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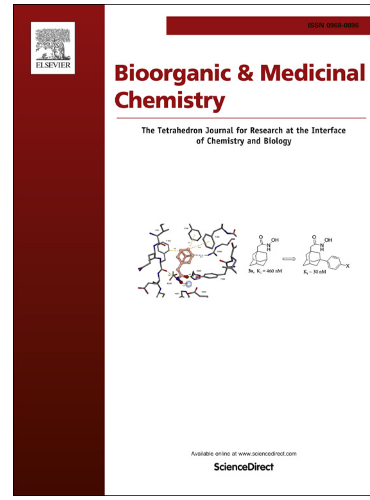

Structure-activity relationships and colorimetric properties of specific probes for the putative cancer biomarker human arylamine *N*-acetyltransferase 1

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Graphical Abstract

Structure-activity relationships and colorimetric properties of specific probes for the putative cancer biomarker human arylamine *N*-acetyltransferase 1

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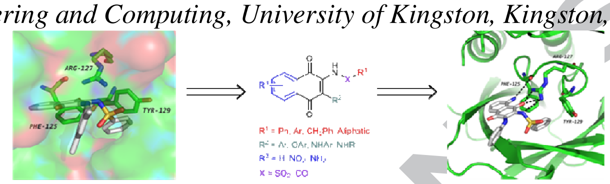

IC_{50} (hNAT1) – 4.10 μM
ε_{CB} = 6590 $M^{-1}cm^{-1}$

IC_{50} (hNAT1) – 0.12 μM
ε_{CB} = 15840 $M^{-1}cm^{-1}$

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ABSTRACT

A naphthoquinone inhibitor of human arylamine *N*-acetyltransferase 1 (hNAT1), a potential cancer biomarker and therapeutic target, has been reported which undergoes a distinctive concomitant color change from red to blue upon binding to the enzyme. Here we describe the use of *in silico* modeling alongside structure-activity relationship studies to advance the hit compound towards a potential probe to quantify hNAT1 levels in tissues. Derivatives with both a fifty-fold higher potency against hNAT1 and a two-fold greater absorption coefficient compared to the initial hit have been synthesized; these compounds retain specificity for hNAT1 and its murine homologue mNat2 over the isoenzyme hNAT2. A relationship between pK_a, inhibitor potency and colorimetric properties has also been uncovered. The high potency of representative examples against hNAT1 in ZR-75-1 cell extracts also paves the way for the development of inhibitors with improved intrinsic sensitivity which could enable detection of hNAT1 in tissue samples and potentially act as tools for elucidating the unknown role hNAT1 plays in ER+ breast cancer; this could in turn lead to a therapeutic use for such inhibitors.

1. Introduction

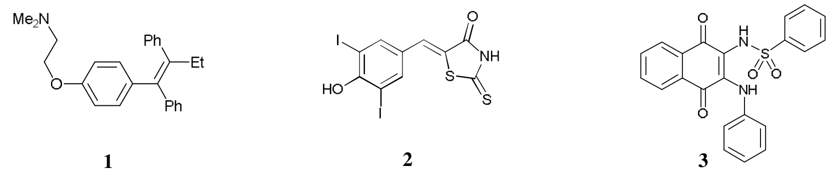
The worldwide annual incidence of breast cancer exceeds one million cases, with statistics from the UK showing that the lifetime risk of a woman developing the disease is 1 in 8.[1] Early detection of tumors is known to be crucial for improving survival rates.[2] The estrogen receptor (ER) is overexpressed in a major subtype of breast cancer, referred to as "ER-positive" (ER+), and was the first protein to be used as a diagnostic and prognostic biomarker for breast cancer.[3] ER+ tumors are often responsive to aromatase inhibitors or to selective ER modulator (SERM) therapies.[3] However, the clinical use of SERM therapies is limited by drug resistance,[4] and the use of immunohistochemical staining to detect ERs for diagnostic purposes suffers from standardization problems.[5] Consequently, new therapeutic leads and diagnostic biomarkers for ER+ tumors are desirable.

Proteomic[6] and microarray[7] studies have identified *human arylamine N-acetyltransferase 1* (*hNAT1*) as one of the ten most highly overexpressed genes in ER+ tumors; furthermore, this overexpression inversely correlates to tumor grade.[8] More recently hNAT1 overexpression in male breast cancers has also been reported.[9] The corresponding enzyme hNAT1 is therefore of interest as a surrogate diagnostic and prognostic biomarker for ER+ tumors. Furthermore, studies suggest that hNAT1 could also be a novel therapeutic target against ER+ breast cancer.[10] Key evidence includes: the discovery of hallmarks of oncogenic potential in the non-cancerous breast cell line HB4a induced to overexpress hNAT1;[6] and the reduction in cell proliferation and invasiveness observed in the MDA-MB-231 breast cancer cell line (which expresses high levels of hNAT1) when either *hNAT1* was knocked down by shRNA or the cells were treated with inhibitors of hNAT1.[11]

NATs are a family of xenobiotic metabolizing enzymes found in both eukaryotic and prokaryotic organisms, which catalyze the transfer of an acetyl group from acetyl coenzyme A (AcCoA) to a xenobiotic substrate, such as arylamines, arylhydroxylamines, arylhydrazines, and *N*-alkylarylamines.[12] The human genome codes for two functional *NAT* genes: *hNAT1* and *hNAT2*. The gene product hNAT2 is implicated in phase II drug metabolism and is abundant in liver and intestinal cells,[12a] whilst hNAT1 has a widespread tissue distribution[13] and is reported to play a role in cofactor homeostasis;[14] studies suggest eukaryotic homologues of hNAT1 are involved in growth and development.[15] Estrogen levels may also have an effect on hNAT1 expression.[16]

Aside from the discovery that the SERM tamoxifen **1** reduces hNAT1 activity *in vitro*,[17] the first compounds identified as hNAT1 inhibitors were found to be non-selective for hNAT1 and to act *via* covalent modification of the catalytic Cys68 residue;[18] these include alkylating agents as well as the widely prescribed chemotherapeutic agent cisplatin.[19]

(a)



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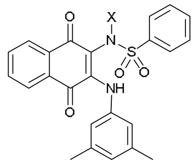
(b)



4 X = H
λ_{max} (pH 8) = 498 nm

5 X = Me
λ_{max} (pH 8) = 496 nm

Compound		Enzyme			
		hNAT1	mNat2 WT	mNat2 R127G	mNat2 R127L
4	IC_{50} (μM)	5.3	1.9	51.7	102.5
	λ_{max} (nm)	585	625	498	498
5	IC_{50} (μM)	5.8	> 150	N.D.	N.D.
	λ_{max} (nm)	476	490	N.D.	N.D.

Figure 1: **(a)** Examples of hNAT1 inhibitors reported in the literature.[6, 20] **(b)** Compound **4** is deprotonated selectively in the presence of hNAT1 with a distinctive concomitant color change; this process is driven by sequestration of the conjugate base of **4** by the Arg127 residue of hNAT1.[21] (N.D. = not determined.)

More recently, rhodanine **2** and naphthoquinone **3** have been identified as inhibitors of hNAT1 (and its murine homologue mNat2)[22] and were found to be selective over a variety of other NATs including hNAT2;[20] additionally both **2** and **3** inhibited hNAT1 activity in cell extracts from the ER+ breast cancer cell line ZR-75-1 (**Figure 1(a)**).[20, 23]

The reversible, competitive naphthoquinone inhibitor **3** proved to be of particular interest, because it was found that **3** undergoes a distinctive color change from red to blue in the presence of hNAT1 and mNat2, but not any other NAT isoform tested.[23] Subsequently spectroscopic, chemical, molecular modeling and biochemical studies have been carried out on a close analogue of **3**, the dimethyl-substituted **4**, which has a similar potency to **3** against hNAT1 and mNat2 (**Figure 1(b)**).[21] These studies demonstrated that this color change is driven by sequestration of the conjugate base of **4** (pK_a ~9.2), mediated by Arg127 in the active site of hNAT1 or mNat2.[21]

This observed color change leads to the possibility that **4** could act as a direct *in vitro* probe for the presence of native hNAT1, without the conventional need for protein tagging or antibody staining.[24] Potentially, any specific small molecule inhibitors of hNAT1 could additionally be valuable leads for a new drug therapy. However, for the potential of these naphthoquinones as probes for hNAT1 to be realized, it is believed that both the binding potency and molar absorption coefficient need to be increased. We describe here our preliminary structure-activity relationship and optimization studies.

2. Results and Discussion

2.1. Quantification of [hNAT1] in Cell Extracts from the Breast Cancer Cell Line ZR-75-1

The approximate level of hNAT1 in a representative breast cancer cell line (ZR-75-1) was determined using immunoblotting, to gain an understanding of the sensitivity required in a probe for hNAT1 detection in cells.

The twelve *C*-terminal residues of hNAT1 and mNat2 are identical and distinct from those of hNAT2. This has allowed generation of a specific antibody which recognizes both hNAT1 and mNat2[13a, 15d, 25] with equal levels of sensitivity.[26]

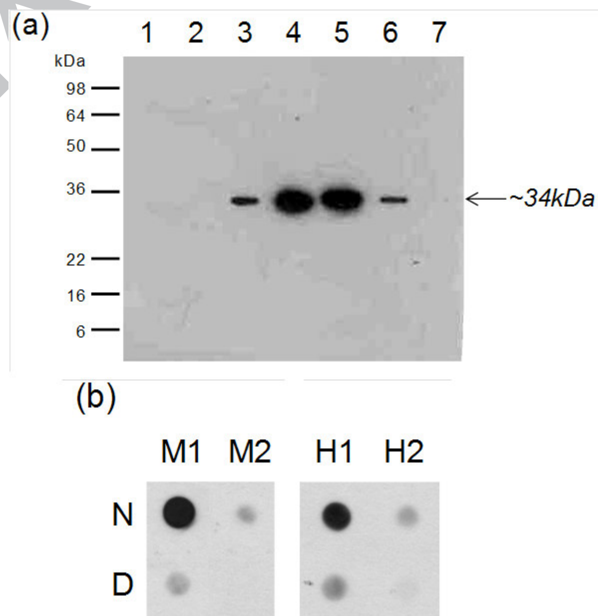


Figure 2: Immunohistochemical quantification of hNAT1 in ZR-75-1 cell lysate. **(a)** Detection of purified recombinant mNat2, and hNAT1 in cell lysate by Western Blotting. Molecular weights and positions of the protein bands in the SeeBlue® Plus2 Pre-Stained Standard used are shown on the left. **Lanes: 1-4** 0.0125, 0.125, 0.625 and 1.25 ng/μL mNat2; **5-7**: 10-, 100- and 1000-fold diluted ZR-75-1 cell lysate of 5 x 10^7 cells/mL. **(b)** Detection of purified recombinant mNat2 (**M**) and hNAT1 in cell lysate (**H**) by dot blotting as native (**N**) and denatured (**D**) proteins. Native protein samples were dissolved in an equal volume of buffer (200 mM Tris.HCl (pH 8)), while denatured proteins were prepared in an equal volume of buffer with 2% (w/v) SDS and heated to 95 °C for 5 min. **Lanes:** **M1** 0.031 ng/μL; **M2** 0.0078 ng/μL; **H1** 500-fold diluted cell lysate of 5 x 10^7 cells/mL; **H2** 2500-fold diluted lysate.

A Western blot was performed with both pure recombinant mNat2 and ZR-75-1 cell extracts, and a single band at ~34 kDa (corresponding to the relevant NAT protein) was observed for all samples **(Figure 2(a))**. A comparison of lanes 3 and 6 suggested that there is less than 62.5 ng/μL of hNAT1 (i.e. less than 62.5 ng hNAT1 per 5 x 10^4 cells).

Subsequently, dot-blotting studies were carried out, varying the concentrations of pure recombinant mNat2 and ZR-75-1 cell extracts, under both native and denaturing conditions **(Figure 2(b))**. Comparing lanes M2 and H2, there appeared to be greater than 19.5 ng of native hNAT1 per 5 x 10^4 cells. It can also be noted that the antibody had a higher affinity for native hNAT1 than denatured hNAT1, a result which is consistent with previous studies.[26]

These experiments therefore showed that the concentration of hNAT1 in the ZR-75-1 cell extract is approximately 0.6-1.2 μM (20-60 ng/μL); this informed us of the level of sensitivity required of a colorimetric probe for detecting hNAT1 in such cell extracts. Bradford assays show that the total cellular protein concentration is 11.5 μg/μL, and hence the percentage of hNAT1 in the ZR-75-1 cell proteome is around 0.2-0.5%.

In separate experiments, the minimum hNAT1:inhibitor ratio to enable the unambiguous determination of a colorimetric shift of **4** spectroscopically was determined. It was predicted that based on the calculated K_d for **4** (4.9 μM) and a 1:1 binding ratio,[23] an excess of the protein would be required for every inhibitor molecule to bind and change color. The required hNAT1:inhibitor ratio would also be expected to decrease with increasing enzyme active site occupancy. With compound **4** this minimum ratio was found to be 1.2:1. Therefore, **4** was added to ZR-75-1 cell extracts at a final concentration ranging from 0.6 μM to 2.5 μM, but the signal-to-noise ratio was poor at these low concentrations and the λ_{max} peak (at 585 nm) could not be detected. This confirmed the hypothesis that the potency and sensitivity of **4** must be increased in order to enable hNAT1 detection in such cell extracts.

