consequence of reduced *N*-substituent flexibility. The benzyl aromatic moiety substituent position was also important with the 3-NH_2 substituted **23** being 5-fold less potent, while the 2-NH_2 analogue **24** was 2-fold more potent than the series lead (**18**). Of more interest, NH_2 removal resulted in the most potent Library 2 analogue (**25**, IC_{50} = 6.9 ± 1.6 μM). Other

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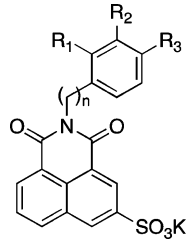
Table 3. Clathrin Inhibition and the PS Stimulated Dynamin I GTPase Activity Inhibition by *N*-(substituted)-3-sulfo-1,8-naphthyl Potassium Salt Analogues 18 and 22−34 (Library 2)



compound	n	R_1	R_2	R_3	clathrin IC_{50} (μM)	DynI IC_{50} (μM)
18	1	H	H	NH_2	18	*a*
22	0	H	H	NH_2	*a*	*a*
23	1	H	NH_2	H	∼100	264[b]
24	1	NH_2	H	H	10 ± 2	108 ± 52[c]
25	1	H	H	H	6.9 ± 1.6	*a*
26	1	H	H	OH	22 ± 1	*a*
27	1	H	H	OCH_3	16 ± 3	*a*
28	1	H	H	CH_3	15 ± 4	*a*
29	1	H	H	$N(CH_3)_2$	∼100	*a*
30	1	H	H	COOH	∼100	185 ± 39[c]
31	1	H	H	NO_2	not active[a]	49[c]
32	1	H	H	F	12 ± 2	306[c]
33	1	H	H	Cl	10 ± 1	111[c]
34	1	H	H	Br	15 ± 4	not active[d]

[a]Not active at 100 μM compound concentration. [b]Not active up to 300 μM compound. [c]n = 1. [d]n = 3 independent experiments.

modifications including bioisosteric manipulations, NH_2 → OH (**26**; IC_{50} = 22 ± 1.0 μM) or NH_2 → CH_3 (**28**; IC_{50} = 15 ± 4 μM) and installation of a 4-OCH_3 moiety (**27**; IC_{50} = 16 ± 3 μM) retained a level of clathrin inhibition comparable with the series lead (**18**). Dimethylation of the 4-NH_2 moiety (**29**; IC_{50} ≈ 100 μM) removed activity, as did replacement with -COOH (**30**, IC_{50} ≈ 100 μM) and -NO_2 (**31**, inactive). These results are consistent with the NH_2 moiety of **18** being sterically but not electronically important. The observations were confirmed on examination of the biological data for a series of halogenated benzyl analogues **32−34** all of which display comparable or improved inhibitory activities relative to the parent compound with IC_{50} values of 12 ± 2, 10 ± 1, and 15 ± 4 μM) respectively, irrespective of the halogen's nature (F, Cl, or Br respectively).

We previously reported a clathrin TD-**18** cocrystal structure.[1] Analogue **18** binds in the "clathrin box motif" region of the clathrin TD (Figure 2). This compound exhibited no dynamin inhibition or inhibition of other key CME pathway proteins or protein−protein interactions examined to date.[1]

The interaction mode of selected naphthalimide analogues was examined by docking into the clathrin TD binding site. All of Library 1 analogues were observed to adopt a similar binding conformation and orientation to that observed for **18** in the clathrin TD−**18** cocrystal structure. However, the inactive analogues failed to engage with Gln89, a consequence of the removal of the C3-SO_3K moiety. The C4 substituted Library 1 analogues **8−12** docked in a largely solvent exposed manner and again failed to engage with Gln89. The minimum distance of a hydrogen bond acceptor, for **13−17**, from Gln89 was 3.4 Å (the 3-NO_2 moiety of **13**), which is 1.3 Å greater than the distance of the sulfonate oxygen of **18** from this residue (Figure 3). The predicted binding pose of **19**, the sole 3,6-substituted analogue, revealed that the 3-NO_2 moiety was positioned too distant from Gln-89 to accept a hydrogen bond from this residue, while the 6-NO_2 moiety was solvent exposed and unable to interact with any residues of the clathrin-TD.

The reduced potency of **2** relative to that of **18** confirmed the importance of substituent positioning. These compounds only differ in the 4-substituent (**18**, R_2 = H; **2**, R_2 = NH_2), and while the binding poses of **2** and **18** are similar, the -SO_3H moiety of **18** is better able to interact with Gln89 (cf. Figures 2 and 3). Compound **2** was the only other analogue predicted to interact with Gln89, and though it is a less potent inhibitor than **18** it further suggests that engagement of Gln89 is required for inhibition of the activity of clathrin.

All Library 2 analogues except **22** and **29−31** adopted a conformation similar to **18** in the clathrin TD binding pocket. Across all clathrin active analogues, the naphthalene moieties are virtually superimposable with only minor variations in the position of the benzyl moiety observed. These variations occur to minimize unfavorable interactions with Ile62. Generally, the larger substituent on the benzyl moiety, the further the benzyl moiety is rotated away from Ile62. This supports our earlier hypothesis that the benzyl ring substituent effects were largely steric and not electronic.

Clathrin inactive analogues **22** and **29−31** did not dock into the clathrin TD binding site but were predicted to bind at the binding site periphery with one side of each analogue being solvent exposed. The absence of a flexible methylene linker in **22** prevents this analogue from adopting the bent conformation observed with **18**. This results in an analogue unable to be accommodated within the binding site (Figure 4). A similar pattern emerged for analogues **29−31** but here the bulky substituent at the 4-position prevented binding a favorable orientation.

These docking studies also helped explain the differences in activity due to the position of amino group on the imide benzyl

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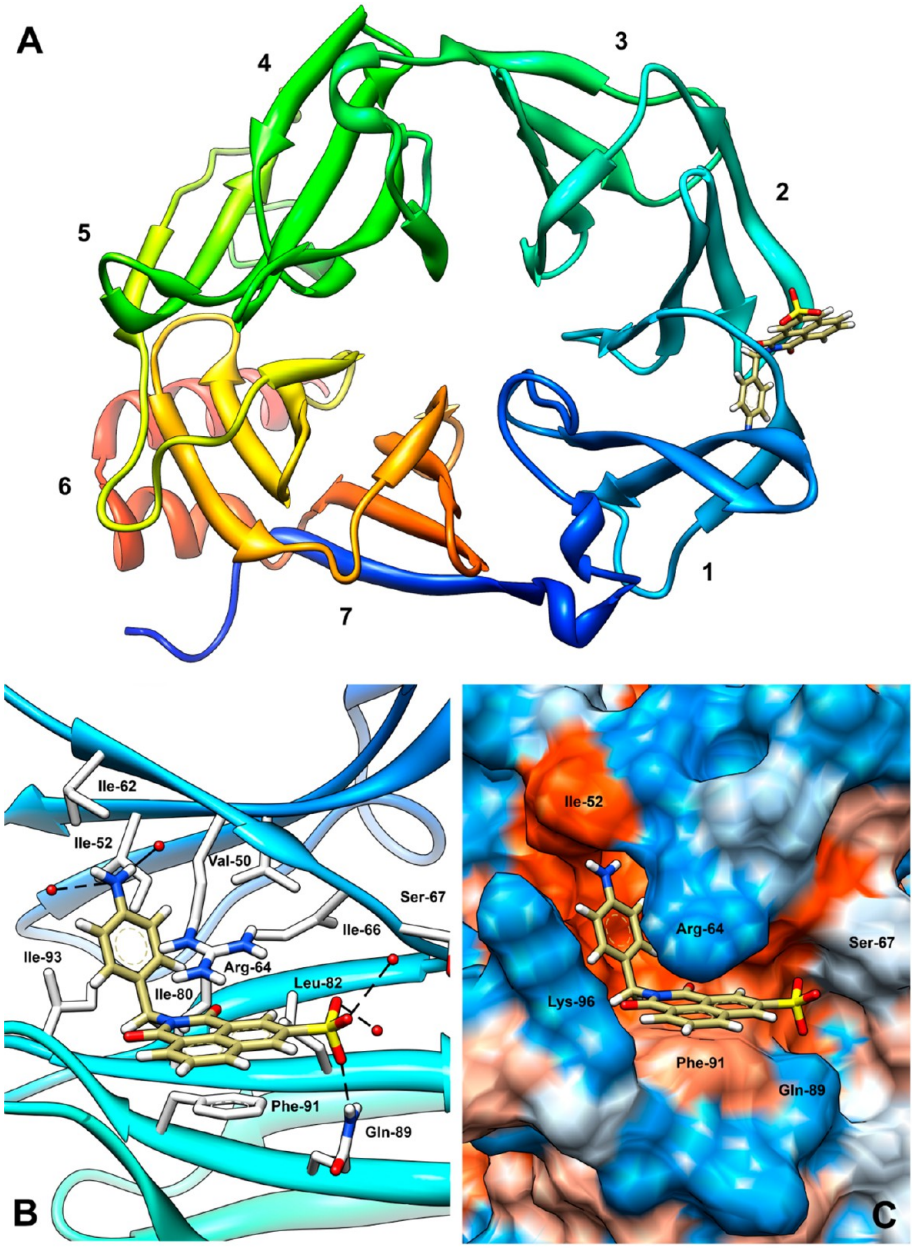