2.2. Overview of SAR Studies

In this study we systematically varied substituents at the C_2, C_3 and C_5-C_8 positions of naphthoquinone **4**, to improve our understanding of inhibitor-hNAT1 interactions which could guide the rational design of colorimetric probes for specific hNAT1 detection and inhibition **(Figure 3)**. Synthesis of novel inhibitors, guided by *in silico* studies, sought to increase both the potency and absorption coefficients of the inhibitor, whilst retaining both selectivity for hNAT1 over hNAT2 and the ability to observe a distinctive color change in the presence of hNAT1.

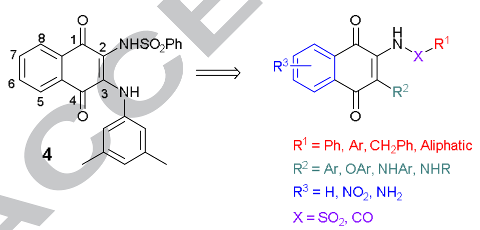


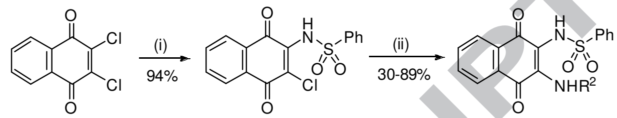
Figure 3: Structure-activity relationships investigated in this study.

2.3. SAR at C_3 of the Naphthoquinone Core

2.3.1. C_3 Amino-Substituted Analogues

Initially, compounds **3**, **4** and **6**, which we have reported previously,[23] were synthesized in a two-step procedure from 2,3-dichloronaphthalene-1,4-dione **7** *via* successive addition-elimination reactions **(Scheme 1; Table 1)**. The reaction conditions were optimized from those previously reported[23] leading to improved overall yields (for optimization see **Scheme S1 and Table S1** in Supporting Information).

A small series of aliphatic amino groups were also introduced at the C_3 position by substitution of chloride from intermediate **8** with aliphatic amines. In comparison to anilines, aliphatic amines possess higher conformational freedom and are unable to form π-π interactions within the NAT active site upon binding. A series of analogues was chosen to allow comparison of effects of chain extension, inclusion of polar heteroatoms and aromatic moieties **(Scheme 1; Table 1)**. Their synthesis was analogous to that of **4** except it was found that step (ii) proceeded in excellent yield within only 6 h in toluene.



7 **8** **3-4, 6, 9-11**

Scheme 1: *Reagents and conditions:* (i) $PhSO_2NH_2$ (1.0 eq.), Cs_2CO_3 (1.4 eq.), DMF, RT, 5 h; (ii) *either* $CeCl_3.7H_2O$ (1.0 eq.), MeOH, RT, 90 min. then requisite aniline (3.0 eq.), 110 °C, 16 h; *or* $CeCl_3.7H_2O$ (1.0 eq.), toluene, RT, 90 min. then requisite amine (3.0 eq.), toluene, 110 °C, 6 h.

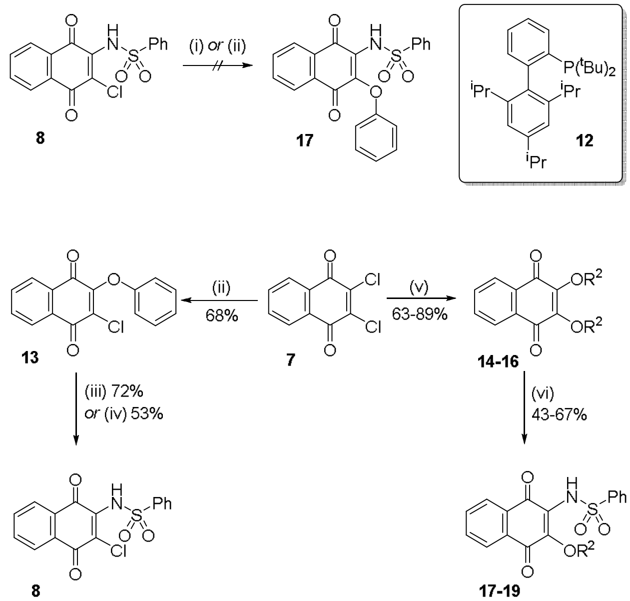
Table 1. Identities of R^2 substituents and yields for the amino analogue series.

Compound	R^2	Yield of step (ii)
3	Ph	63%
4	3'',5''-Me_2-C_6H_3	57%
6	4''-Br-C_6H_4	30%
9	2''-Methoxyethyl	87%
10	Cyclopentyl	89%
11	Benzyl	42%

2.3.2. C_3 Aryloxy-Substituted Analogues

Aryloxy analogues of **4** were also synthesized, since these were predicted to have similar conformational preferences to anilino substituents whilst possessing H-bond acceptor but not donor ability. Initially, intermediate **8** was pre-treated with $CeCl_3.7H_2O$ before addition to sodium phenoxide (generated by treatment of phenol with NaH) in toluene; however, none of the desired product was obtained **(Scheme 2)**. A Buchwald-type cross-coupling reaction involving the bulky electron-rich ligand di^{t}BuXPhos **12** between **8** and phenol was subsequently attempted,[27] but returned only starting materials. The same Buchwald conditions applied to the dichloro precursor **7** yielded the monophenoxy-substituted derivative **13** in moderate yield. Subsequent treatment of **13** with $CeCl_3.7H_2O$ and benzenesulfonamide, however, resulted in the sulfonamide displacing the phenoxy substituent rather than the desired chloride leading to formation of **8**. Attempted cross-coupling between **13** and $PhSO_2NH_2$ with $Pd(dppf)_2Cl_2$ as a catalyst also afforded **8**.

Given the susceptibility of phenoxy substituents to displacement by benzenesulfonamide, an alternative route to aryloxy analogues of **4** *via* the corresponding diaryloxy naphthoquinones was developed. Dichloride **7** was treated with the requisite aryl alcohol and cesium carbonate in THF at reflux to give the corresponding diaryloxy-substituted naphthoquinones **14-16**, which were subsequently treated with benzenesulfonamide and cesium carbonate in THF at 80 °C for 1 h. Recrystallisation from toluene gave the desired C_3 aryloxy-substituted analogues **17-19 (Scheme 2; Table 2)**.



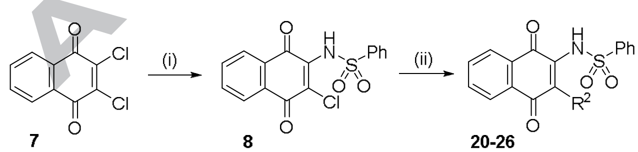
Scheme 2: *Reagents and conditions:* (i) $CeCl_3.7H_2O$ (1.0 eq.), toluene, RT, 90 min., then NaOPh (1.0 eq.), reflux, 16 h; (ii) PhOH (1.2 eq.), $Pd(OAc)_2$ (0.02 eq.), **12** (0.03 eq.), K_3PO_4 (2.0 eq.), toluene, reflux, 16 h; (iii) $CeCl_3.7H_2O$ (1.0 eq.), toluene, RT, 90 min., then $PhSO_2NH_2$ (3.0 eq.), reflux, 18 h; (iv) $PhSO_2NH_2$ (1.2 eq.), $Pd(dppf)_2Cl_2$ (0.02 eq.), Cs_2CO_3 (2.0 eq.), dioxane, reflux, 1 h; (v) ArOH (2.2 eq.), Cs_2CO_3 (2.2 eq.), THF, reflux, 16 h; (vi), $PhSO_2NH_2$ (1.2 eq.), Cs_2CO_3 (1.2 eq.), THF, reflux, 1 h.

Table 2: Identities of R^2 substituents and yields for the aryloxy analogue series.

Compound	R^2	Isolated Yield step (v)	Isolated Yield step (vi)
17	Ph	63%	67%
18	3'',5''-Me_2-C_6H_3	89%	57%
19	4''-Br-C_6H_4	72%	43%

2.3.3. C_3 Aryl-Substituted Analogues

A library of C_3 aryl-substituted analogues, which are predicted to have different conformational preferences to the anilino derivatives and lack both H-bond donor and acceptor properties, was synthesized. Suzuki cross-coupling reactions of chloride **8** and the requisite boronic acid with $Pd(PPh_3)_2Cl_2$ in 4:1 THF:sat. aq. $NaHCO_3$ proceeded in 70-80% yield; partial purification by column chromatography followed by recrystallisation from toluene led to isolation of analogues **20-26** in moderate to low yield **(Scheme 3; Table 3)**.



Scheme 3: *Reagents and conditions:* (i) $PhSO_2NH_2$ (1.0 eq.), Cs_2CO_3 (1.4 eq.), DMF, RT, 5 h; (ii) Requisite boronic acid (2.0 eq.), $Pd(PPh_3)_2Cl_2$ (0.1 eq.), 4:1 THF:sat. aq. $NaHCO_3$, reflux, 18 h.

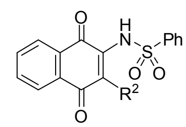
Table 3: Identities of R^2 substituents and yields for the aryl analogue series.

Compound	R^2	Isolated Yield step (ii)
20	2''-Cl-C_6H_4	48%
21	3''-Cl-C_6H_4	35%
22	4''-Cl-C_6H_4	47%
23	3''-CHO-C_6H_4	31%
24	4''-CHO-C_6H_4	24%
25	2''-furanyl	45%
26	3''-furanyl	23%

2.3.4. Pharmacological Evaluation of the C_3-Substituted Analogues

All of the synthesized analogues of compound **4** were initially tested for inhibitory potency against recombinant mNat2 and hNAT1, which were expressed and purified as outlined in **Section 4.2.1.**.[22, 28] NAT activity in the presence of an inhibitor was determined by measuring the rate of acetyl coenzyme A (AcCoA) hydrolysis using a previously described method.[29] The IC_{50} value of each synthesized compound is given in **Table 4**; squared correlation coefficients for these data are presented in **Table S2** in Supporting Information.

Table 4: IC_{50} values for the C_3 analogue library.



Compound	R^2	IC_{50} (mNat2) (μM)	IC_{50} (hNAT1) (μM)
3	NHPh	1.9[23]	1.7[23]
4	NH-3'',5''-Me_2-C_6H_3	2.4	4.1
6	NH-4''-Br-C_6H_4	1.7	0.9
9	$NHCH_2CH_2OMe$	> 30	> 30
10	NH-Cyclopentyl	> 30	> 30
11	$NHCH_2Ph$	> 30	> 30
17	OPh	1.8	2.8
18	O-3'',5''-Me_2-C_6H_3	5.2	12.1
19	O-4''-Br-C_6H_4	1.1	1.9
20	2''-Cl-C_6H_4	3.7	8.0
21	3''-Cl-C_6H_4	5.1	14.5
22	4''-Cl-C_6H_4	8.2	12.1
23	3''-CHO-C_6H_4	9.2	8.4
24	4''-CHO-C_6H_4	6.9	10.3
25	Furan-2''-yl	4.3	9.6
26	Furan-3''-yl	1.4	10.0

A wide variety of substituents appears to be tolerated at the C_3 position, with examples of anilino, aryloxy and aryl groups at this position all exhibiting inhibition of hNAT1 and mNat2. This suggests that the N-H hydrogen bond donor capability of anilino-substituted species such as **4** is not essential for binding to the enzyme, since ligands with hydrogen bond acceptor properties at C_3 (aryloxy-substituted species such as **17**) and ligands with neither hydrogen-bond-acceptor nor -donor properties at C_3 (aryl-substituted species such as **26**) bind with similar potencies to that of compound **4**. *In silico* modeling was carried out on compounds **3**, **4**, **6** and **17-26** and suggests that the binding modes of all the anilino-, aryloxy- and aryl-substituted species are similar, supporting the experimental observations; representative examples of modeling solutions are shown in **Figure 4(a)**.

The other key observation from the pharmacological data is that all derivatives bearing an aliphatic substituent at C_3 (**9-11**) display poor levels of inhibition *vs.* hNAT1 and mNat2. Docking suggests that no residues around the entrance to the active site are capable of forming π-π interactions with the C_3 moiety; however, any of the conformational preferences, electronic properties or hydrophobicity of aromatic substituents may be a requisite of binding **(Figure 4(b))**. Examples of both electron-rich and electron-poor aromatic moieties at C_3 have shown similar levels of potency to the hit compound **4**.

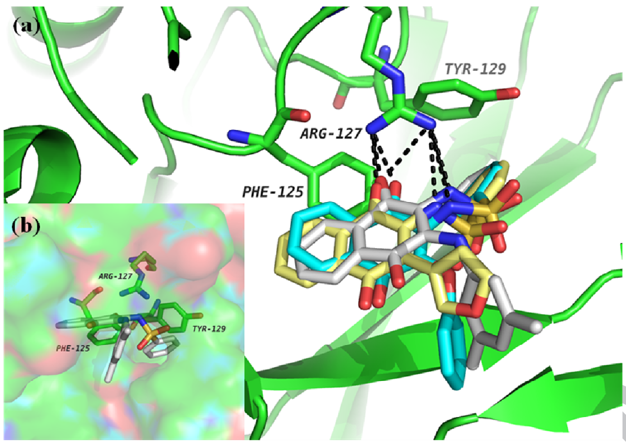


Figure 4: (a) *In silico* modeling of representative compounds **4** (carbons in grey), **17** (carbons in cyan) and **26** (carbons in yellow) in the active site of hNAT1 (pdb: 2PQT)[12e]. **(b)** Surface-filled model for interaction of compound **4** with hNAT1, depicting electrostatic surfaces and illustrating the active site pocket. Analysis performed using GOLD® software.[30]

The ground state rotational freedom of amino-substituted species **9** and **11** and the concomitant loss of entropy on binding to hNAT1 could also contribute to the low inhibitory potency of these compounds. However, the less conformationally flexible cyclopentylamine **10** is also a poor inhibitor, suggesting that an *N*-, *O*-, or directly linked aromatic substituent is indeed preferred at the C_3 position.

2.3.5. Spectrophotometric Evaluation of the C_3-Substituted Analogues

For a probe to be clinically useful for quantification of hNAT1 in biological samples, it not only needs to be a potent and selective binder of hNAT1, but it must also possess appropriate colorimetric properties, namely: the probe must have a pK_a value for the acidic sulfonamide proton above the assay pH of 8 but below the pK_{aH} of the Arg127 residue; the color change should be distinct, so that it can be unambiguously determined from visible spectra; and the conjugate base of the probe must have a high absorption coefficient (ε_{CB}) to enable a high sensitivity of detection.

The colorimetric properties of species **3**, **4**, **6**, **9-11** and **17-26** were evaluated and are outlined in **Tables 5 and 6**. pK_a values were determined *via* titration experiments (outlined in **Section 4.2.4.2.**). ε Values for both the neutral (ε_N) and conjugate base (ε_{CB}) forms of each compound were calculated following the experimental procedure described in **Section 4.2.4.3.**.

Despite the promising inhibitory potency of both the C_3 aryloxy- and aryl-substituted species, the utility of both of these series of compounds as colorimetric probes are limited by their low pK_a values, which are below that of physiological pH.

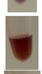
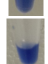
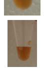
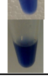
Conversely, all the anilino-substituted and amino-substituted species synthesised show clear changes in λ_{max} between pH 8 and pH 13. The anilino-substituted species **3**, **4** and **6** also show a color change in the presence of mNat2, whereas the amino-substituted species **9-11** do not; this is likely to be attributable to the weak binding of the amino-substituted species against the enzyme (IC_{50} > 30 μM). However, none of the conjugate bases display significantly enhanced absorption coefficient values over that of the conjugate base of **4**.

Therefore, attention was next turned to modifying the substituent at the C_2 position on the naphthoquinone core instead.

Table 5: pK_a values for the C_3 library of inhibitors.

Compound	3	4	6	9	10	11	17	18	19	20	21	22	23	24	25	26
pK_a	9.5	9.2	10.4	9.8	10.1	10.4	5.0	5.0	5.0	4.9	4.5	5.0	5.0	4.6	5.1	5.0

Table 6: Spectrophotometric properties of the anilino- and amino-substituted inhibitors **3**, **4**, **6** and **9-11**. N.D. = not determined.

Compound	R^2	Compound at pH 8	Compound at pH 13	$\Delta\lambda_{max}$ pH 13 (nm)	$\Delta\lambda_{max}$ mNat2 (nm)	ε_{CB} ($M^{-1}cm^{-1}$)	ε_{CB} / ε_{CB}(4)
3	NHPh			+ 71	+ 121	7900	1.20
4	NH-3'',5''-Me_2-C_6H_3			+ 79	+ 112	6590	1.00
6	NH-4''-Br-C_6H_4			+ 70	+ 93	7790	1.18
9	$NHCH_2CH_2OMe$			+ 89	N.D.	1010	0.15
10	NH-Cyclopentyl			+ 82	+ 3	4470	0.68
11	$NHCH_2Ph$			+ 74	+ 5	5770	0.88

2.4. SAR at C_2 of the Naphthoquinone Core

2.4.1. C_2 Sulfonamido-Substituted Species

In silico docking of compounds **48** and **49** in hNAT1 as representative examples of new analogues suggested that at the C_2 position, there are key interactions between the sulfonamide group and Arg127 (a hypothesis supported by our previous work)[21] and between the R^1 substituent and Tyr129 **(Figure 5)**. This potential interaction was investigated by synthesis of a library of analogues of **4** containing examples of both aliphatic and aromatic R^1 substituents **(Scheme 4; Table 7)**. All sulfonamides were commercially available except for **29** and **30**, which were prepared from the corresponding sulfonyl chlorides using standard methods.[31]

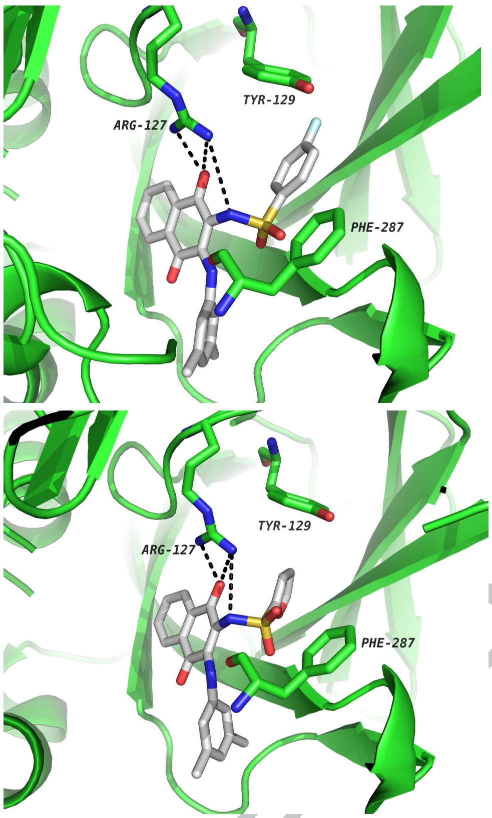
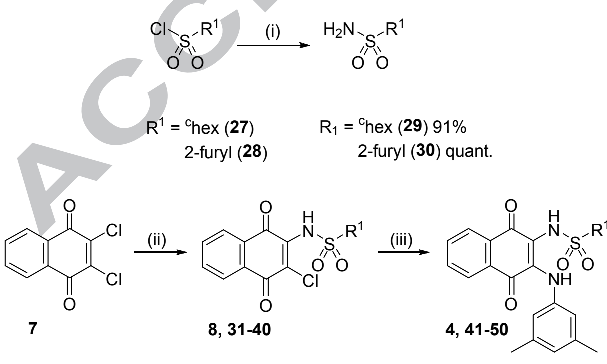


Figure 5: *In silico* modeling of **48** and **49** in the active site of hNAT1 (pdb: 2PQT)[12e], highlighting the potentially key interactions between the inhibitors and enzyme. Distances between atoms highlighted by black dashed lines are given in Angstroms. Analysis performed using GOLD® software.[30]



Scheme 4: *Reagents and conditions:* (i) NH_3 (aq., 33%), RT, 30-100 min.; (ii) requisite sulfonamide (1.0 eq.), Cs_2CO_3 (1.4 eq.), DMF, RT, 5-16 h; (iii) $CeCl_3.7H_2O$ (1.0 eq.), MeOH, RT, 90 min., then 3,5-dimethylaniline (3.0 eq.), 90 °C, 16 h.

Table 7: Identities of R_1 and yields for the C_2 sulfonamide library.

Compound	R_1	Isolated Yield Step (ii)	Isolated Yield Step (iii)
4	Ph	91%	57%
41	Me	39%	65%
42	^{c}Hex	64%	32%
43	2'-Me-C_6H_4	70%	49%
44	2'-NO_2-C_6H_4	44%	20%
45	2',6'-F_2-C_6H_3	67%	33%
46	3'-NO_2-C_6H_4	Quant.	26%
47	4'-Me-C_6H_4	66%	25%
48	4'-F-C_6H_4	63%	59%
49	Furan-2'-yl	85%	66%
50	Benzyl	81%	53%

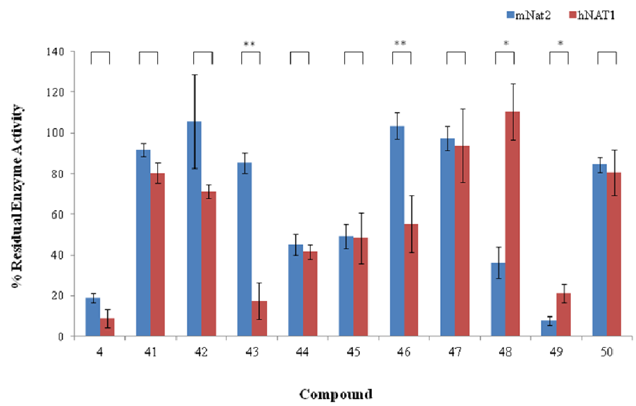
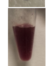
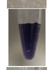
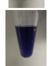
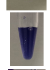


Figure 6: The % residual activity of mNat2 and hNAT1 when dosed with 30 µM of compounds **4** and **41-50**. Experiments were conducted in triplicate and results are shown as averages ± one standard deviation. The stars and brackets above the bars show the significance of the difference between the mNat2 and hNAT1 data when a Student's T-Test was performed: no stars = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

An analysis of the potency of these compounds against hNAT1 and mNat2 **(Figure 6)** shows that many of these compounds are poor inhibitors of both enzymes. IC_{50} values were subsequently determined for species showing > 50% inhibition at 30 µM. The only compounds in this series which display comparable potency to phenyl-substituted **4** against one or both proteins are the bioisosteric 4'-fluorophenyl-substituted **48** ($IC_{50, mNat2}$ = 3.9 µM; $IC_{50, hNAT1}$ = > 30 µM), 2',6'-difluorophenyl-substituted **45** ($IC_{50, mNat2}$ = 10.0 µM; $IC_{50, hNAT1}$ = 10.0 µM) and furan-2'-yl-substituted **49** ($IC_{50, mNat2}$ = 1.9 µM; $IC_{50, hNAT1}$ = 7.0 µM). 2'-methylphenyl-substituted **43** and 2'-nitrophenyl-substituted **44** show weak activity with IC_{50} values against hNAT1 of 23.1 µM and 21.8 µM respectively.

First, these results suggest that at the C_2 position, an aryl or heteroaryl substituent appears to be required for binding to hNAT1; compound **42**, which incorporates a fully saturated analogue of the phenyl group within **4**, shows poor levels of hNAT1 and mNat2 inhibition. This is possibly because π-π interactions are instrumental in achieving high inhibitor potency, a conclusion which supports the binding mode predicted by *in silico* modeling **(Figure 5)** which suggests an interaction between an aromatic C_2 substituent and the Tyr129 residue of hNAT1.

Table 8: Spectrophotometric properties of the C_2 sulfonamide-substituted inhibitors **4** and **41-50**. N.D. = not determined.

Compound	R_1	pK_a	Compound at pH 8	Compound at pH 13	$\Delta\lambda_{max}$ pH 13 (nm)	$\Delta\lambda_{max}$ mNat2 (nm)	ε_{CB} ($M^{-1}cm^{-1}$)	ε_{CB} / ε_{CB}(4)
4	Ph	9.2			+ 79	+ 112	6590	1.00
41	Me	9.5			+ 87	N.D.	6770	1.03
42	^{c}Hex	10.6			+ 53	- 7	6790	1.03
43	2'-Me-C_6H_4	9.3			+ 17	N.D.	6430	0.98
44	2'-NO_2-C_6H_4	7.9			+ 32	N.D.	7610	1.16
45	2',6'-F_2-C_6H_3	8.4			+ 39	+ 79	7780	1.18
46	3'-NO_2-C_6H_4	8.6			+ 42	N.D.	5670	0.86
47	4'-Me-C_6H_4	9.7			+ 73	- 8	7170	1.09
48	4'-F-C_6H_4	9.9			+ 59	+ 122	3490	0.53
49	Furan-2'-yl	8.3			+ 32	+ 99	7770	1.18
50	Benzyl	10.0			+ 61	+ 57	6660	1.01

Furthermore, the results show that substitution on the aromatic R_1 moiety is poorly tolerated, with examples of both electron-donating and electron-withdrawing *ortho*-, *meta*- and *para*- substituted species showing poor levels of inhibition. The only substitution tolerated on an aromatic R^1 moiety was found to be fluoro substitution (compounds **45** and **48**), which suggests that the R^1 aromatic group occupies a tight pocket within the enzyme active site. This hypothesis was further supported by the synthesis of the benzyl substituted analogue **50**, which also shows much poorer levels of inhibition than **4**.

The importance of the Tyr129 residue of hNAT1/mNat2 in binding of these naphthoquinone ligands was further investigated in a study on the homologous enzyme from the Syrian hamster, shNat2. hNAT1 and shNat2 share an 81% sequence homology and homology in substrate specificity,[32] but in shNat2, Tyr129 is replaced by Leu (see **Figure S1** in Supporting Information for sequence alignment). Other key active site residues, in particular Arg127, Phe125 and the catalytic Cys-His-Asp triad, are conserved between shNat2, hNAT1 and mNat2. Compound **4** was found to be a poor inhibitor of shNat2 (IC_{50} = 89 μM) although some evidence of a color change was observed upon binding; this suggests that Tyr129 does indeed play a crucial role in inhibitor recognition.