Figure 2. Compound **18** competes with clathrin box ligands for a common site on the clathrin TD. (A) Ribbon representation of the clathrin TD, with the blades of the TD-β-propeller numbered from 1 to 7. **18** binds to the clathrin box-binding site, located between blades 1 and 2 of the clathrin TD. (B) Close-up view of the binding site for **18**. Hydrogen bonds formed at both ends of **18** are indicated by broken lines. (C) Hydrophobicity surface. Blue areas are the most hydrophilic, while orange-red indicates hydrophobic regions.

ring (**18**, **23**, and **24**). While the naphthalene core remained virtually superimposable, subtle differences were predicted in the positioning of the benzyl moiety for each variation (Figure 5). Examination of the clathrin TD-**18** cocrystal structure (Figure 2) had revealed a potential unfavorable interaction between the 4-NH_2 moiety and the Ile62 side chain of the binding site. In the case of **24**, the 2-NH_2 substituent was predicted to lie on the more solvent-exposed side of the binding site, allowing the benzyl ring to better interact with binding site hydrophobic residues, specifically Ile52 and Ile62 (which is the residue that clashes with the 4-NH_2 of **18**). Conversely, the 3-NH_2 moiety of **23** is positioned within a highly hydrophobic binding site region (surrounded by five hydrophobic residues), particularly close to Val50 and Ile52. This presented an unfavorable interaction that drives the benzyl ring further from these residues, weakening the favorable hydrophobic interactions observed for both **18** and **24**. These docking observations were consistent with the observed inhibitory activities, with potency improving in the rank order **23** > **18** > **24**, which matches the order of improved hydrophobic contacts within the binding site. Docking of the three halogenated analogues, **32**−**34**, revealed only subtle differences to accommodate the different sized halogen atoms (Figure 5).

The binding pose of **25**, the most active analogue, was again virtually superimposable with that of **18** (Figure 6). Many of the binding interactions observed for **18** were also observed for **25**. Removal of the 4-NH_2 benzyl moiety substituent appears to have a dual effect of both removing an unfavorable Ile62 interaction with the amine group and allowing more favorable

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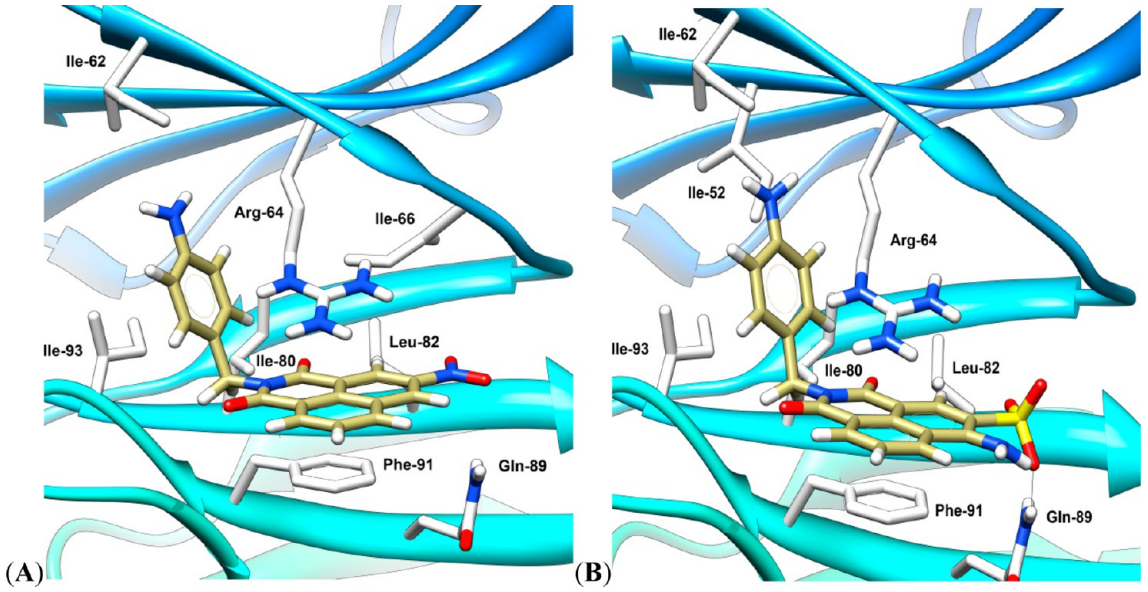

Figure 3. (A) Predicted binding pose of **13** in the clathrin-TD binding site, revealing a lack of hydrogen bonding interactions with the binding site. (B) Predicted binding pose of **2** in the clathrin-TD domain binding site. Only one hydrogen bond is observed, which is donated from the side chain of Gln89 to the sulfonate of **2**. For clarity, only polar binding site hydrogen atoms are shown.

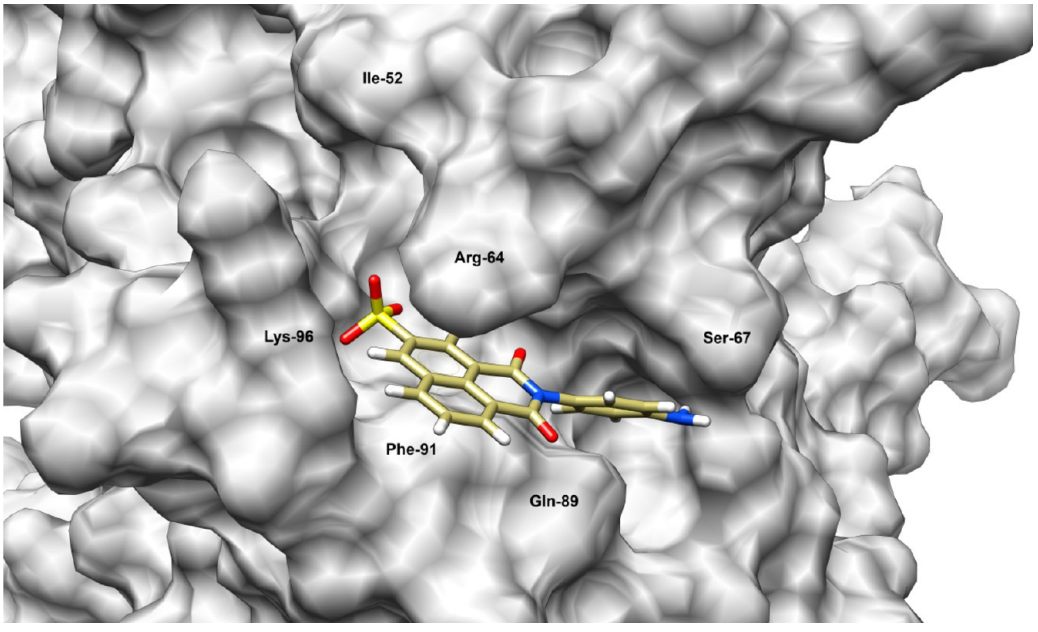

Figure 4. Predicted binding pose of **22** in the clathrin-TD binding site, revealing the potential structural reason for the inactivity of this analogue. The compound binds at the periphery of the binding site and is largely solvent exposed, preventing favorable interactions with binding site residues.

hydrophobic interactions of the benzene ring with surrounding residues. This may explain the almost 3-fold increased clathrin inhibitory activity of **25** (IC_{50} = 6.9 ± 1.6 μM) compared to **18** (IC_{50} = 18 μM).

We previously reported that **18** was not cell permeable but inhibited CME when directly microinjected into cells.[1] To determine whether the new analogues were cell permeable, all compounds in Table 3 were examined for their ability to inhibit endocytosis of transferrin in U2OS cells after 30 min preincubation with the compound.[22] None were able to inhibit CME after 30 or 120 min (data not shown). This lack of in-cell CME activity was attributed to the highly polar nature of the sulfonic acid moiety present with all clathrin active analogues. This is expected to hinder cell permeability giving rise to only very low levels of in cell CME inhibition.