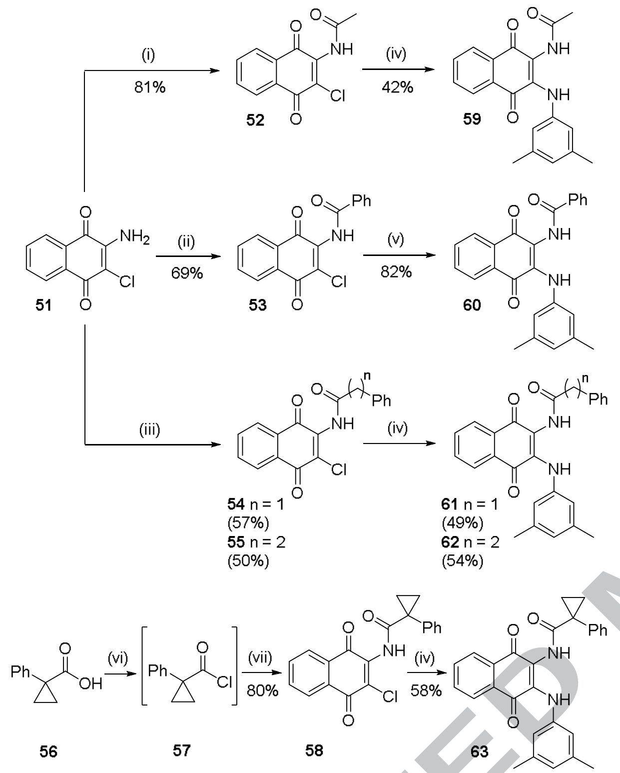
The colorimetric properties of this series of inhibitors were also evaluated **(Table 8)**. Varying the R^1 substituent appears to have little effect on the λ_{max} value of the neutral species; however, electron-donating or electron-withdrawing groups can have a significant effect on both the pK_a and ε_{CB} of the inhibitor. Inhibitors with lower pK_a values (close to 8) are unsurprisingly found to exhibit smaller shifts in λ_{max} in the presence of either base or enzyme relative to λ_{max} at pH 8. 2',6'-Difluorophenyl- and 2-furyl-substituted species **45** and **49** have undesirably low pK_a values (and hence $\Delta\lambda_{max}$), despite showing improved ε_{CB} values and similar potencies to those of the hit compound **4**.

2.4.2. C_2 Amido-Substituted Species

On the basis of the *in silico* studies carried out **(Figures 4 and 5)**, which was supported by and consistent with our initial SAR data, we hypothesised that an amide group might be a suitable alternative to the sulfonamide as a binding partner for the guanidinium group of Arg127. An amide group would be expected to have a different preferred conformation to a sulfonamide group,[33] which is likely to affect the binding to hNAT1. Replacement of the sulfonamide functionality might also be expected to alter the colorimetric properties of the molecules, such as λ_{max} and ε values. It was also anticipated the sulfonamide to amide switch would increase the pK_a by ~7-8 log units.[34] Since amides have shorter bond lengths than their sulfonamide counterparts,[35] it was predicted that a larger R^1 substituent than phenyl might be required to retain the important π-π interactions in the binding site; in particular, computational modeling studies support the proposal of a benzyl amide substituent as an isostere for a phenyl sulfonamide substitutent (see **Figure S2** in Supporting Information).

Initially, a library of amides bearing a 3,5-dimethylanilino group at C_3 were synthesized for direct comparison with hit compound **4**. Literature procedures to generate the known intermediates **52** and **53** were followed **(Scheme 5)**.[36] However, the attempted synthesis of **54** proceeded in low yield and thus a general coupling procedure to access these intermediates was developed, using the conversion of **51** to **54** as the model reaction (see **Scheme S2 and Table S2** in Supporting Information). The optimum conditions were found to involve Lewis acid catalysis with $BF_3.OEt_2$ in toluene; these improved conditions were subsequently used to synthesize intermediates **54**, **55** and **58** in good yield. From intermediates **52**, **54**, **55** and **58**, the desired final products could be obtained by substitution with 3,5-dimethylaniline (under our standard conditions); however intermediate **53** was inert under these reaction conditions. Final product **60** was therefore obtained using a Buchwald-Hartwig coupling between naphthoquinone intermediate **53** and 3,5-dimethylaniline.[37]

The SAR at C_2 for the amide series is steep, with only the benzyl amide **61** showing a moderate level of mNat2 inhibition ($IC_{50,\ mNat2}$ = 28.1 µM), and all of the series have IC_{50} values of > 30 µM against hNAT1 (**Figure 7**). The inactivity of phenyl amide **60** is possibly attributable to the restricted rotation imposed on the molecule through the use of the amide linker, which might prevent the key π-π interactions with Tyr129 between the aryl rings from forming. Introduction of a flexible $–CH_2–$ linker in benzyl amide **61** while improving inhibitory activity still however does not lead to levels of potency which are equivalent to sulfonamide **4**.



Scheme 5: *Reagents and conditions:* (i) MeCOCl (excess), c. H_2SO_4 (cat.), 50 °C, 1 h; (ii) NaH (3.3 eq.), THF, RT, 30 min. then PhCOCl (1.3 eq.), RT, 1 h; (iii) requisite acyl chloride (4.0 eq.), $BF_3.OEt_2$ (1.0 eq.), toluene, 90 °C, 4 h; (iv) $CeCl_3.7H_2O$ (1.0 eq.), MeOH, RT, 90 min. then 3,5-dimethylaniline (3.0 eq.), 110 °C, 16 h; (v) 3,5-dimethylaniline (1.2 eq.), $Pd(OAc)_2$ (0.01 eq.), XPhos (0.03 eq.), K_2CO_3 (1.4 eq.), H_2O (0.04 eq.), ^{t}BuOH, 100 °C, 16 h; (vi) $(COCl)_2$ (1.2 eq.), DMF, CH_2Cl_2, RT, 3 h; (vii) naphthoquinone **51** (0.25 eq.), $BF_3.OEt_2$ (0.25 eq.), toluene, 90 °C, 4 h.

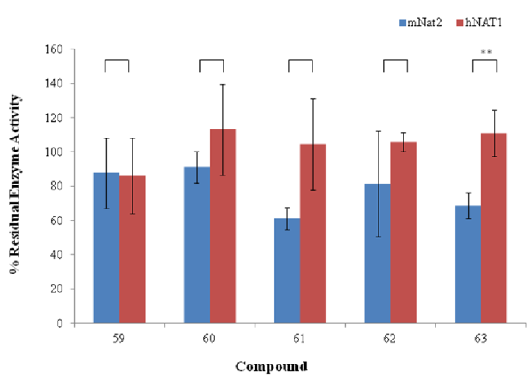
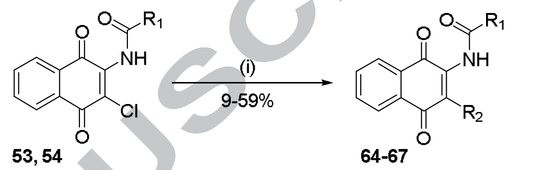


Figure 7: The % residual activity of mNat2 and hNAT1 when dosed with 30 µM of compounds **59-63**. Experiments were conducted in triplicate and results are shown as averages ± one standard deviation. The stars and brackets above the bars show the significance of the difference between the mNat2 and hNAT1 data when a Student's T-Test was performed: no stars = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

One alternative explanation for the lack of activity of these amides is that all of these species **59-63** possess pK_a values higher than 14, which is likely to preclude formation of their conjugate bases in the presence of hNAT1/mNat2 in an assay buffer of pH 8. We have previously established that the color change of sulfonamide inhibitors such as **4** in the hNAT1 active site is due to selective recognition of the conjugate base species by the enzyme.[21] If hNAT1 and mNat2 were to have higher affinities for the conjugate base species of any given naphthoquinone inhibitor than the respective neutral species, then one might expect naphthoquinones with very high pK_a values to be poor inhibitors.

Therefore, a small number of species with an amide substituent at C_2 but with an aryl substituent replacing the aniline substituent at C_3 were synthesized, to test the inhibitory potency and colorimetric properties of amides with pK_a values predicted to be within a more appropriate range. These species were synthesized *via* a Suzuki coupling reaction **(Scheme 6; Table 9)**.

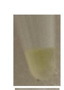


Scheme 6: *Reagents and conditions:* (i) Requisite boronic acid (2.0 eq.), $Pd(PPh_3)_2Cl_2$ (0.1 eq.), 4:1 THF:sat. aq. $NaHCO_3$, reflux, 18 h. For identities of R_1 and R_2, refer to **Table 9**.

Table 9: Yields in the synthesis of amides **64-67**.

Compound	R_1	R_2	Isolated Yield step (i)
64	Ph	3''-CHO-C_6H_4	15%
65	Ph	Furan-3''-yl	59%
66	Bn	3''-CHO-C_6H_4	44%
67	Bn	Furan-3''-yl	9%

Table 10: Pharmacological and spectrophotometric properties of amides **64-67**.

Compound	64	65	66	67
IC_{50} (mNat2) (µM)	30.4	14.5	36.8	23.5
IC_{50} (hNAT1) (µM)	10.0	19.0	22.1	19.3
pK_a	10.9	12.1	11.4	12.5
Compound at pH 8				
Compound at pH 13				
λ_{max} shift at pH 13?	Yes	Yes	Yes	Yes
λ_{max} shift with mNat2?	Yes	No	No	No
ε_{CB} ($M^{-1}cm^{-1}$)	2672	4817	8491	2449
ε_{CB} / ε_{CB}(4)	0.41	0.73	1.29	0.37

This small series of amide compounds were indeed found to have lower pK_a values than their C_3 anilino-substituted counterparts (**Table 10**). Furthermore, all examples displayed improved IC_{50} values against hNAT1 relative to the analogues with an anilino substituent at C_3. This supports a potential link between pK_a and potency which is currently under further investigation. The compound in this series with the lowest pK_a value, **64**, is shown to undergo a shift of λ_{max} in the presence of enzyme **(Figure 8)**. Compounds **65**, **66** and **67** do not show corresponding $\Delta\lambda_{max}$ shifts; this could be due to their higher pK_a values or because they have a different binding mode in the enzyme active site; *in silico* modeling does propose a feasible alternative mode of binding for the furanyl-substituted species **65** in which the furanyl oxygen is capable of binding to Arg127 in place of the amide carbonyl oxygen **(Figure 9)**, which would preclude a color change driven by recognition between the conjugate base and Arg127.

Whilst studies on these compounds give an interesting mechanistic insight into molecular interactions with the enzyme active site, colorimetric studies show that they suffer from lower ε_{CB} values than the corresponding sulfonamides **(Table 10)**. Furthermore, when tested against hNAT2, it was found that amide species **64** and **65** were far less selective for hNAT1 over hNAT2 than their sulfonamide analogues, with **64** and **65** showing > 50% inhibition of hNAT2 at 30 µM.

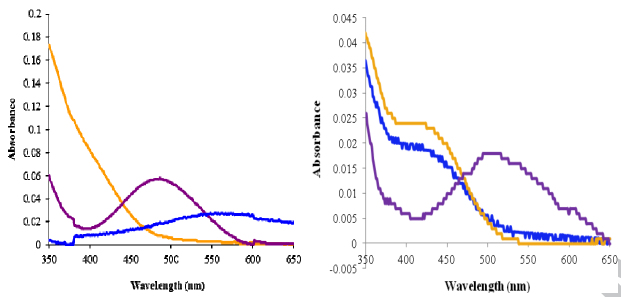


Figure 8: Visible spectra of compound **64** (left) and **65** (right), showing the compounds at a final concentration of 15 µM in 20 mM Tris.HCl buffer solution, pH 8 (yellow line), 4 M NaOH solution, pH 13.75 (purple line) and in 20 mM Tris.HCl buffer solution, pH 8, with 30 µM mNat2 (blue line).

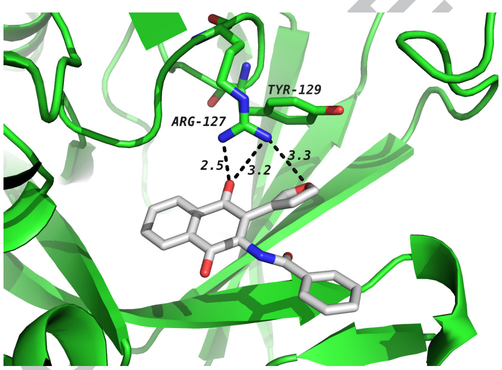


Figure 9: *In silico* modeling of **65** in the active site of hNAT1 (pdb: 2PQT)[12e], highlighting a potential alternative binding mode for this furanyl analogue. Distances between atoms highlighted by black dashed lines are given in Angstroms. Analysis performed using GOLD® software.[30] Docking was repeated 10 times giving consistent results.

2.5. SAR at C_5-C_8 of the Naphthoquinone Core

Since the sulfonamide inhibitors generally appear to have more favorable properties than their amide analogues, a series of nitro- and amino-substituted species at the C_5-C_8 positions was synthesized *via* a similar preparation to **4** but starting from 5- or 6-nitro-2,3-dichloronaphtho-1,4-quinone **68** or **69**, and utilizing 10% Pd on carbon as a catalyst for the reduction in the final step **(Schemes 7 and 8)**. It was also thought that the introduction of substituents at the 5-, 6-, 7- or 8-positions might allow exploitation of new inhibitor-enzyme interactions, which could lead to an increase in inhibitory potency against hNAT1. *In silico* modeling studies suggest that amino substituents at the 7- or 8-positions may be able to interact with the backbone carbonyls of residues Gly124 or Phe125 **(Figure 10)**; conversely it was hypothesized that nitro substituents at these positions might not be tolerated. Docking did not reveal any obvious additional polar contacts which might be gained by substitution at the 5- or 6-positions.

In addition, it was hypothesized that amino substitution could increase the absorption coefficient, ε, of the ligands relative to that of the unsubstituted species **4**, since the amino group is auxochromic and could donate electron density into the naphthoquinone core.

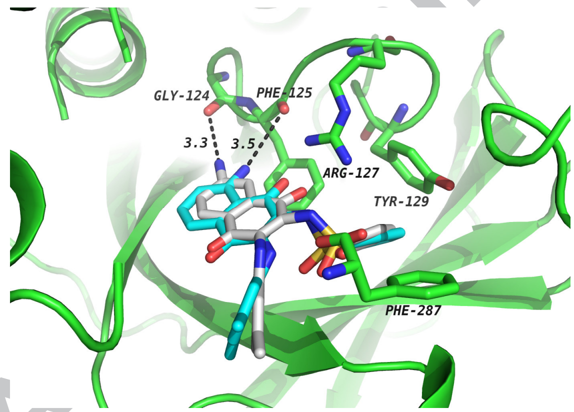
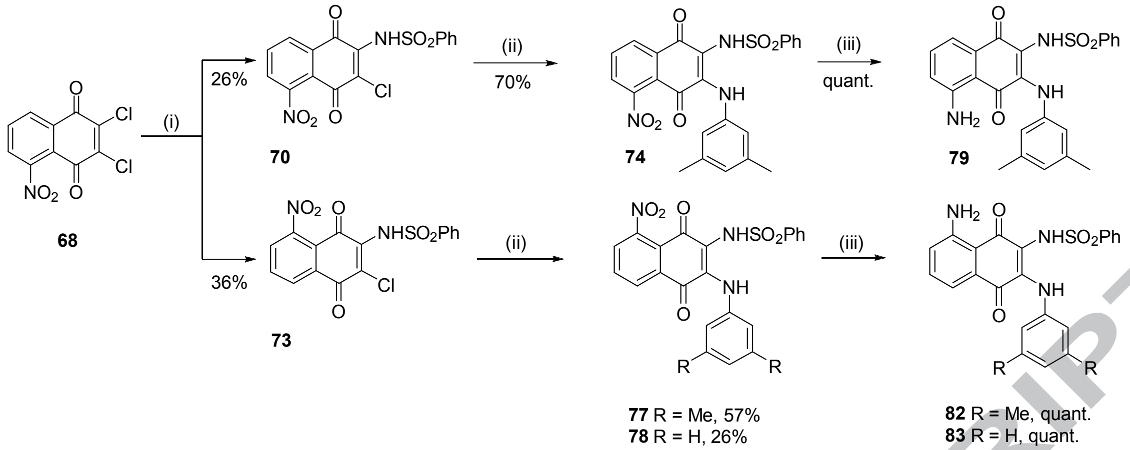


Figure 10: *In silico* modeling of **81** (carbons in grey) and **82** (carbons in cyan) in the active site of hNAT1 (pdb: 2PQT)[12e], highlighting potential interactions between the 7- or 8-amino substituent and the backbone carbonyl oxygen of Gly124 or Phe125. Distances between atoms highlighted by red dashed lines are given in Angstroms. Analysis performed using GOLD® software.[30]

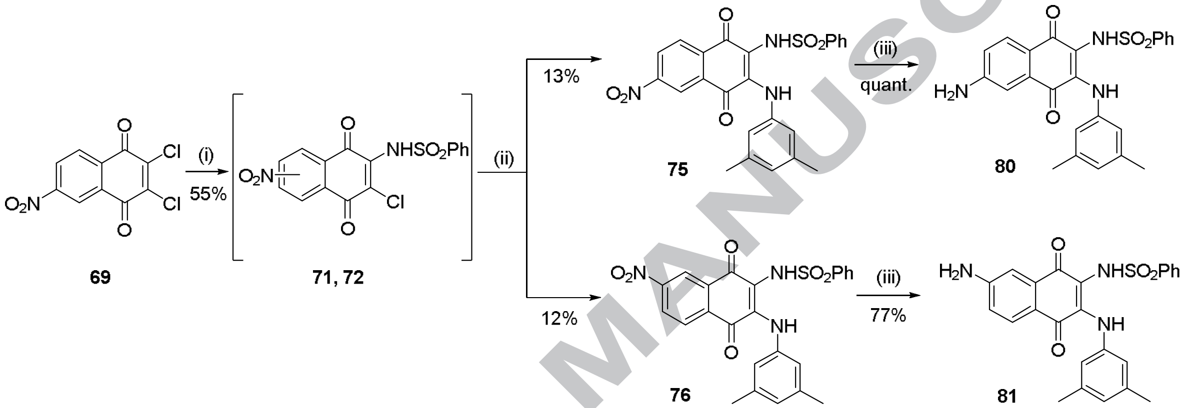
When substituents were introduced directly onto the naphthoquinone core at positions 5-8, some clear SAR patterns emerged. A 5-nitro substituent **(74)** displayed similar IC_{50} values to those of compound **4** against hNAT1 and mNat2, whilst those with 6-nitro, 7-nitro or 8-nitro substituents **(75-77)** were significantly less active (**Table 11**; for squared correlation coefficients for IC_{50} data see **Table S2** in Supporting Information). For the amino-substituted series, 5-amino derivative **79** and 7-amino derivative **81** had potencies comparable to that of **4**, whilst the 6-amino derivative **80** showed low potency and the 8-amino derivative **82** possessed IC_{50} values one order of magnitude more potent than **4**. These experimental results correlate well with the predictions from *in silico* modeling.

Given the high potency of compound **82** against both hNAT1 and mNat2, with IC_{50} values of 540 nM and 430 nM respectively against each enzyme, the analogous species **83** was also synthesized. With IC_{50} values against hNAT1 and mNat2 of 120 and 270 nM respectively, **83** represents the most potent inhibitor of hNAT1 and mNat2 synthesized in this study. Its nitro-substituted precursor **78** had low activity (> 30 µM) against both enzymes.

Crucially, both 8-amino substituted species **82** and **83** possess significantly improved absorption coefficients compared to compound **4**, whilst still leading to a discernible color change in the presence of mNat2; compound **82** was also found to be highly selective for hNAT1 and mNat2 over hNAT2 **(Table 11; Figure 11)**. Compounds **82** and **83** were therefore subjected to further studies in lysates from ZR-75-1 cells.

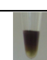
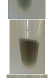
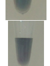


Scheme 7: *Reagents and conditions:* (i) $PhSO_2NH_2$ (1.0 eq.), Cs_2CO_3 (1.4 eq.), DMF, RT, 5 h; (ii) $CeCl_3.7H_2O$ (1.0 eq.), MeOH, RT, 90 min. then requisite aniline (3.0 eq.), 110 °C, 16 h; (iii) Pd/C 10% (0.1 eq.), H_2, MeOH, RT, 16 h.



Scheme 8: *Reagents and conditions:* (i) $PhSO_2NH_2$ (1.0 eq.), Cs_2CO_3 (1.4 eq.), DMF, RT, 5 h; (ii) $CeCl_3.7H_2O$ (1.0 eq.), MeOH, RT, 90 min. then requisite aniline (3.0 eq.), 110 °C, 16 h; (iii) Pd/C 10% (0.1 eq.), H_2, MeOH, RT, 16 h.

Table 11: Pharmacological and spectrophotometric properties of nitro- and amino-substituted naphthoquinones **74-83**. N.D. = not determined. *Not possible to determine pK_a *via* this method.

Compound	IC_{50} (mNat2) (µM)	IC_{50} (hNAT1) (µM)	Compound at pH 8	Compound at pH 13	$\Delta\lambda_{max}$ pH 13 (nm)	$\Delta\lambda_{max}$ mNat2 (nm)	pK_a	ε_{CB}	ε_{CB}/ ε_{CB}(4)
74	2.7	6.6			+ 23	+ 95	8.2	4517	0.69
75	> 30	> 30			+ 73	+ 74	7.5	2241	0.34
76	> 30	N.D.			+ 87	N.D.	8.0	3879	0.59
77	> 30	> 30			+ 58	N.D.	8.6	2737	0.42
78	> 30	> 30			+ 74	+ 107	8.4	3012	0.46
79	5.7	4.2			+ 16	+ 16	*	15796	2.40
80	> 30	N.D.			- 9	N.D.	*	12542	1.90
81	2.5	2.6			+10	0	*	21892	3.32
82	0.43	0.54			- 63	- 62	8.4	12384	1.88
83	0.27	0.12			- 28	- 12	8.2	15842	2.40

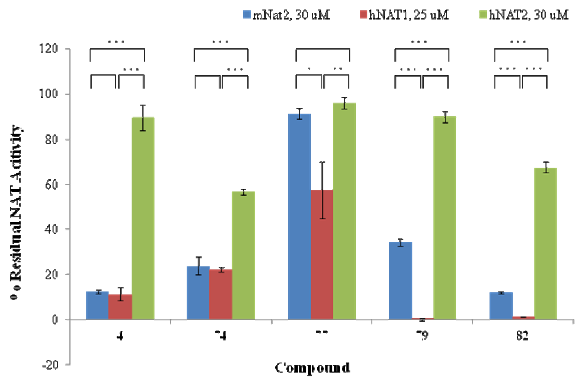


Figure 11: The % residual activity of mNat2, hNAT1 and hNAT2 when dosed with compounds **74**, **77**, **79** and **82** at 30 µM. Hit compound **4** is also included as a control. Experiments were conducted in triplicate and results are shown as averages ± one standard deviation. The stars and brackets above the bars show the significance of the difference between pairs of data when a Student's T-Test was performed: no stars = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

2.6. Evaluation in Cell Extracts from the Breast Cancer Cell Line ZR-75-1

Representative compounds from this study possessing a range of functionality were tested for inhibitory activity of hNAT1 in ZR-75-1 lysate at concentrations of 30 µM, 10 µM and 1 µM **(Figure 12)**. All the representative sulfonamides which showed high levels of potency against recombinant hNAT1 also showed high levels of inhibition against hNAT1 in the lysate, with compounds **4**, **48**, **81** and **82** exhibiting > 50% inhibition of hNAT1 at an inhibitor concentration of 1 µM.

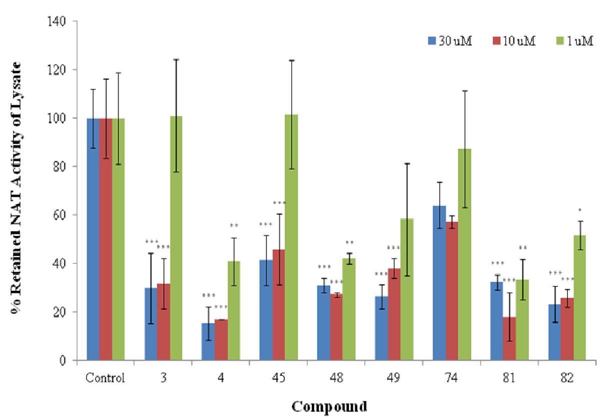


Figure 12: The % residual activity of hNAT1 in ZR-75-1 cell lysate when dosed with representative compounds **3**, **4**, **45, 48, 49, 77, 81** and **82** at 30 µM, 10 µM and 1 µM, relative to a control experiment (vehicle only). Experiments were conducted in triplicate and results are shown as averages ± one standard deviation. The stars above the bars show the significance of the difference between the test data and its respective control when a Student's T-Test was performed: no stars = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

8-amino substituted species **82** and **83** possessing a tenfold and fiftyfold improved potency against hNAT1 over **4** respectively, and with twofold improved ε_{CB}, were subsequently examined in spectrophotometric experiments with the ZR-75-1 cell extracts in an attempt to detect the hNAT1 present in the cells. The probes were tested at final concentrations ranging from 0.6 µM to 2.5 µM, but again due to poor signal-to-noise ratios no λ_{max} peak could be detected at these low concentrations. A negative control concentration of 20 µM was also used, clearly showing λ_{max} corresponding to the neutral (protonated) species in each case. This demonstrates that further improvements to the sensitivity of the probes are required for their clinical use; however, this study has provided key underpinning insights to demonstrate that there is a scope for variation around the naphthoquinone core of the original hit compound **5** in order to achieve this.

3. Conclusions and Future Work

In this study, we have designed and synthesized a family of naphthoquinones which are capable of rapidly and unambiguously detecting pure recombinant hNAT1 as they change color in the presence of the protein through a mechanism which we have previously elucidated. As no similar colorimetric probes have been reported to date in the literature, it proved necessary to determine the key requirements for such probes to be clinically useful. This study verifies that the potency, pK_a and absorption coefficient of the conjugate base (ε_{CB}) are crucial parameters of probe design. We have developed extensive SAR around the naphthoquinone core of our hit compound **4**, and have built up an *in silico* docking model which is consistent with our experimental observations; this should prove valuable for any future attempts to develop hNAT1 inhibitors and is summarized in **Figure 13**. Compounds **82** and **83** show ten- and fifty-fold increases respectively in potency and a two-fold increase in ε_{CB} over initial hit compound **4**; **82** is also shown to retain selectivity for hNAT1 over its isoenzyme hNAT2. Although the conjugate bases of **82** and **83** could not be clearly detected in absorption spectra with ZR-75-1 extracts, they offer promising insights which aid ongoing work focused on increasing the ε_{CB} of these probes whilst retaining high potency and selectivity for hNAT1. The high potency of representative examples of the sulfonamide probes against hNAT1 in ZR-75-1 cell extracts also pave the way for the development of inhibitors with improved physical properties such as solubility and cell permeability which could potentially act as tools for elucidating the currently unknown role which hNAT1 plays in ER+ breast cancer progression; these studies are also ongoing. Such selective inhibitors of hNAT1 with appropriate proerties for application *in vivo* might also possess a valuable therapeutic use.