CONCLUSIONS

Scaffold simplification of a HTS hit, followed by screening of an existing library, identified substituted 1,8,-naphthalimides as clathrin inhibitors. The most potent analogue in Library 1 was 3-sulfo-*N*-(4-aminobenzyl)-1,8-naphthalimide, potassium salt (**18**) with an IC_{50} = 18 μM. Subsequent evaluation of the naphthyl core substituents identified the 3-SO_3K substituent as being crucial for clathrin inhibition. Hydrogen bonding of this substituent with Gln89, along with $\pi-\pi$ and π-cation interaction with the naphthalene core, gave rise to excellent

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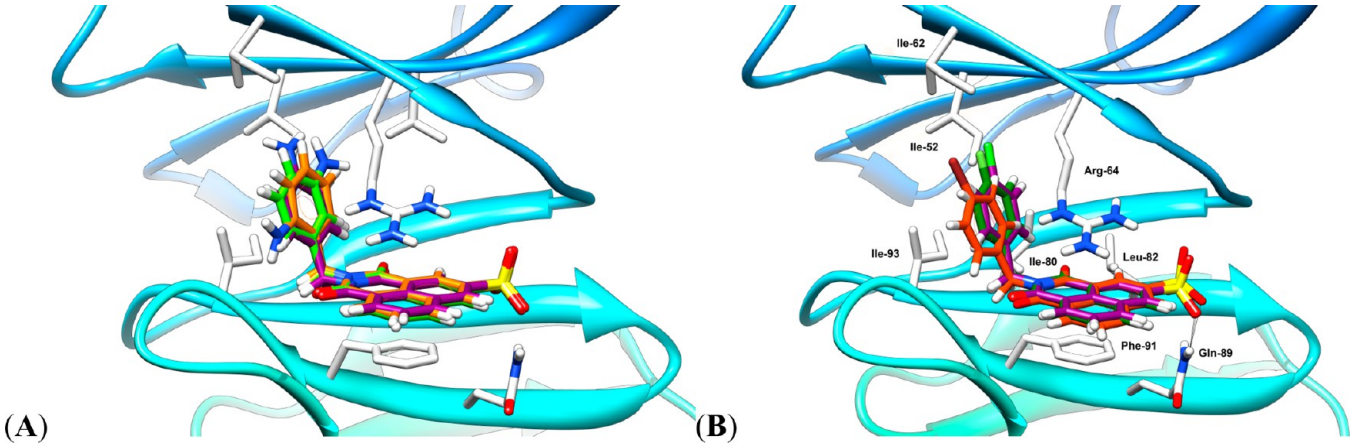

Figure 5. Effect of substitution on the benzyl moiety on predicted binding poses. (A) Effect of amine substitution position on the benzyl moiety. Predicted binding poses with 4-NH_2 [**18** (purple)], 3-NH_2 [**23** (green)], and 2-NH_2 [**24** (orange)]. (B) Effect of halogen substitution at the 4-position. Predicted binding poses with F, [**32** (green)], Cl [**33** (purple)], and Br [**34** (orange)]. For clarity, only polar binding site hydrogen atoms are shown.

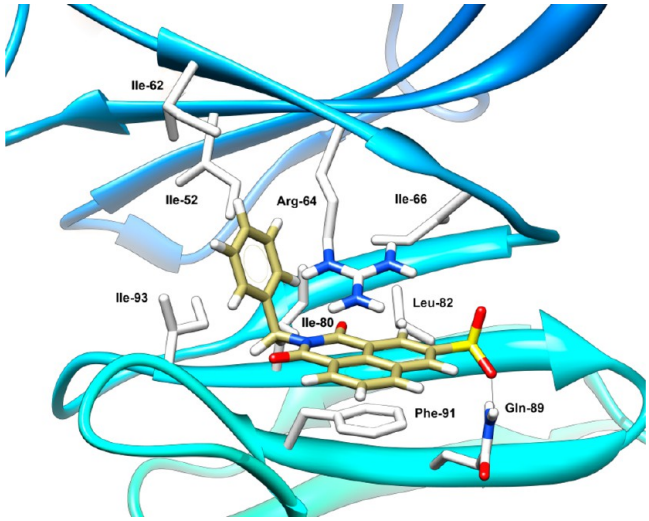

Figure 6. Predicted binding pose of **25** in the clathrin-TD binding site. Compound **25** is predicted to adopt a binding pose identical to that of **18**, differing only in the removal of the clash with Ile62. This is predicted to be responsible for the improved inhibitory activity of **25**.

binding interactions of these compounds in CTD binding site. This interaction with Gln89 was subsequently found to be missing in docking studies with the other Library 1 compounds.

The importance of the 4-aminobenzyl imide on clathrin inhibition was explored through the synthesis of Library 2. The phenyl equivalent (**22**) of **18** was inactive, suggesting the need for flexibility, confirmed by docking studies, to allow the correct binding pose to be accessed. Introduction of small polar moieties was well tolerated with the -OH, -OCH_3, -CH_3, -F, -Cl, -Br analogues **26**–**28** and **32**–**34** respectively displaying similar levels of clathrin inhibition to **18**. Bulkier substituents were poorly tolerated with the 4-$N(CH_3)_2$ (**29**), 4-COOH (**30**), and 4-NO_2 (**31**) inactive. Removal of the 4-amino moiety afforded the most active clathrin inhibitor to date (**25**, IC_{50} 6.9 ± 1.6 μM).

None of these new clathrin inhibitors were however able to inhibit CME. Each of these compounds retains the same sulfonic acid functionality as **18**, which is expected to hinder cell permeability. Nonetheless, **18** was highly effective in inhibiting synaptic vesicle endocytosis when microinjected into the lamprey reticulospinal synapse.[1,26] A challenge for a future study is to render these compounds more cell permeable, such as using a pro-drug approach.

Our studies also highlight the importance of *in vitro* counter-screening against other key enzymes such as dynamin GTPase in the CME pathway when developing clathrin inhibitors for endocytosis. As CME has a dynamin-dependent vesicle scission step, we also examined these clathrin inhibitors for their ability to inhibit dynamin GTPase. Six of the novel clathrin-active naphthylimide analogues reported herein displayed no dynamin 1 GTPase activity at concentrations up to 300 μM, while two, **24** and **33**, displayed weak dynamin inhibition. This work also reports on the discovery of **25**, which with an IC_{50} = 6.9 ± 1.6 μM, represents the most potent clathrin inhibitor yet reported.

EXPERIMENTAL SECTION

ELISA-Based Clathrin Inhibitor Binding Assay. The clathrin inhibitor assay was based on that of our previous report.[1] Bacterially expressed recombinant purified His_6-tagged amphiphysin 1 (amino acids 250–578) in screening buffer (20 mM HEPES, pH 7.4, 50 mM NaCl, 1 mM DTT, 1 mM PMSF) was added to a 384 well ELISA plate (high-binding PS Microplate, Greiner Bio-One) and bound to the plastic for 1 h at room temperature. Nonspecific binding was reduced by overnight incubation with 50 μL blocking buffer (20 mM HEPES, pH 7.4, 50 mM NaCl, 1 mM PMSF, 2% BSA, 2.5% skim milk) at 4 °C. Following extensive washes (with 20 mM HEPES, pH 7.4, 50 mM NaCl, 0.05% Tween 20), chemical compounds diluted in DMSO (10 μL) were added and incubated together with bacterially expressed recombinant GST-tagged clathrin heavy chain TD (amino acids 1–364) for 1 h at room temperature in screening buffer. After three washes, horseradish peroxidase-coupled anti-GST antibodies were added in screening buffer, and the plate was incubated for 15 min at room temperature. Following additional washes, 50 μL of TMB (3,3′,5,5′-tetramethylbenzidine) (Pierce Biotechnology) chromogenic substrate of horseradish peroxidase was added, and the plate was incubated for 20 min before the reaction was terminated by adding 50 μL of 1 N sulfuric acid to produce a yellow color. The amount of bound protein was determined by spectrophotometric measurement in a plate reader. Relative binding was calculated as a percentage of DMSO control.

Dynamin GTPase Assay. Native dynamin I was purified from sheep brain by extraction from the peripheral membrane fraction of whole brain and affinity purification on GST-Amph2-SH3-sepharose as previously described.[27] The GTPase assay was based on

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colorimetric detection of phosphate release from GTP using the Malachite Green method as previously described.[22,27] except that the GTPase assay buffer contained 5 mM Tris-HCl, 10 mM NaCl, 2 mM Mg^{2+}, pH 7.4, 1 μg/mL leupeptin, 0.1 mM PMSF, and 0.3 mM GTP. Dynamin (20 nM) activity was stimulated by 4 μg/mL sonicated phosphatidylserine in the presence of test compounds for 30 min at 37 °C.

CME Assay (Texas red-Tf Uptake). Tf uptake was analyzed in U2OS cells based on methods previously described.[1,28] Cells were exposed to test inhibitors (from 1 to 300 μM) or vehicle for 30 min prior to addition of 4 μg/mL Tf-A594 for 8 min at 37 °C. Quantitative analysis of the inhibition of TxR-Tf endocytosis in U2OS cells was performed on large numbers of cells by an automated acquisition and analysis system (Image Xpress Micro, Molecular Devices, Sunnyvale, CA). The average number of cells for each data point was ∼1200. IC_{50} values were calculated using Graphpad Prism v5 and data were expressed as mean ±95% confidence interval (CI) for three wells and ∼1200 cells.