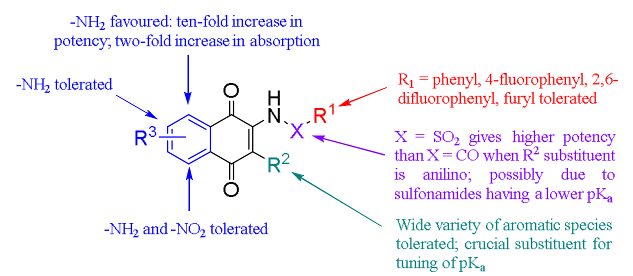


Figure 13: Summary of SAR elucidated around the naphthoquinone core of hit compound **4** in this study.

4. Experimental

4.1. Chemistry

4.1.1. General Experimental

Chemicals were purchased from Sigma-Aldrich UK, TCI UK, Apollo Scientific UK, Alfa Aesar UK, Fluorochem UK or Fisher Scientific UK and used without further purification. Where appropriate, all reactions involving moisture-sensitive reagents were carried out under a nitrogen or argon atmosphere using standard vacuum line techniques and glassware that was flame-dried before use. Anhydrous DMF, anhydrous MeOH and anhydrous dioxane were purchased from Sigma-Aldrich UK in

SureSeal™ bottles and used without further purification; other anhydrous solvents were dried following the procedure outlined by Grubbs and co-workers.[38] Water was purified by an Elix® UV-10 system. Organic layers were dried over anhydrous $MgSO_4$. Brine refers to a saturated aqueous solution of sodium chloride. *In vacuo* refers to the use of a rotary evaporator attached to a diaphragm pump. Pet ether refers to the fraction of petroleum spirit boiling between 30 and 40 °C. Thin layer chromatography was performed on Merck silica gel 60 F_{254} aluminium-supported thin layer chromatography sheets. Plates were visualised using UV light (254 nm), or thermal development after dipping in 1% aq. $KMnO_4$. Flash column chromatography was performed on Kieselgel 60 silica in a glass column, or on a Biotage SP4 flash column chromatography platform.

Melting points were recorded on a Gallenkamp Hot Stage apparatus and are uncorrected. Where relevant, the recrystallisation solvent is reported in parentheses. Infrared spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer, neat or as KBr discs. Selected characteristic peaks are reported in wavenumbers (cm^{-1}). NMR spectra were recorded on Bruker Avance spectrometers (DPX400, DQX400, AVII 500 or DRX500) in the deuterated solvent stated. The field was locked by external referencing to the relevant deuteron resonance. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) are quoted in Hz; both are reported to one decimal place. The coupling constants were determined by analysis using ACD Labs software. Low-resolution mass spectra were recorded on either a VG MassLab 20-250 or a Micromass Platform 1 spectrometer, operating in positive or negative mode, from solutions of MeOH. Accurate mass measurements were run on either a Bruker MicroTOF internally calibrated with polyalanine, or a Micromass GCT instrument fitted with a Scientific Glass Instruments BPX5 column (15 m × 0.25 mm) using amyl acetate as a lock mass, by the mass spectrometry department of the Chemistry Research Laboratory, University of Oxford, UK. *m/z* Values are reported in Daltons and followed by their percentage abundance in parentheses.

Reverse-phase high-performance liquid chromatography (RP-HPLC) was performed by one of two methods, as stated. Method A: RP-HPLC was performed using a 1525 pump, 2707 autosampler and 2849 detector, all from Waters. Separations were performed on a Phenomonex Luma C18 (analytical) column (5 µm particle size, 250.0 mm x 4.6 mm). Experiments were performed under gradient elution (eluent H_2O containing 0.1% (v/v) TFA:MeCN 95:5 to 5:95 over 20 min. then isocratic for 15 min.). Sample injections consisted of 40 µL of 2 mg/mL sample solution in MeCN. The flow rate was 1 mL/min. and detection was at a wavelength of 215 nm. Method B: RP-HPLC was performed on a Gilson instrument equipped with Gilson 306 pumps, a Gilson 811C dynamic mixer, a Gilson 806 manometric module with automated sample injection on a Gilson 215 Liquid Handler, configured with a Gilson 819 valve actuator. Separations were performed on a Varian Omnisphere 5 C18 (analytical) column (5 µm particle size, 150.0 mm x 4.6 mm). Experiments were performed under gradient elution (eluent H_2O containing 0.1% (v/v) TFA:MeCN 95:5 to 5:95 over 8 min. then isocratic for 4 min.). Sample injections consisted of 20 µL of 1 mg/mL sample solution in DMSO. The flow rate was 1 mL/min. Detection was at wavelengths of 220 and 254 nm using a Gilson 170 Diode Array Detector. For each compound, retention times (t_R) are quoted to the nearest minute and are followed by the % purity.

Compounds are characterized throughout this experimental section by numbering aromatic carbons and any attached hydrogen nuclei as shown in **Figure 14**.

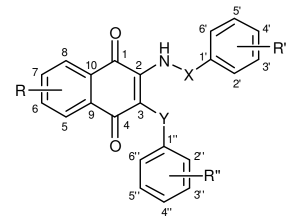


Figure 14: Numbering system employed throughout based on a generic representative naphthoquinone species.

4.1.2. Representative Procedures

4.1.2.1. Representative Procedure 1: Substitution of 2,3-dichloronaphthalene-1,4-diones with sulfonamides. 2,3-Dichloronaphthalene-1,4-dione (1.0 eq.), the requisite sulfonamide (1.0 eq.) and Cs_2CO_3 (1.4 eq.) were stirred in DMF in a sealed microwave vial at RT for 5 h. 1 M aq. HCl (50 mL) was added and the organic product extracted with EtOAc (3 x 20 mL). The organic washings were combined, washed with brine (3 x 20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the crude product.

4.1.2.2. Representative Procedure 2: Substitution of *N*-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)sulfonamides with anilines. The requisite sulfonamide (1.0 eq.) was stirred with cerium trichloride heptahydrate (1.0 eq.) in MeOH at RT in a microwave vial for 1.5 h. The requisite aniline (3.0 eq.) was added, the vial sealed and the reaction mixture heated to 90 °C for 16 h, unless otherwise stated. The solution was cooled to RT, sat. aq. NH_4Cl (30 mL) was added and the organic product was extracted with EtOAc (3 x 20 mL). The organic washings were combined, washed with brine (3 x 20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the crude product.

4.1.2.3. Representative Procedure 3: Substitution of *N*-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)sulfonamide with aliphatic amines. The requisite sulfonamide (1.0 eq.) was stirred with cerium trichloride heptahydrate (0.4 or 1.0 eq., as stated) in toluene at RT in a microwave vial for 1.5 h. The requisite aniline (3.0 eq.) was added, the vial sealed and the reaction mixture heated to 110 °C for 6 h. The solution was cooled to RT, sat. aq. NH_4Cl (30 mL) was added and the organic product was extracted with EtOAc (3 x 20 mL). The organic washings were combined, washed with brine (3 x 20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the crude product.

4.1.2.4. Representative Procedure 4: Disubstitution of 2,3-dichloronaphthalene-1,4-dione with phenols. 2,3-Dichloronaphthalene-1,4-dione (1.0 eq.), the requisite phenol (2.2 eq.) and Cs_2CO_3 (2.2 eq.) were refluxed in THF for 16 h. The solution was cooled to RT and partitioned between EtOAc (50 mL) and 0.1 M aq. NaOH (30 mL). The organic layer was washed with sat. aq. NH_4Cl (2 x 20 mL) and brine (3 x 20 mL), before being dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the crude product.

4.1.2.5. Representative Procedure 5: Substitution of 2,3-diaryloxynaphthalene-1,4-diones with benzenesulfonamide. The requisite diphenol (1.0 eq.), benzenesulfonamide (1.0 eq.) and Cs_2CO_3 (1.2 eq.) were refluxed in THF for 1 h, unless otherwise stated. The solution was cooled to RT and partitioned between EtOAc (50 mL) and 1 M aq. HCl (30 mL). The organic layer was washed with brine (3 x 20 mL), before being dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the crude product.

4.1.2.6. Representative Procedure 6: Suzuki coupling of *N*-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-arylsulfonamide with arylboronic acids. The requisite sulfonamide (1.0 eq.), the requisite boronic acid (2.0 eq.),

$Pd(PPh_3)_2Cl_2$ (0.1 eq.) and sat. aq. $NaHCO_3$ were stirred in THF in a sealed microwave vial under N_2 and heated to 100 °C for 16 h. The solution was cooled to RT and partitioned between EtOAc (50 mL) and sat. aq. NH_4Cl (50 mL). The organic layer was collected, washed with brine (3 x 20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the crude product.

4.1.2.7. Representative Procedure 7: Reduction of nitronaphthalene-1,4-diones to aminonapthahlene-1,4-diones. The aromatic nitro species (1.0 eq.) and 10% Pd/C catalyst (0.2 eq.) were stirred in MeOH in a sealed microwave vial under H_2 (1 atm.) at RT for 16 h. The mixture was filtered through Celite® to remove the Pd/C catalyst, and the filtrate concentrated *in vacuo* to give the crude product.

4.1.3. *N*-(3-Phenylamino-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (3)[23]

Following *Representative Procedure 2*, using naphthoquinone **8** (100 mg, 0.29 mmol), aniline (79 µL, 0.84 mmol) and $CeCl_3.7H_2O$ (108 mg, 0.29 mmol) in MeOH (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 85:15) gave 3-anilinonaphthoquinone **3** as a red solid (80 mg, 63%). mp 215-221 °C; HPLC (method A) t_R 18 min., >99%; δ_H (400 MHz, DMSO-d_6) 6.99-7.09 (3H, m, $H_{2''}$, $H_{4''}$ and $H_{6''}$), 7.23 (2H, app. t, *J* 7.9, $H_{3''}$ and $H_{5''}$), 7.33-7.41 (2H, m, $H_{3'}$ and $H_{5'}$), 7.45-7.52 (1H, m, $H_{4'}$), 7.52-7.58 (2H, m, $H_{2'}$ and $H_{6'}$), 7.73-7.84 (3H, m, H_6, H_7 and H_5 or H_8), 7.99-8.04 (1H, m, H_5 or H_8), 9.02 (2H, s, aniline-N*H* and sulfonamide-N*H*); *m/z* (ESI⁻) 403 ([M-H]⁻, 100%); λ_{max} (pH 8) 489 nm (ε_N 8700 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 561 nm (ε_{CB} 7900 $M^{-1}cm^{-1}$); pK_a 9.5.

4.1.4. *N*-(3-(3'',5''-Dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (4)[21, 23]

Following *Representative Procedure 2*, using naphthoquinone **8** (1.30 g, 3.75 mmol), 3,5-dimethylaniline (1.40 mL, 11.25 mmol) and $CeCl_3.7H_2O$ (1.39 g, 3.75 mmol) in MeOH (10 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 50:50) and subsequent recrystallisation from toluene gave 3-anilinonaphthoquinone **4** as a red solid (919 mg, 57%). mp 188-192 °C (toluene); HPLC (method A) t_R 17 min., 97%; δ_H (400 MHz, DMSO-d_6) 2.22 (6H, s, 2 x Ar-*Me*), 6.59 (2H, s, $H_{2''}$ and $H_{6''}$), 6.67 (1H, s, $H_{4''}$), 7.34-7.40 (2H, m, $H_{3'}$ and $H_{5'}$), 7.47-7.51 (1H, m, $H_{4'}$), 7.51-7.56 (2H, m, $H_{2'}$ and $H_{6'}$), 7.74-7.83 (3H, m, H_6, H_7 and H_5 or H_8), 8.00-8.04 (1H, m, H_5 or H_8), 8.79 (1H, s, N*H*), 9.06 (1H, s, N*H*); *m/z* (ESI⁻) 431 ([M-H]⁻, 100%); λ_{max} (pH 8) 498 nm (ε_N 11960 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 577 nm (ε_{CB} 6590 $M^{-1}cm^{-1}$); pK_a 9.2.

4.1.5. *N*-(3-(4''-Bromophenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (6)[23]

Following *Representative Procedure 2,* using naphthoquinone **8** (300 mg, 0.86 mmol), 4-bromoaniline (446 mg, 2.59 mmol) and $CeCl_3.7H_2O$ (322 mg, 0.86 mmol) in MeOH (7 mL). Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 90:10 to 50:50) gave 3-anilinonaphthoquinone **6** as a red solid (133 mg, 30%). mp 246-247 °C; HPLC (method A) t_R 17 min., 96%; δ_H (500 MHz, DMSO-d_6) 6.98 (2H, d, *J* 8.4, $H_{2''}$ and $H_{6''}$), 7.36-7.41 (4H, m, $H_{3'}$, $H_{5'}$, $H_{3''}$ and $H_{5''}$), 7.51 (1H, t, *J* 7.0, $H_{4'}$), 7.57 (2H, d, *J* 7.0, $H_{2'}$ and $H_{6'}$), 7.75-7.83 (3H, m, H_6, H_7 and H_8), 8.02 (1H, d, *J* 8.4, H_5), 9.06 (1H, s, sulfonamide-N*H*), 9.13 (1H, s, aniline-N*H*); *m/z* (ESI⁻) 481 ([M(^{79}Br)-H]⁻, 83%), 483 ([M(^{81}Br)-H]⁻, 100%); λ_{max} (pH 8) 500 nm (ε_N 13330 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 570 nm (ε_{CB} 7790 $M^{-1}cm^{-1}$); pK_a 10.4.