Molecular Modeling and Docking Studies. Molecular modeling was conducted using ICM Version 3.6. The **18**-clathrin-TD cocrystal structure was used as the modeling base (PDB Code: 2ZXG). The binding site was defined by the bound ligand, which was then removed, and the receptor was converted to an ICM object and optimized using the internal software. Docking was carried out using default settings with a flexible ligand and 0.5 Å grid potential representation of the receptor accounting for hydrophobicity, van der Waals boundaries, hydrogen-bonding profile, and electrostatic potential of the defined binding site. The resulting conformation was then optimized with flexible ligand and receptor side chains using the refine and dock function within ICM v.3.1. A score was calculated for each compound according to its fit with the receptor, taking into account factors such as steric fit, hydrogen bonding, solvation electrostatics, hydrophobic interactions, and the ligand entropy change upon binding. The docking was carried out in triplicate, and the binding conformation producing the most negative score was retained (the more negative the score, the more favorable the interaction). The receptor and docked ligand complex were then exported in PDB format. The molecular graphics images were produced from these files using the USCF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR001081).[29]

Chemistry General Procedures. All reactions were performed using standard laboratory equipment and standard laboratory glassware. Solvents and reagents were purchased from Sigma Aldrich, Lancaster International or TCI and used as received, with the exception of some substituted 1,8-naphthalic anhydrides, which were synthesized in-house as described below. Organic solvents were bulk quality and were distilled from glass prior to use. Flash chromatography was carried out using silica gel 200−400 mesh (60 Å). Organic solvent extracts were dried with magnesium sulfate ($MgSO_4$) and dried under reduced pressure with either Büchi or Heidolph rotary evaporators. Lithium acetate buffer was made by making a 1 M solution of lithium acetate dihydrate in water and adjusting the pH to 5 with glacial acetic acid. Melting points were recorded in open capillaries on a Stuart SMP11 Melting Point Apparatus. Temperatures are expressed in degrees Celsius (°C) and are uncorrected. Where available, literature values are provided and appropriately referenced. Electrospray mass spectra were recorded using 10% DMSO/H_2O or HPLC-grade methanol or acetonitrile as carrier solvents on a Shimadzu LC-MS spectrometer. All compounds were ≥95% purity as determined by HPLC and LCMS analysis.

NMR spectroscopy was performed on a Bruker Avance 300 MHz spectrometer, where proton NMR (^{1}H NMR) spectra and carbon NMR (^{13}C NMR) spectra were acquired at 300 and 75 MHz respectively, or a Bruker Avance III 400 MHz spectrometer, where ^{1}H NMR and ^{13}C NMR were acquired at 400 and 100 MHz respectively. All spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6), obtained from Sigma Aldrich or Cambridge Isotope Laboratories Inc., unless otherwise stated, with the residual solvent peaks used as the internal reference (δ 2.49 (quintet) and δ 39.7 (septet) for ^{1}H NMR and ^{13}C NMR respectively. Chemical shifts (δ) were measured in parts per million (ppm) and referenced against the internal reference peaks. Coupling constants (J) were measured in Hertz (Hz). Multiplicities are denoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), triplet (t), triplet of doublets (td), doublet of triplets (dt), quartet (q), quintet (quin), and multiplet (m). Peaks are listed in increasing chemical shift in the following format: chemical shift (integration (^{1}H), multiplicity (^{1}H), coupling constant (^{1}H)).

General Procedure 1. To a stirring suspension of the required 1,8-naphthalic anhydride (0.95 mmol) in ethanol (15 mL) were added TEA (10 drops) and the required amine (3.09 mmol, 3 equiv). An excess of amine was used to minimize formation of the di-imide. The resulting suspension is then stirred at 100 °C for 18 h before cooling, with stirring, to room temperature. The solid product is collected by vacuum filtration on a sintered funnel, washed sequentially with ethanol, and ether, and dried under a vacuum.

General Procedure 2. To a stirring suspension of the required 1,8-naphthalic anhydride (potassium salt) (0.79 mmol) in 1 M lithium acetate buffer adjusted to pH 5 by the addition of glacial acetic acid (5 mL) was added the required amine (1.58 mmol, 2 equiv). The resulting cream-brown suspension was then heated at 130 °C for 18 h. The reaction mixture was then diluted to 15 mL with water, and KCl (∼0.5 g) was added. After cooling to room temperature with stirring, the resulting cream-brown suspension was allowed to stand at 4 °C overnight, to increase product precipitation. The precipitated product was then collected by vacuum filtration on a sintered funnel, washed sequentially with cold water, ethanol, and ether, and dried under a vacuum.

*Procedure for Synthesis of 1,8-naphthalic Anhydride Scaffolds. 4-Amino-1,8-naphthalic Anhydride (**10a**).* A solution of tin(II) chloride (4.015 g, 21.2 mmol) in concentrated HCl (32%, 3.5 mL) was added dropwise to a stirring suspension of 4-nitro-1,8-naphthalic anhydride (1.009 g, 4.1 mmol) in ethanol (2 mL), and the resulting suspension was stirred at reflux for 2 h, before being cooled to room temperature. The precipitated product was collected by filtration, washed sequentially with water, ethanol, and ether, and dried under a vacuum. An orange-red solid was obtained.

Yield 0.761 g (86%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 6.86 (1H, d, J = 8.4 Hz), 7.67 (1H, dd, J = 7.2, 8.4 Hz), 7.77 (2H, br s, NH_2), 8.17 (1H, d, J = 8.4 Hz), 8.42 (1H, dd, J = 0.9, 7.2 Hz), 8.67 (1H, dd, J = 0.9, 8.4 Hz).

^{13}C NMR (DMSO-d_6) δ 102.4, 108.9, 118.5, 119.5, 124.6, 130.9, 132.8, 133.2, 136.1, 154.1, 160.5, 162.2 ppm.

*3-Amino-1,8-naphthalic Anhydride (**16a**).* Synthesized using the same general procedure as for **10a**, commencing with 3-nitro-1,8-naphthalic anhydride. A yellow-orange solid was obtained.

Yield 0.727 g (83%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 6.11 (2H, br, s, NH_2), 7.36 (d, J = 2.2 Hz, 1H), 7.65 (dd, J = 8.2, 7.2 Hz, 1H), 7.96 (d, J = 2.2 Hz, 1H), 8.09 (d, J = 7.2 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H).

^{13}C NMR (DMSO-d_6) δ 113.6, 118.8, 119.6, 123.4, 123.5, 127.8, 127.9, 133.1, 133.9, 148.0, 161.3, 161.4 ppm.

*4-Amino-3-sulfo-1,8-naphthalic Anhydride, Potassium Salt (**2a**).* 4-Amino-1,8-naphthalic anhydride (**10a**) (0.204 g, 1.0 mmol) was dissolved in oleum (4 mL), and the resulting solution was stirred at 50 °C for 3 h. The mixture was then cooled to room temperature and poured into water (20 mL). Aqueous saturated potassium chloride (25 mL) was then added, resulting in the precipitation of the off-yellow product. The product was collected by filtration, washed sequentially with water, ethanol, and ether, and dried under a vacuum.

Yield 0.248 g (78%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 4.54 (2H, br, s, NH_2), 7.72 (1H, dd, J = 7.2, 8.4 Hz), 8.43 (1H, dd, J = 0.9, 7.2 Hz), 8.57 (1H, s), 8.78 (1H, dd, J = 0.9, 8.4 Hz).

^{13}C NMR (DMSO-d_6) δ 102.1, 118.9, 121.1, 125.4, 125.9, 131.6, 132.7, 133.5, 134.6, 149.7, 160.8, 162.3 ppm.

*3-Methoxy-1,8-naphthalic Anhydride (**17a**).* To a stirred suspension of 3-hydroxy-1,8-naphthalic anhydride (0.509 g, 2.38 mmol) and potassium carbonate (2.006 g, 14.51 mmol) in acetone (30

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mL) was added dimethyl sulfate (1.00 mL, 5.95 mmol). The resulting colorless solution was stirred at reflux overnight. The resulting white suspension was cooled to room temperature with stirring, and then the acetone was removed *in vacuo*. The pale yellow residue was dissolved in water (100 mL) and acidified to pH 3 with 2 M HCl. The clear colorless solution was allowed to stand at room temperature overnight, resulting in precipitation of the product. The product was collected by filtration, washed with water, and dried under a vacuum. A pale yellow solid was obtained.

Yield 0.434 g (80%). MP 248−249 °C (Lit. 249−250 °C).[30]

^{1}H NMR (DMSO-d_6) δ 3.99 (3H, s, OCH_3), 7.85 (1H, dd, J = 7.2, 8.1 Hz), 8.00 (1H, d, J = 2.7 Hz), 8.05 (1H, d, J = 2.7 Hz), 8.34 (1H, dd, J = 1.2, 7.2 Hz), 8.40 (1H, dd, J = 1.2, 8.1 Hz).

^{13}C NMR (DMSO-d_6) δ 55.9, 110.8, 121.9, 122.1, 126.2, 127.6, 129.5, 131.2, 131.7, 135.9, 156.2, 168.0, 168.7 ppm.

*3-Sulfo-1,8-naphthalic Anhydride (Potassium Salt) (**21**).* 1,8-Naphthalic anhydride (**20**, 0.990 g, 5.0 mmol) was dissolved in oleum (6 mL). The resulting solution was stirred at 120 °C for 1 h (until a drop of the mixture, when added to water, did not precipitate), and then cooled to room temperature. The cooled solution was then poured into water (30 mL). Addition of aqueous saturated potassium chloride (30 mL) resulted in precipitation of the product. The white solid was collected by filtration, washed sequentially with water, ethanol, and ether, and dried under a vacuum.

Yield 1.580 g (96%). MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 7.91 (1H, dd, J = 7.2, 8.4 Hz), 8.51 (1H, dd, J = 0.9, 7.2 Hz), 8.62 (1H, d, J = 1.5 Hz), 8.64 (1H, dd, J = 0.9, 8.4 Hz), 8.73 (1H, d, J = 1.5 Hz).

^{13}C NMR (DMSO-d_6) δ 119.7, 128.4, 130.0, 130.3, 131.4, 131.6, 133.1, 136.5, 147.7, 161.1, 161.2 ppm.

*Synthesis of Initial Lead Candidates. 4-Amino-3-sulfo-N-(4-aminobenzyl)-1,8-naphthalimide, Potassium Salt (**2**).* Synthesized using general procedure 1, commencing with 4-amino-3-sulfo-1,8-naphthalic anhydride (potassium salt **2a**) and 4-aminobenzylamine. A bright yellow solid was obtained.

Yield 0.245 g (94%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 4.92 (2H, br s, NH_2), 5.02 (2H, s), 6.45 (2H, d), 7.04 (2H, d, J = 8.4 Hz), 7.68 (1H, dd, J = 7.2), 7.82 (2H, br s, NH_2), 8.42 (1H, dd), 8.63 (1H, s), 8.68 (1H, dd, J = 1.2, 8.4 Hz).

^{13}C NMR (DMSO-d_6) δ 42.3, 106.8, 113.8, 120.8, 122.1, 124.8, 125.1, 125.2, 129.2, 129.6, 130.0, 131.4, 133.0, 147.8, 148.4, 163.2, 163.9 ppm.

MS (ESI-) m/z: 396 (M − K) (100%).

*4-Amino-3-sulfo-N-ethyl-1,8-naphthalimide, Potassium Salt (**3**).* Synthesized using general procedure 1, commencing with 4-amino-3-sulfo-1,8-naphthalic anhydride (potassium salt **2a**) and ethylamine. A yellow solid was obtained.

Yield 0.187 g (85%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 6.9 Hz), 4.04 (2H, q, J = 6.9 Hz), 7.68 (1H, dd, J = 7.2, 8.4 Hz), 7.80 (2H, br s, NH_2), 8.42 (1H, dd, J = 0.6, 7.2 Hz), 8.62 (1H, s), 8.68 (1H, dd, J = 0.6, 8.4 Hz).

^{13}C NMR (DMSO-d_6) δ 13.5, 34.5, 106.8, 120.8, 122.1, 124.7, 125.1, 129.6, 129.9, 131.2, 132.8, 148.3, 162.9, 163.7 ppm.

MS (ESI-) m/z: 319 (M − K) (100%).

*4-Amino-3-sulfo-N-propyl-1,8-naphthalimide, Potassium Salt (**4**).* Synthesized using general procedure 1, commencing with 4-amino-3-sulfo-1,8-naphthalic anhydride (potassium salt **2a**) and *n*-propylamine. A yellow solid was obtained.

Yield 0.104 g (48%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 0.89 (3H, t, J = 7.5 Hz), 1.61 (2H, sextet, J = 7.5 Hz), 3.97 (2H, t, J = 7.5 Hz), 7.68 (1H, dd, J = 7.2, 8.4 Hz), 7.80 (2H, br s, NH_2), 8.42 (1H, d, J = 7.2 Hz), 8.61 (1H, s), 8.68 (1H, d, J = 8.4 Hz); ^{13}C NMR (DMSO-d_6) δ 11.6, 21.1, 41.0, 106.8, 120.8, 122.0, 124.7, 125.2, 129.6, 129.9, 131.2, 132.8, 148.3, 163.1, 163.9 ppm.

MS (ESI-) m/z: 333 (M − K) (100%).

*3-Nitro-N-(2-carboxy-4-hydroxyphenyl)-1,8-naphthalimide (**5**).* Synthesized using general procedure 1, commencing with 3-nitro-1,8-naphthalic anhydride and 2-amino-5-hydroxybenzoic acid. Product was isolated as the triethylamine salt. A yellow-brown solid was obtained.

Yield 0.246 g (64%); MP 224−226 °C.

^{1}H NMR (DMSO-d_6) δ 1.00 (9H, Et_3N, t, J = 7.2 Hz), 2.59 (6H, Et_3N, q, J = 7.2 Hz), 7.32 (1H, d, J = 8.1 Hz), 7.47 (1H, dd, J = 1.5, 8.1 Hz), 7.55 (1H, d, J = 1.5 Hz), 8.09 (1H, dd, J = 7.2, 8.4 Hz), 8.70 (1H, dd, J = 0.9, 7.2 Hz), 8.83 (1H, dd, J = 0.9, 8.1 Hz), 8.98 (1H, d, J = 2.4 Hz), 9.53 (1H, d, J = 2.4 Hz).

^{13}C NMR (100 MHz) (DMSO-d_6) δ 10.3 (3C, Et_3N,), 45.5 (3C, Et_3N,), 117.4, 120.0, 123.1, 123.3, 124.7, 125.1, 129.5, 129.8, 130.1, 130.2, 131.2, 134.1, 136.7 (2C), 146.1, 153.3, 162.2, 162.7, 168.3 ppm.

MS (ESI-) m/z: 377 (M − H) (100%), 755 (2 M − H).

*3-Nitro-N-(4-carboxy-2-hydroxyphenyl)-1,8-naphthalimide (**6**).* Synthesized using general procedure 1, commencing with 3-nitro-1,8-naphthalic anhydride and 4-amino-3-hydroxybenzoic acid. Product was isolated as the triethylamine salt. A brown solid was obtained.

Yield 0.254 g (64%); MP 230−232 °C.

^{1}H NMR (DMSO-d_6) δ 0.87 (9H, Et_3N, t, J = 7.2 Hz), 2.58 (6H, q, Et_3N, J = 7.2 Hz), 6.93 (1H, dd, J = 2.7, 8.4 Hz), 7.12 (1H, d, J = 8.4 Hz), 7.44 (1H, d, J = 2.7 Hz), 8.06 (1H, dd, J = 7.2, 8.4 Hz), 8.64 (1H, dd, J = 0.9, 7.2 Hz), 8.79 (1H, dd, J = 0.9, 8.4 Hz), 8.92 (1H, d, J = 2.4 Hz), 9.49 (1H, d, J = 2.4 Hz), 9.83 (1H, br s, OH).

^{13}C NMR (100 MHz) (DMSO-d_6) δ 9.2 (3C, Et_3N), 45.1 (3C, Et_3N), 117.5, 117.9, 122.8, 123.5, 125.0, 126.5, 129.4, 129.7, 130.1, 130.9, 131.2, 133.9, 134.9, 136.3, 146.0, 157.3, 162.9, 163.4, 167.5 ppm.

MS (ESI-) m/z: 377 (M − H) (100%), 755 (2 M − H).

*Library **1**. N-(4-Aminobenzyl)-1,8-naphthalimide (**7**).* Synthesized using general procedure 1, commencing with 1,8-naphthalic anhydride (**20**) and 4-aminobenzylamine. A yellow solid was obtained.

Yield 0.359 g (95%); MP > 250 °C; ^{1}H NMR (DMSO-d_6) δ 4.96 (2H, br s, NH_2), 5.06 (2H, s), 6.46 (2H, d, J = 8.4 Hz), 7.08 (2H, d, J = 8.4 Hz), 7.85 (2H, dd, J = 7.2, 8.4 Hz), 8.43 (2H, dd, J = 1.5, 8.4 Hz), 8.49 (2H, dd, J = 1.5, 7.2 Hz).

^{13}C NMR (DMSO-d_6) δ 42.7, 113.8 (2C), 122.1 (2C), 124.6, 127.4 (3C), 129.4 (2C), 131.0 (2C), 131.4, 134.5 (2C), 148.0, 163.5 (2C) ppm.

MS (ESI+) m/z: 303 (M + H) (calc.).

*4-Nitro-N-(4-aminobenzyl)-1,8-naphthalimide (**8**).* Synthesized using general procedure 1, commencing with 4-nitro-1,8-naphthalic anhydride and 4-aminobenzylamine. A pink-brown solid was obtained.

Yield 0.313 g (87%); MP 218−220 °C (Lit. 214−216 °C).

^{1}H NMR (DMSO-d_6) δ 4.89 (2H, br s, NH_2), 5.01 (2H, s), 6.47 (2H, d, J = 8.4 Hz), 7.06 (2H, d, J = 8.4 Hz), 7.97 (1H, dd, J = 7.5, 8.7 Hz), 8.42 (1H, d, J = 7.8 Hz), 8.52 (1H, d, J = 7.8 Hz), 8.53 (1H, dd, J = 0.9, 7.5 Hz), 8.58 (1H, dd, J = 0.9, 8.7 Hz).

^{13}C NMR (DMSO-d_6) δ 43.1, 113.8 (2C), 122.7, 122.8, 124.0, 124.4, 126.5, 128.3, 128.9, 129.5 (2C), 129.9, 130.2, 132.0, 148.2, 149.2, 162.2, 163.0 ppm.

*4-Bromo-N-(4-aminobenzyl)-1,8-naphthalimide (**9**).* Synthesized using general procedure 1, commencing with 4-bromo-1,8-naphthalic anhydride and 4-aminobenzylamine. A yellow solid was obtained.

Yield 0.316 g (91%); MP 238−240 °C.

^{1}H NMR (DMSO-d_6) δ 4.97 (2H, br s, NH_2), 5.04 (2H, s), 6.47 (2H, d, J = 8.4 Hz), 7.08 (2H, d, J = 8.4 Hz), 7.94 (1H, dd, J = 7.2, 8.4 Hz), 8.16 (1H, d, J = 7.8 Hz), 8.30 (1H, d, J = 7.8 Hz), 8.49 (1H, dd, J = 1.2, 8.4 Hz), 8.54 (1H, dd, J = 1.2, 8.4 Hz).