4.1.6. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (8)[23]

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (1.00 g, 4.40 mmol), benzenesulfonamide (0.69 g, 4.40 mmol) and Cs_2CO_3 (2.00 g, 6.16 mmol) in DMF (10 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) gave sulfonamide **8** as a yellow solid (1.39 g, 91%). mp 219-223 °C; δ_H (400 MHz, DMSO-d_6) 7.56-7.64 (2H, m, $H_{3'}$ and $H_{5'}$), 7.66 (1H, app. t, *J* 7.1, $H_{4'}$), 7.81-7.90 (2H, m, H_6 and H_7), 7.91-7.98 ($H_{2'}$, $H_{6'}$ and H_5 or H_8), 8.01-8.06 (1H, m, H_5 or H_8); *m/z* (ESI⁻) 346 ([M(^{35}Cl)-H]⁻, 100%).

4.1.7. *N*-(3-((2''-Methoxyethyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (9)

Following *Representative Procedure 3*, using naphthoquinone **8** (200 mg, 0.58 mmol), 3-methoxyethylamine (150 µL, 1.74 mmol) and $CeCl_3.7H_2O$ (86 mg, 0.23 mmol) in toluene (10 mL). Purification *via* column chromatography (eluent pet ether:acetone 70:30) gave 3-aminonaphthoquinone **9** as an orange solid (195 mg, 87%). mp 167-169 °C; HPLC (method B) t_R 7 min., 97%; υ_{max} (KBr) 3312 (N-H), 3235 (N-H), 1676 (C=O), 1610 (C=O); δ_H (400 MHz, $CDCl_3$) 3.43 (3H, s, O*Me*), 3.67 (2H, t, *J* 5.2, 2 x $H_{2''}$), 4.16 (2H, q, *J* 5.2, 2 x $H_{1''}$), 6.64 (1H, br. s, sulfonamide-N*H*), 6.72 (1H, br. t, *J* 5.2, amine-N*H*), 7.32-7.39 (2H, m, $H_{3'}$ and $H_{5'}$), 7.45-7.52 (1H, m, $H_{4'}$), 7.55-7.64 (2H, m, H_6 and H_7), 7.66-7.71 (1H, m, H_5 or H_8), 7.79 (2H, d, *J* 7.3, $H_{2'}$ and $H_{6'}$), 8.00-8.06 (1H, m, H_5 or H_8); δ_C (100 MHz, $CDCl_3$) 44.2, 58.8, 70.6, 109.3, 125.9, 126.8, 127.8, 128.6, 130.1, 131.8, 132.4, 133.1, 134.9, 138.2, 143.9, 178.6, 181.7; *m/z* (ESI⁻) 385 ([M-H]⁻, 100%); HRMS (ESI⁺) $C_{19}H_{18}N_2NaO_5S^+$ ([M+Na]⁺) requires 409.0829, found 409.0829; λ_{max} (pH 8) 469 nm (ε_N 1330 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 558 nm (ε_{CB} 1010 $M^{-1}cm^{-1}$); pK_a 9.8.

4.1.8. *N*-(3-(Cyclopentylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (10)

Following *Representative Procedure 3*, using naphthoquinone **8** (200 mg, 0.58 mmol), cyclopentylamine (172 µL, 1.74 mmol) and $CeCl_3.7H_2O$ (86 mg, 0.23 mmol) in toluene (10 mL). Purification *via* column chromatography (eluent pet ether:acetone 70:30) gave 3-aminonaphthoquinone **10** as a red solid (204 mg, 89%). mp 199-201 °C; HPLC (method B) t_R 9 min., 99%; υ_{max} (KBr) 3318 (N-H), 3243 (N-H), 1673 (C=O), 1612 (C=O); δ_H (400 MHz, $CDCl_3$) 1.49-1.61 (2H, m, 1 x $H_{2''}$ and 1 x $H_{5''}$), 1.66-1.82 (4H, m, 2 x $H_{3''}$ and 2 x $H_{4''}$), 2.08-2.21 (2H, m, 1 x $H_{2''}$ and 1 x $H_{5''}$), 5.02 (1H, app. sext, *J* 7.1, $H_{1''}$), 6.41 (1H, d, *J* 7.1, amine-N*H*), 6.68 (1H, s, sulfonamide-N*H*), 7.32-7.39 (2H, m, $H_{3'}$ and $H_{5'}$), 7.45-7.52 (1H, m, $H_{4'}$), 7.55-7.64 (2H, m, H_6 and H_7), 7.66-7.72 (1H, m, H_5 or H_8), 7.80 (2H, d, *J* 7.8, $H_{2'}$ and $H_{6'}$), 7.99-8.06 (1H, m, H_5 or H_8); δ_C (100 MHz, $CDCl_3$) 24.1, 34.5, 55.0, 109.1, 125.9, 126.7, 127.8, 128.6, 130.1, 131.9, 132.3, 133.1, 134.9, 138.3, 143.5, 178.5, 182.0; *m/z* (ESI⁻) 395 ([M-H]⁻, 100%); HRMS (ESI⁺) $C_{21}H_{20}N_2NaO_4S^+$ ([M+Na]⁺) requires 419.1036, found 419.1036; λ_{max} (pH 8) 471 nm (ε_N 10080 $M^{-1}cm^{-1}$) λ_{max} (pH 13.75) 553 nm (ε_{CB} 4470 $M^{-1}cm^{-1}$); pK_a 10.1.

4.1.9. *N*-(3-(Benzylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (11)

Following *Representative Procedure 3*, using naphthoquinone **8** (100 mg, 0.29 mmol), benzylamine (100 µL, 0.86 mmol) and $CeCl_3.7H_2O$ (107 mg, 0.29 mmol) in toluene (4 mL). Purification *via* column chromatography (eluent pet ether:acetone 85:15) and subsequent recrystallisation from toluene gave 3-aminonaphthoquinone **11** as an orange solid

(50 mg, 42%). mp 206-211 °C (toluene); HPLC (method A) t_R 16 min., 99%; υ_{max} (neat) 3342 (N-H), 1675 (C=O), 1612 (C=O); δ_H (500 MHz, $CDCl_3$) 5.12 (2H, d, *J* 5.8, benzyl-C*H*$_2$), 6.56 (1H, s, sulfonamide-N*H*), 6.59 (1H, br. s, amine-N*H*), 7.30-7.35 (1H, m, $H_{4''}$), 7.35-7.40 (6H, m, $H_{3'}$, $H_{5'}$, $H_{2''}$, $H_{3''}$, $H_{5''}$ and $H_{6''}$), 7.50 (1H, t, *J* 7.7, $H_{4'}$), 7.57-7.64 (2H, m, H_6 and H_7), 7.67-7.71 (1H, m, H_8), 7.82 (2H, d, *J* 7.6, $H_{2'}$ and $H_{6'}$), 8.02-8.05 (1H, m, H_5); δ_C (125 MHz, $CDCl_3$) 48.9, 109.8, 126.0, 126.8, 127.8, 127.9, 128.1, 128.7, 128.9, 130.0, 131.3, 132.5, 133.1, 134.9, 137.8, 138.3, 143.6, 178.8, 181.8; *m/z* (ESI$^-$) 417 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{23}H_{18}N_2NaO_4S^+$ ([M+Na]$^+$) requires 441.0879, found 441.0865; λ_{max} (pH 8) 486 nm (ε_N 10480 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 560 nm (ε_{CB} 5770 $M^{-1}cm^{-1}$); pK_a 10.4.

4.1.10. *N*-(3-Phenoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (17)

Following *Representative Procedure 4,* using 2,3-dichloronaphthalene-1,4-dione **7** (454 mg, 2.00 mmol), phenol (414 mg, 4.40 mmol) and Cs_2CO_3 (1434 mg, 4.40 mmol) in THF (30 mL) gave intermediate diphenoxy **14** as an orange solid (430 mg, 63%). mp 188-193 °C; υ_{max} (neat) 1662 (C=O), 1591 (C=O); δ_H (400 MHz, $CDCl_3$) 6.88-6.94 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$ and $H_{6''}$), 7.02-7.09 (2H, m, $H_{4'}$ and $H_{4''}$), 7.21-7.28 (4H, m, $H_{3'}$, $H_{5'}$, $H_{3''}$ and $H_{5''}$), 7.75-7.81 (2H, m, H_6 and H_7), 8.10-8.16 (2H, m, H_5 and H_8); δ_C (100 MHz, $CDCl_3$) 116.6, 123.6, 126.8, 129.4, 130.8, 134.3, 146.1, 156.5, 180.4; *m/z* (ESI$^+$) 343 ([M+H]$^+$, 80%), 365 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{22}H_{14}NaO_4^+$ ([M+Na]$^+$) requires 365.0784, found 365.0768. Then, following *Representative Procedure 5*, using naphthoquinone **14** without further purification (400 mg, 1.17 mmol), benzenesulfonamide (184 mg, 1.17 mmol) and Cs_2CO_3 (458 mg, 1.40 mmol) in THF (30 mL) for 16 h and subsequent recrystallisation from toluene gave 2-sulfonamidonaphthoquinone **17** as a yellow solid (175 mg, 67%). mp 216-219 °C (toluene); HPLC (method A) t_R 18 min., 95%; υ_{max} (neat) 3235 (N-H), 1668 (C=O), 1655 (C=O); δ_H (500 MHz, DMSO-d_6) 6.82-6.86 (2H, m, $H_{2''}$ and $H_{6''}$), 6.99-7.04 (1H, m, $H_{4''}$), 7.18-7.24 (2H, m, $H_{3''}$ and $H_{5''}$), 7.46-7.51 (2H, m, $H_{3'}$ and $H_{5'}$), 7.53-7.57 (1H, m, $H_{4'}$), 7.81-7.92 (5H, m, H_5, H_6, H_7, $H_{2'}$ and $H_{6'}$), 8.03-8.06 (1H, m, H_8), 10.30 (1H, br. s, N*H*); δ_C (125 MHz, DMSO-d_6) 116.4, 122.7, 126.1, 126.1, 126.3, 128.7, 129.1, 130.6, 130.6, 132.3, 132.4, 134.4, 134.5, 141.7, 145.1, 156.4, 179.1, 180.6; *m/z* (ESI$^-$) 405 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{22}H_{15}NNaO_5S^+$ ([M+Na]$^+$) requires 428.0563, found 428.0565; λ_{max} (pH 8) < 400 nm, λ_{max} (pH 13.75) 473 nm (ε_{CB} 7080 $M^{-1}cm^{-1}$); pK_a 5.0.

4.1.11. *N*-(3-(3'',5''-Dimethylphenoxy)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (18)

Following *Representative Procedure 4,* using 2,3-dichloronaphthalene-1,4-dione **7** (454 mg, 2.00 mmol), 3,5-dimethylphenol (537 mg, 4.40 mmol) and Cs_2CO_3 (1434 mg, 4.40 mmol) in THF (30 mL) gave intermediate diaryloxy **15** as an orange solid (709 mg, 89%). mp 175-177 °C; υ_{max} (neat) 1665 (C=O), 1590 (C=O); δ_H (500 MHz, $CDCl_3$) 2.18 (12H, s, 4 x Ar-*Me*), 6.65 (2H, s, $H_{4'}$ and $H_{4''}$), 6.73 (4H, s, $H_{2'}$, $H_{6'}$, $H_{2''}$ and $H_{6''}$), 7.88-7.93 (2H, m, H_6 and H_7), 8.01-8.06 (2H, m, H_5 and H_8); δ_C (125 MHz, $CDCl_3$) 20.7, 113.6, 124.3, 126.0, 131.0, 134.3, 138.8, 146.2, 156.7, 180.2; *m/z* (ESI$^+$) 399 ([M+H]$^+$, 100%), 421 ([M+Na]$^+$, 20%); HRMS (ESI$^+$) $C_{26}H_{22}NaO_4^+$ ([M+Na]$^+$) requires 421.1410, found 421.1391. Then, following *Representative Procedure 5*, using naphthoquinone **15** without further purification (400 mg, 1.01 mmol), benzenesulfonamide (158 mg, 1.01 mmol) and Cs_2CO_3 (393 mg, 1.21 mmol) in THF (30 mL) and subsequent recrystallisation from toluene gave 2-sulfonamidonaphthoquinone **18** as an orange-brown solid (248 mg, 57%). mp 218-223 °C (toluene); HPLC (method A) t_R 18 min., 96%; υ_{max} (neat) 3242 (N-H), 1662 (C=O), 1641 (C=O); δ_H (500 MHz, $CDCl_3$) 2.22 (6H, s, 2 x Ar-*Me*), 6.28 (2H, s, $H_{2''}$ and $H_{6''}$), 6.66 (1H, s, $H_{4''}$), 7.32-7.37 (2H, m, $H_{3'}$ and $H_{5'}$), 7.43-7.49 (1H, m, $H_{4'}$), 7.72-7.77 (3H, m, H_6, H_7 and N*H*), 7.80-7.85 (2H, m, $H_{2'}$ and $H_{6'}$), 7.94-7.99 (1H, m, H_5), 8.12-8.18 (1H, m, H_8); δ_C (125 MHz, $CDCl_3$) 21.2, 114.5, 125.3, 126.8, 126.9, 127.1, 128.5, 129.8, 131.1, 131.1, 132.7, 133.9, 134.9, 138.8, 140.1, 141.2, 156.0, 178.8, 180.5; *m/z* (ESI$^+$) 434 ([M+H]$^+$, 100%), 456 ([M+Na]$^+$, 60%); HRMS (ESI$^+$) $C_{24}H_{19}NNaO_5S^+$ ([M+Na]$^+$) requires 456.0876, found 456.0857; λ_{max} (pH 8) < 400 nm, λ_{max} (pH 13.75) 474 nm (ε_{CB} 7200 $M^{-1}cm^{-1}$); pK_a 5.0.