^{13}C NMR (DMSO-d_6) δ 42.8, 113.7 (2C), 121.9, 122.7, 124.3, 128.2, 128.8, 129.3, 129.6 (2C), 129.8, 131.1, 131.4, 131.8, 132.7, 148.1, 162.8, 162.9 ppm.

MS (ESI+) m/z: 381 (M + H) (calc.).

*4-Amino-N-(4-aminobenzyl)-1,8-naphthalimide (**10**).* Synthesized using general procedure 1, commencing with 4-amino-1,8-naphthalic anhydride (**10a**) and 4-aminobenzylamine. A yellow solid was obtained.

Yield 0.342 g (91%); MP > 250 °C (Lit. 287−288 °C).[31]

^{1}H NMR (DMSO-d_6) δ 4.92 (2H, br s, NH_2), 5.01 (2H, s), 6.45 (2H, d, J = 8.4 Hz), 6.83 (1H, d, J = 8.4 Hz), 7.04 (2H, d, J = 8.4 Hz), 7.42 (2H, br s, NH_2), 7.63 (1H, dd, J = 7.2, 8.4 Hz), 8.18 (1H, d, J = 8.4 Hz), 8.41 (1H, dd, J = 1.2, 7.2 Hz), 8.59 (1H, dd, J = 1.2, 8.4 Hz).

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^{13}C NMR (DMSO-d_6) δ 42.2, 107.8, 108.4, 113.7 (2C), 119.6, 122.0, 124.2, 125.3, 129.3 (2C), 129.5, 129.8, 131.3, 134.2, 147.8, 152.9, 163.1, 163.9 ppm.

*4-Chloro-N-(4-aminobenzyl)-1,8-naphthalimide (**11**).* Synthesized using general procedure 1, commencing with 4-chloro-1,8-naphthalic anhydride and 4-aminobenzylamine. A yellow solid was obtained.

Yield 0.325 g (88%); MP 228−230 °C (Lit. 195−196 °C).[31]

^{1}H NMR (DMSO-d_6) δ 4.97 (2H, br s, NH_2), 5.04 (2H, s), 6.47 (2H, d, J = 8.4 Hz), 7.08 (2H, d, J = 8.4 Hz), 7.93 (1H, dd, J = 7.5, 8.4 Hz), 7.96 (1H, d, J = 7.8 Hz), 8.38 (1H, d, J = 7.8 Hz), 8.52 (1H, dd, J = 1.2, 7.5 Hz), 8.53 (1H, dd, J = 1.2, 8.4 Hz).

^{13}C NMR (DMSO-d_6) δ 42.8, 113.7 (2C), 121.9, 122.7, 124.3, 127.8, 128.3, 128.5, 128.7, 129.5 (2C), 130.2, 131.1, 131.8, 137.7, 148.1, 162.7, 163.0 ppm.

*4-Sulfo-N-(4-aminobenzyl)-1,8-naphthalimide, Potassium Salt (**12**).* Synthesized using general procedure 1, commencing with 4-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-aminobenzylamine. A yellow solid was obtained.

Yield 0.314 g (94%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 4.95 (2H, br s, NH_2), 5.06 (2H, s), 6.46 (2H, d, J = 8.4 Hz), 7.07 (2H, d, J = 8.4 Hz), 7.87 (1H, dd, J = 7.2, 8.4 Hz), 8.21 (1H, d, J = 7.5 Hz), 8.47 (1H, d, J = 7.5 Hz), 8.49 (1H, dd, J = 1.2, 7.2 Hz), 9.25 (1H, dd, J = 1.2, 8.4 Hz).

^{13}C NMR (DMSO-d_6) δ 42.7, 113.8 (2C), 122.2, 122.9, 124.5, 125.2, 127.0, 127.7, 128.2, 129.3 (2C), 130.5, 130.8, 134.4, 148.0, 150.1, 163.3, 163.7 ppm.

MS (ESI-) m/z: 381 (M − K) (100%).

*3-Nitro-N-(4-aminobenzyl)-1,8-naphthalimide (**13**).* Synthesized using general procedure 1, commencing with 3-nitro-1,8-naphthalic anhydride and 4-aminobenzylamine. A light orange-brown solid was obtained.

Yield 0.296 g (83%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 4.99 (2H, br s, NH_2), 5.05 (2H, s), 6.47 (2H, d, J = 8.4 Hz), 7.09 (2H, d, J = 8.4 Hz), 7.99 (1H, dd, J = 7.2, 8.4 Hz), 8.63 (1H, dd, J = 1.2, 7.2 Hz), 8.70 (1H, dd, J = 1.2, 8.4 Hz), 8.91 (1H, d, J = 2.4 Hz), 9.41 (1H, d, J = 2.4 Hz).

^{13}C NMR (DMSO-d_6) δ 43.0, 113.7 (2C), 122.5, 123.1, 123.9, 124.0, 129.3, 129.4, 129.5 (2C), 129.8, 130.9, 134.2, 136.4, 145.9, 148.2, 162.3, 162.7 ppm.

MS (ESI+) m/z: 348 (M + H) (calc.).

*3-Hydroxy-N-(4-aminobenzyl)-1,8-naphthalimide (**14**).* Synthesized using general procedure 1, commencing with 3-hydroxy-1,8-naphthalic anhydride and 4-aminobenzylamine. A yellow solid was obtained.

Yield 0.312 g (83%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 4.96 (2H, br s, NH_2), 5.03 (2H, s), 6.46 (2H, d, J = 8.4 Hz), 7.06 (2H, d, J = 8.4 Hz), 7.62 (1H, d, J = 2.4 Hz), 7.71 (1H, dd, J = 7.2, 8.4 Hz), 8.01 (1H, d, J = 2.4 Hz), 8.20 (1H, dd, J = 1.2, 8.4 Hz), 8.23 (1H, dd, J = 1.2, 7.2 Hz), 10.45 (1H, br s, OH).

^{13}C NMR (DMSO-d_6) δ 42.7, 113.7 (2C), 116.0, 122.0, 122.1, 122.2, 123.5, 124.6, 127.5, 127.7, 129.3 (2C), 132.8, 133.5, 148.0, 156.4, 163.3, 163.7 ppm.

MS (ESI+) m/z: 319 (M + H) (calc.).

*3-Bromo-N-(4-aminobenzyl)-1,8-naphthalimide (**15**).* Synthesized using general procedure 1, commencing with 3-bromo-1,8-naphthalic anhydride and 4-aminobenzylamine. A pale yellow solid was obtained.

Yield 0.316 g (91%); MP 220−222 °C.

^{1}H NMR (DMSO-d_6) δ 4.98 (2H, br s, NH_2), 5.03 (2H, s), 6.46 (2H, d, J = 8.4 Hz), 7.07 (2H, d, J = 8.4 Hz), 7.86 (1H, dd, J = 7.2, 8.4 Hz), 8.35 (1H, dd, J = 1.2, 8.4 Hz), 8.41 (1H, d, J = 1.8 Hz), 8.47 (1H, dd, J = 1.2, 7.2 Hz), 8.70 (1H, d, J = 1.8 Hz).

^{13}C NMR (100 MHz) (DMSO-d_6) δ 43.0, 113.9 (2C), 120.4, 122.5, 124.2, 124.5, 126.2, 128.8, 129.5 (2C), 131.5, 133.0 (2C), 133.8, 136.1, 148.2, 162.7, 163.3 ppm.

MS (ESI+) m/z: 381 (M + H) (calc.).

*3-Amino-N-(4-aminobenzyl)-1,8-naphthalimide (**16**).* Synthesized using general procedure 1, commencing with 3-amino-1,8-naphthalic anhydride (**16a**) and 4-aminobenzylamine. A yellow solid was obtained.

Yield 0.320 g (86%); MP 248−250 °C.

^{1}H NMR (DMSO-d_6) δ 4.95 (2H, br s, NH_2), 5.03 (2H, s), 5.97 (2H, br s, NH_2), 6.46 (2H, d, J = 8.4 Hz), 7.04 (2H, d, J = 8.4 Hz), 7.27 (1H, d, J = 2.4 Hz), 7.60 (1H, dd, J = 7.2, 8.4 Hz), 7.96 (1H, d, J = 2.4 Hz), 8.02 (1H, dd, J = 1.2, 8.4 Hz), 8.07 (1H, dd, J = 1.2, 7.2 Hz).

^{13}C NMR (DMSO-d_6) δ 42.5, 112.0, 113.7 (2C), 120.7, 121.9, 122.0, 122.7, 124.7, 125.7, 127.1, 129.2 (2C), 131.7, 133.7, 147.9, 148.1, 163.7, 163.9 ppm.

MS (ESI+) m/z: 318 (M + H) (calc.).

*3-Methoxy-N-(4-aminobenzyl)-1,8-naphthalimide (**17**).* Synthesized using general procedure 1, commencing with 3-methoxy-1,8-naphthalic anhydride (**17a**) and 4-aminobenzylamine. A pale yellow solid was obtained.

Yield 0.266 g (73%); MP 238−240 °C.

^{1}H NMR (DMSO-d_6) δ 3.97 (3H, s), 4.96 (2H, br s), 5.04 (2H, s), 6.46 (2H, d, J = 8.4 Hz), 7.07 (2H, dd, J = 8.4 Hz), 7.79 (1H, dd, J = 7.2, 8.4 Hz), 7.89 (1H, d, J = 2.7 Hz), 8.03 (1H, d, J = 2.7 Hz), 8.32 (2H, m).

^{13}C NMR (DMSO-d_6) δ 42.7, 56.1, 113.5, 113.7 (2C), 121.9, 122.1, 122.9, 123.7, 124.5, 127.9, 128.4, 129.4 (2C), 133.2, 133.3, 148.0, 157.9, 163.2, 163.6 ppm.

MS (ESI+) m/z: 333 (M + H) (calc.).

*3-Sulfo-N-(4-aminobenzyl)-1,8-naphthalimide, Potassium Salt (**18**) [Pitstop-1].* Synthesized using general procedure 1, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) (**20**, 0.302 g, 0.95 mmol) and 4-aminobenzylamine (350 μL, 3.09 mmol). A cream solid was obtained.

Yield 0.401 g (85%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 4.97 (2H, br s, NH_2), 5.07 (2H, s), 6.46 (2H, d, J = 8.4 Hz), 7.07 (2H, d, J = 8.4 Hz), 7.87 (1H, dd, J = 7.5, 8.1 Hz), 8.49 (1H, dd, J = 0.9, 7.5 Hz), 8.55 (1H, dd, J = 0.9, 8.1 Hz), 8.65 (1H, d, J = 1.5 Hz), 8.68 (1H, d, J = 1.5 Hz).

^{13}C NMR (100 MHz) (DMSO-d_6) δ 42.9, 114.0 (2C), 122.3 (2C), 124.7, 127.4, 128.1, 128.9, 129.3 (2C), 130.4, 131.3, 131.6, 135.4, 147.0, 148.0, 163.6, 163.7 ppm.

MS (ESI-) m/z: 381 (M − K) (100%).

*3,6-Dinitro-N-(4-aminobenzyl)-1,8-naphthalimide (**19**).* Synthesized using general procedure 1, commencing with 3,6-dinitro-1,8-naphthalic anhydride and 4-aminobenzylamine. The crude mixture was purified by column chromatography using a gradient 10% pet. Spirit/EtOAc to 50% pet spirit/EtOAc. A dark brown solid was obtained.

Yield 0.102g (52%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.01 (2H, br s, NH_2), 5.10 (2H, s), 6.47 (2H, d, J = 8.1 Hz), 7.11 (2H, d, J = 8.1 Hz), 9.10 (2H, s), 9.77 (2H, s).

^{13}C NMR (100 MHz) (DMSO-d_6) δ 43.4, 113.8 (2C), 123.7, 124.5 (2C), 126.2 (2C), 129.6 (2C), 130.9, 131.6 (2C), 131.7, 147.2 (2C), 148.3, 161.8 (2C) ppm.

MS (ESI+) m/z: 393 (M + H) (100%).

*Library **2**. 3-Sulfo-N-(4-aminophenyl)-1,8-naphthalimide, Sodium Salt (**22**).* Synthesized using general procedure 1, commencing with 3-sulfo-1,8-naphthalic anhydride (sodium salt) and 1,4-phenylenediamine. An off-white solid was obtained.

Yield 0.281 g (86%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.25 (2H, br s), 6.63 (2H, d, J = 8.7 Hz), 6.94 (2H, d, J = 8.7 Hz), 7.88 (1H, dd, J = 7.2, 8.4 Hz), 8.46 (1H, dd, J = 0.9, 7.2 Hz), 8.57 (1H, dd, J = 0.9, 8.4 Hz), 8.63 (1H, d, J = 1.8 Hz), 8.66 (1H, d, J = 1.8 Hz).

^{13}C NMR (DMSO-d_6) δ 113.9 (2C), 122.8, 122.9, 124.0, 127.7 (2C), 128.6, 129.4 (2C), 130.0, 131.1, 131.2, 135.1, 147.1, 148.8, 164.0, 164.2 ppm.

MS (ESI-) m/z: 367 (M − Na) (100%).

*3-Sulfo-N-(3-aminobenzyl)-1,8-naphthalimide, Potassium Salt (**23**).* Synthesized using general procedure 1, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 3-aminobenzylamine. A pale yellow solid was obtained.

Yield 0.271 g (83%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 4.96 (2H, br s), 5.12 (2H, s), 6.39 (1H, ddd, J = 1.2, 2.1, 7.8 Hz), 6.46 (1H, d, J = 2.1 Hz), 6.49 (1H, dd, J = 1.2, 7.8 Hz), 6.92 (1H, t, J = 7.8 Hz), 7.88 (1H, dd, J = 7.2, 8.1 Hz),

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8.50 (1H, dd, J = 1.2, 7.2 Hz), 8.56 (1H, dd, J = 1.2, 8.1 Hz), 8.67 (1H, d, J = 1.8 Hz), 8.70 (1H, d, J = 1.8 Hz).

^{13}C NMR (DMSO-d_6) δ 43.2, 112.4, 112.8, 115.0, 122.1 (2C), 127.4, 128.0, 129.0 (2C), 130.4, 131.2, 131.5, 135.4, 138.0, 147.1, 149.0, 163.5 (C_{10}), 163.6 (C_9) ppm.

MS (ESI-) m/z: 381 (M − K) (100%).

*3-Sulfo-N-(2-aminobenzyl)-1,8-naphthalimide, Potassium Salt (**24**).* Synthesized using general procedure 1, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 2-aminobenzylamine. A yellow solid was obtained.

Yield 0.292 g (86%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.02 (2H, s, $H_{1'}$), 5.15 (2H, br s), 6.43 (1H, m), 6.65 (1H, dd, J = 1.2, 8.1 Hz), 6.87 (1H, dd, J = 1.5, 7.5 Hz), 6.92 (1H, m), 7.89 (1H, dd, J = 7.5, 8.1 Hz), 8.50 (1H, dd, J = 1.2, 7.5 Hz), 8.56 (1H, dd, J = 1.2, 8.1 Hz), 8.68 (1H, d, J = 1.5 Hz), 8.69 (1H, d, J = 1.5 Hz).

^{13}C NMR (DMSO-d_6) δ 40.3, 115.3, 116.5, 120.0, 122.1, 122.2, 127.5, 127.6, 128.0, 128.1, 129.1, 130.6, 131.3, 131.8, 135.6, 146.3, 147.0, 163.9, 164.0 ppm.

MS (ESI-) m/z: 381 (M − K) (100%).

*3-Sulfo-N-(benzyl)-1,8-naphthalimide, potassium salt (**25**) [Pitstop-1−25].* Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) (0.251 g, 0.79 mmol) and benzylamine (173 μL, 1.58 mmol). An off-white solid was obtained.

Yield 0.267 g (83%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.26 (2H, s), 7.28 (5H, m), 7.88 (1H, dd, J = 7.2, 8.4 Hz), 8.50 (1H, dd, J = 1.2, 7.2, Hz), 8.57 (1H, dd, J = 1.2, 8.4 Hz), 8.68 (1H, d, J = 1.8 Hz), 8.70 (1H, d, J = 1.8 Hz).

^{13}C NMR (DMSO-d_6) δ 43.1, 122.1 (2C), 127.2, 127.4, 127.6 (2C), 127.9, 128.6 (2C), 129.0, 130.4, 131.2, 131.4, 135.4, 137.5, 147.2, 163.6, 163.7 ppm.

MS (ESI-) m/z: 366 (M − K) (100%).

*3-Sulfo-N-(4-hydroxybenzyl)-1,8-naphthalimide, Potassium Salt (**26**).* Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-hydroxybenzylamine (1.2 equiv). A cream-brown solid was obtained.

Yield 0.293 g (88%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.14 (2H, s), 6.67 (2H, d, J = 8.7 Hz), 7.20 (2H, d, J = 8.7 Hz), 7.87 (1H, dd, J = 7.2, 8.1 Hz), 8.49 (1H, dd, J = 1.2, 7.2 Hz), 8.55 (1H, dd, J = 1.2, 8.1 Hz), 8.65 (1H, d, J = 1.5 Hz), 8.69 (1H, d, J = 1.5 Hz), 9.27 (1H, br s).

^{13}C NMR (DMSO-d_6) δ 42.6, 115.2 (2C), 122.1 (2C), 127.3, 127.8 (2C), 128.9, 129.4 (2C), 130.3, 131.2, 131.3, 135.3, 147.2, 156.6, 163.5, 163.6 ppm.

MS (ESI-) m/z: 382 (M − K) (100%).

*3-Sulfo-N-(4-methoxybenzyl)-1,8-naphthalimide, Potassium Salt (**27**).* Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-methoxybenzylamine (1.05 equiv). An off-white solid was obtained.

Yield 0.286 g (83%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 3.69 (3H, s), 5.19 (2H, s), 6.84 (2H, d, J = 8.7 Hz), 7.32 (2H, d, J = 8.7 Hz), 7.87 (1H, dd, J = 7.2, 8.1 Hz), 8.49 (1H, dd, J = 1.2, 7.2 Hz), 8.55 (1H, dd, J = 1.2, 8.1 Hz), 8.66 (1H, d, J = 1.8 Hz), 8.69 (1H, d, J = 1.8 Hz).

^{13}C NMR (DMSO-d_6) δ 42.5, 55.2, 113.9 (2C), 122.0, 122.1, 127.3, 127.8, 128.9, 129.4 (2C), 129.5, 130.3, 131.2, 131.3, 135.3, 147.3, 158.5, 163.5, 163.6 ppm.

MS (ESI-) m/z: 396 (M − K) (100%).

*3-Sulfo-N-(4-methylbenzyl)-1,8-naphthalimide, Potassium Salt (**28**).* Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-methylbenzylamine (0.6 equiv). An off-white solid was obtained.

Yield 0.365 g (100%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 2.23 (3H, s), 5.21 (2H, s), 7.09 (2H, d, J = 8.1 Hz), 7.25 (2H, d, J = 8.1 Hz), 7.88 (1H, dd, J = 7.2, 8.4 Hz), 8.49 (1H, dd, J = 1.2, 7.2 Hz), 8.56 (1H, dd, J = 1.2, 8.4 Hz), 8.67 (1H, d, J = 1.8 Hz), 8.69 (1H, d, J = 1.8 Hz).

^{13}C NMR (DMSO-d_6) δ 20.8, 42.9, 122.0, 122.1, 127.4, 127.7 (2C), 127.8, 128.9, 129.1 (2C), 130.3, 131.2, 131.4, 134.5, 135.4, 136.3, 147.2, 163.5, 163.6 ppm.

MS (ESI-) m/z: 380 (M − K) (100%).

*3-Sulfo-N-(4-(dimethylamino)benzyl)-1,8-naphthalimide, Potassium Salt (**29**).* Synthesized using general procedure 1, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-(dimethylamino)benzylamine (dihydrochloride salt) (1.2 equiv). An excess of TEA was added to react with liberated HCl. A pale yellow solid was obtained.

Yield 0.271 g (95%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 2.81 (6H, s), 5.14 (2H, s), 6.63 (2H, d, J = 8.7 Hz), 7.23 (2H, d, J = 8.7 Hz), 7.87 (1H, dd, J = 7.5, 8.1 Hz), 8.49 (1H, dd, J = 1.2, 7.5 Hz), 8.55 (1H, dd, J = 1.2, 8.1 Hz), 8.65 (1H, d, J = 1.5 Hz), 8.69 (1H, d, J = 1.5 Hz).

^{13}C NMR (DMSO-d_6) δ 40.3 (2C), 42.6, 112.4 (2C), 122.1 (2C), 125.1, 127.3, 127.8, 128.9, 129.2 ($2C_3$), 130.2, 131.2, 131.3, 135.3, 147.2, 149.9, 163.4, 163.5 ppm.

MS (ESI-) m/z: 409 (M − K) (100%).

*3-Sulfo-N-(4-carboxybenzyl)-1,8-naphthalimide, Potassium Salt (**30**).* Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-(aminomethyl)-benzoic acid (1.2 equiv). A pale yellow solid was obtained.

Yield 0.261 g (91%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.32 (2H, s), 7.46 (2H, d, J = 8.4 Hz), 7.86 (2H, d, J = 8.4 Hz), 7.89 (1H, dd, J = 7.5, 8.1 Hz), 8.50 (1H, dd, J = 1.2, 7.5 Hz), 8.58 (1H, dd, J = 1.2, 8.1 Hz), 8.68 (1H, d, J = 1.8 Hz), 8.70 (1H, d, J = 1.8 Hz).

^{13}C NMR (DMSO-d_6) δ 43.1, 122.0, 122.1, 127.5 (3C), 127.8, 129.0, 129.6 (2C), 129.7, 130.4, 131.2, 131.4, 135.4, 142.5, 147.2, 163.6, 163.7, 167.3 ppm.

MS (ESI-) m/z: 410 (M − K).

*3-Sulfo-N-(4-nitrobenzyl)-1,8-naphthalimide, Potassium Salt (**31**).* Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-nitrobenzylamine (hydrochloride salt) (1.2 equiv). A light brown solid was obtained.

Yield 0.250 g (88%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.37 (2H, s), 7.63 (2H, d, J = 8.7 Hz), 7.89 (1H, dd, J = 7.2, 8.4 Hz), 8.15 (2H, d, J = 8.7 Hz), 8.50 (1H, dd, J = 1.2, 7.2 Hz), 8.58 (1H, dd, J = 1.2, 8.4 Hz), 8.69 (2H, s).

^{13}C NMR (DMSO-d_6) δ 43.0, 122.0 (2C), 123.7 (2C), 127.6, 127.9, 128.6 (2C), 129.0, 130.5, 131.2, 131.5, 135.5, 145.4, 146.8, 147.2, 163.6, 163.7 (C_9) ppm.

MS (ESI-) m/z: 411 (M − K) (100%).

*3-Sulfo-N-(4-fluorobenzyl)-1,8-naphthalimide, Potassium Salt (**32**).* Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-fluorobenzylamine (0.1 equivalents). An off-white solid was obtained.

Yield 0.049 g (73%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.23 (2H, s), 7.10 (2H, t, J = 8.7 Hz), 7.42 (2H, dd, J = 5.7, 8.7 Hz), 7.88 (1H, dd, J = 7.2, 8.4 Hz), 8.50 (1H, dd, J = 1.2, 7.2 Hz), 8.57 (1H, dd, J = 1.2, 8.4 Hz), 8.67 (1H, d, J = 1.5 Hz), 8.69 (1H, d, J = 1.5 Hz, H_2).

^{13}C NMR (DMSO-d_6) δ 42.5 ($C_{1'}$), 115.2 ($^2J_{CF}$ = 21.3 Hz, $2C_4$), 122.0, 122.1, 127.4, 127.8, 129.0, 129.9 ($^3J_{CF}$ = 8.2 Hz, 2C), 130.3, 131.2, 131.4, 133.7 ($^4J_{CF}$ = 2.1 Hz), 135.4, 147.2, 161.4 ($^1J_{CF}$ = 242.9 Hz), 163.5, 163.6 ppm.

MS (ESI-) m/z: 384 (M − K) (100%).

*3-Sulfo-N-(4-chlorobenzyl)-1,8-naphthalimide, Potassium Salt (**33**).* Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-chlorobenzylamine (0.1 equiv). An off-white solid was obtained.

Yield 0.057 g (83%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.24 (2H, s), 7.34 (2H, d, J = 8.7 Hz), 7.40 (2H, d, J = 8.7 Hz), 7.88 (1H, dd, J = 7.2, 8.4 Hz), 8.50 (1H, dd, J = 1.2, 7.2 Hz), 8.57 (1H, dd, J = 1.2, 8.4 Hz), 8.67 (1H, d, J = 1.5 Hz), 8.69 (1H, d, J = 1.5 Hz).

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^{13}C NMR (DMSO-d_6) δ 42.6, 122.0, 122.1, 127.4, 127.8, 128.5 (2C), 129.0, 129.6 (2C), 130.3, 131.2, 131.4, 131.8, 135.4, 136.5, 147.3, 163.6, 163.7 ppm.

MS (ESI-) m/z: 400 (M − K) (100%).

3-Sulfo-N-(4-bromobenzyl)-1,8-naphthalimide, Potassium Salt (34). Synthesized using general procedure 2, commencing with 3-sulfo-1,8-naphthalic anhydride (potassium salt) and 4-bromobenzylamine (0.1 equiv). An off-white solid was obtained.

Yield 0.037 g (77%); MP > 250 °C.

^{1}H NMR (DMSO-d_6) δ 5.22 (2H, s), 7.33 (2H, d, J = 8.4 Hz), 7.48 (2H, d, J = 8.4 Hz), 7.88 (1H, dd, J = 7.2, 8.4 Hz), 8.50 (1H, dd, J = 1.2, 7.2 Hz), 8.57 (1H, dd, J = 1.2, 8.4 Hz), 8.67 (1H, d, J = 1.8 Hz), 8.69 (1H, d, J = 1.8 Hz).

^{13}C NMR (DMSO-d_6) δ 42.6, 120.3, 122.0, 122.1, 127.4, 127.8, 129.0, 129.9 (2C), 130.3, 131.2, 131.4 (3C), 135.4, 137.0, 147.2, 163.6, 163.7 ppm.

MS (ESI-) m/z: 444 (M − K) (100%).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}CNMR spectra for compounds **10a**, **16a**, **2a**, **17a**, **21**, **2**−**19**, and **22**−**34**; and HRMS data for compounds **2**−**7**, **9**, **12**−**19**, and **22**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): We have entered into a commercial agreement with Abcam Biochemicals (Bristol, UK) for the supply of our dynamin and clathrin inhibitors. This includes some of the compounds listed in this paper. #Pitstop is a registered trademark of Childrens Medical Research Institute and Newcastle Innovation Ltd.

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■ ABBREVIATIONS

CME: clathrin mediated endocytosis; CCV: clathrin coated vesicle; TD: terminal domain; TD-A: terminal domain with amphiphysin; HTS: High throughput screening

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