4.1.12. *N*-(3-(4''-Bromophenoxy)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (19)

Following *Representative Procedure 4,* using 2,3-dichloronaphthalene-1,4-dione **7** (454 mg, 2.00 mmol), 4-bromophenol (761 mg, 4.40 mmol) and Cs_2CO_3 (1434 mg, 4.40 mmol) in THF (30 mL) gave intermediate diaryloxy **16** as a yellow solid (715 mg, 72%). mp 182-185 °C; υ_{max} (neat) 1677 (C=O), 1659 (C=O); δ_H (500 MHz, $CDCl_3$) 6.78-6.82 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$ and $H_{6''}$), 7.34-7.39 (4H, m, $H_{3'}$, $H_{5'}$, $H_{3''}$ and $H_{5''}$), 7.78-7.82 (2H, m, H_6 and H_7), 8.10-8.15 (2H, m, H_5 and H_8); δ_C (125 MHz, $CDCl_3$) 116.4, 118.3, 126.9, 130.6, 132.4, 134.5, 145.7, 155.4, 180.0; *m/z* (FI) 498 ([M(^{79}Br^{79}Br)]$^+$, 50%), 500 ([M(^{79}Br)(^{81}Br)]$^+$, 100%), 502 ([M(^{81}Br)(^{81}Br)]$^+$, 50%); HRMS (FI) $C_{22}H_{12}O_4Br_2$ ([M(^{79}Br^{79}Br)]$^+$) requires 497.9092, found 497.9102. Then, following *Representative Procedure 5*, using naphthoquinone **16** without further purification (400 mg, 0.80 mmol), benzenesulfonamide (126 mg, 0.80 mmol) and Cs_2CO_3 (313 mg, 0.96 mmol) in THF (30 mL) and subsequent recrystallisation from toluene gave 2-sulfonamidonaphthoquinone **19** as a yellow solid (210 mg, 43%). mp 215-218 °C (toluene); HPLC (method A) t_R 19 min., 97%; υ_{max} (neat) 3251 (N-H), 1675 (C=O), 1649 (C=O); δ_H (500 MHz, $CDCl_3$) 6.57-6.62 (2H, m, $H_{2''}$ and $H_{6''}$), 7.29-7.34 (2H, m, $H_{3''}$ and $H_{5''}$), 7.36-7.42 (2H, m, $H_{3'}$ and $H_{5'}$), 7.48-7.53 (1H, m, $H_{4'}$), 7.72-7.84 (5H, m, H_6, H_7, $H_{2'}$, $H_{6'}$ and N*H*), 7.93-7.98 (1H, m, H_5), 8.13-8.18 (1H, m, H_8); δ_C (125 MHz, $CDCl_3$) 116.1, 118.7, 126.9, 126.9, 127.0, 128.8, 129.7, 130.9, 131.0, 132.1, 133.0, 134.1, 135.1, 140.2, 140.4, 155.0, 178.5, 180.3; *m/z* (ESI$^-$) 482 ([M(^{79}Br)-H]$^-$, 100%), 484 ([M(^{81}Br)-H]$^-$, 100%); HRMS (ESI$^+$) $C_{22}H_{14}BrNNaO_5S^+$ ([M(^{81}Br)+Na]$^+$) requires 507.9649, found 507.9666; λ_{max} (pH 8) < 400 nm, λ_{max} (pH 13.75) 473 nm (ε_{CB} 8550 $M^{-1}cm^{-1}$); pK_a 5.0.

4.1.13. *N*-(3-(2''-Chlorophenyl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (20)

Following *Representative Procedure 6*, using naphthoquinone **8** (200 mg, 0.58 mmol), 2-chlorophenylboronic acid (181 mg, 1.14 mmol), $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) and sat. aq. $NaHCO_3$ (5 mL) in THF (22 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) and subsequent recrystallisation from toluene gave 3-arylnaphthoquinone **20** as a yellow solid (119 mg, 48%). mp 194-196 °C (toluene); HPLC (method B) t_R 10 min., 96%; υ_{max} (KBr) 3206 (N-H), 1673 (C=O), 1656 (C=O); δ_H (400 MHz, Acetone-d_6) 7.21-7.36 (4H, m, $H_{3''}$, $H_{4''}$, $H_{5''}$ and $H_{6''}$) 7.44-7.53 (2H, m, $H_{3'}$ and $H_{5'}$), 7.59 (1H, s, $H_{4'}$), 7.67-7.76 (2H, m, $H_{2'}$ and $H_{6'}$), 7.85-7.97 (2H, m, H_6 and H_7), 8.05-8.14 (2H, m, H_5 and H_8), 8.61 (1H, br. s, N*H*); δ_C (100 MHz, Acetone-d_6) 126.7, 126.8, 126.8, 126.9, 129.1, 129.4, 130.8, 131.2, 131.6, 132.4, 132.6, 132.9, 134.3, 134.4, 134.8, 135.2, 140.7, 141.8, 181.2, 182.5; *m/z* (ESI$^-$) 422 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{22}H_{14}ClNNaO_4S^+$ ([M+Na]$^+$) requires 446.0224, found 446.0225; λ_{max} (pH 8) < 400 nm, λ_{max} (pH 13.75) 474 nm (ε_{CB} 8860 $M^{-1}cm^{-1}$); pK_a 4.9.

4.1.14. ***N*-(3-(3''-Chlorophenyl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (21)**

Following *Representative Procedure 6*, using naphthoquinone **8** (200 mg, 0.58 mmol), 3-chlorophenylboronic acid (181 mg, 1.14 mmol), $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) and sat. aq. $NaHCO_3$ (5 mL) in THF (22 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) and subsequent recrystallisation from toluene gave 3-arylnaphthoquinone **21** as a yellow solid (87 mg, 35%). mp 168-172 °C (toluene); HPLC (method B) t_R 11 min., 99%; υ_{max} (KBr) 3229 (N-H), 1670 (C=O), 1651 (C=O); δ_H (400 MHz, Acetone-d_6) 7.26-7.31 (3H, m, $H_{4''}$, $H_{5''}$ and $H_{6''}$), 7.33 (1H, s, $H_{2''}$), 7.44-7.51 (2H, m, $H_{3'}$ and $H_{5'}$), 7.57-7.63 (1H, m, $H_{4'}$), 7.63-7.69 (2H, m, $H_{2'}$ and $H_{6'}$), 7.84-7.94 (2H, m, H_6 and H_7), 8.04-8.13 (2H, m, H_5 and H_8), 8.53 (1H, br. s, N*H*); δ_C (100 MHz, Acetone-d_6) 126.6, 126.7, 126.9, 128.9, 129.1, 129.6, 129.7, 130.9, 131.2, 132.5, 133.1, 133.3, 134.3, 134.4, 135.1, 136.1, 139.7, 141.7, 181.6, 183.4; *m/z* (ESI^-) 422 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{22}H_{13}ClNO_4S^-$ ($[M-H]^-$) requires 422.0259, found 422.0258; λ_{max} (pH 8) < 400 nm, λ_{max} (pH 13.75) 473 nm (ε_{CB} 3890 $M^{-1}cm^{-1}$); pK_a 4.5.

4.1.15. ***N*-(3-(4''-Chlorophenyl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (22)**

Following *Representative Procedure 6*, using naphthoquinone **8** (200 mg, 0.58 mmol), 4-chlorophenylboronic acid (181 mg, 1.14 mmol), $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) and sat. aq. $NaHCO_3$ (5 mL) in THF (22 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) and subsequent recrystallisation from toluene gave 3-arylnaphthoquinone **22** as a yellow solid (115 mg, 47%). mp 222-223 °C (toluene); HPLC (method B) t_R 11 min., 95%; υ_{max} (KBr) 3240 (N-H), 1627 (C=O), 1655 (C=O); δ_H (400 MHz, DMSO-d_6) 7.21-7.27 (2H, m, $H_{2''}$ and $H_{6''}$ or $H_{3''}$ and $H_{5''}$), 7.30-7.38 (2H, m, $H_{2''}$ and $H_{6''}$ or $H_{3''}$ and $H_{5''}$), 7.43-7.51 (2H, m, $H_{3'}$ and $H_{5'}$), 7.54-7.66 (3H, m, $H_{2'}$, $H_{4'}$ and $H_{6'}$), 7.80-7.93 (2H, m, H_6 and H_7), 7.97-8.05 (2H, m, H_5 and H_8), 9.93 (1H, br. s, N*H*); δ_C (100 MHz, DMSO-d_6) 126.4, 126.7, 127.0, 127.2, 128.4, 129.5, 129.8, 131.2, 131.5, 132.4, 133.1, 134.2, 135.1, 135.5, 140.0, 142.7, 182.0, 184.0; *m/z* (ESI^-) 422 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{22}H_{13}ClNO_4S^-$ ($[M-H]^-$) requires 422.0259, found 422.0260; λ_{max} (pH 8) < 400 nm, λ_{max} (pH 13.75) 477 nm (ε_{CB} 6810 $M^{-1}cm^{-1}$); pK_a 5.0.

4.1.16. ***N*-(3-(3''-Formylphenyl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (23)**

Following *Representative Procedure 6*, using naphthoquinone **8** (200 mg, 0.58 mmol), 3-formylphenylboronic acid (174 mg, 1.14 mmol), $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) and sat. aq. $NaHCO_3$ (5 mL) in THF (22 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 70:30) and subsequent recrystallisation from toluene gave 3-arylnaphthoquinone **23** as a yellow solid (74 mg, 31%). mp 200-201 °C (toluene); HPLC (method B) t_R 9 min., >99%; υ_{max} (KBr) 3229 (N-H), 1698 (C=O), 1671 (C=O), 1651 (C=O); δ_H (400 MHz, Acetone-d_6) 7.37-7.45 (2H, m, two of $H_{2''}$, $H_{4''}$, $H_{5''}$ and $H_{6''}$), 7.49-7.55 (4H, m, $H_{3'}$, $H_{5'}$ and two of $H_{2''}$, $H_{4''}$, $H_{5''}$ and $H_{6''}$), 7.63-7.70 (1H, m, $H_{4'}$), 7.75-7.85 (2H, m, $H_{2'}$ and $H_{6'}$), 7.87-7.97 (2H, m, H_6 and H_7), 8.06-8.16 (2H, m, H_5 and H_8), 8.69 (1H, br. s, N*H*), 9.89 (1H, s, C*H*O); δ_C (100 MHz, Acetone-d_6) 126.5, 126.7, 126.9, 128.7, 129.2, 129.8, 131.2, 132.1, 132.5, 133.0, 133.4, 134.4, 135.2, 136.2, 136.5, 137.2, 139.6, 141.7, 181.7, 183.6, 192.0; *m/z* (ESI^-) 416 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{23}H_{14}NO_5S^-$ ($[M-H]^-$) requires 416.0598, found 416.0596; λ_{max} (pH 8) < 400 nm, λ_{max} (pH 13.75) 474 nm (ε_{CB} 5720 $M^{-1}cm^{-1}$); pK_a 5.0.

4.1.17. ***N*-(3-(4''-Formylphenyl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (24)**

Following *Representative Procedure 6*, using naphthoquinone **8** (200 mg, 0.58 mmol), 4-formylbenzeneboronic acid (174 mg, 1.14 mmol), $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) and sat. aq. $NaHCO_3$ (5 mL) in THF (22 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 70:30) and subsequent recrystallization from toluene gave 3-arylnaphthoquinone **24** as a yellow solid (59 mg, 24%). mp 200-202 °C (toluene); HPLC (method B) t_R 9 min., 99%; υ_{max} (KBr) 3234 (N-H), 1698 (C=O), 1668 (C=O), 1650 (C=O); δ_H (400 MHz, DMSO-d_6) 7.40-7.47 (4H, m, $H_{3'}$, $H_{5'}$ and $H_{2''}$ and $H_{6''}$ or $H_{3''}$ and $H_{5''}$), 7.52-7.58 (1H, m, $H_{4'}$), 7.58-7.63 (2H, m, $H_{2'}$ and $H_{6'}$), 7.76-7.84 (2H, m, $H_{2''}$ and $H_{6''}$ or $H_{3''}$ and $H_{5''}$), 7.85-7.96 (2H, m, H_6 and H_7), 7.98-8.08 (2H, m, H_5 and H_8), 10.02 (2H, s, C*H*O and N*H*); δ_C (100 MHz, DMSO-d_6) 126.7, 127.0, 127.2, 129.3, 129.5, 131.5, 132.1, 132.4, 133.1, 135.1, 135.6, 136.5, 138.7, 139.1, 140.0, 142.5, 182.0, 183.9, 193.8; *m/z* (ESI^-) 416 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{23}H_{14}NO_5S^-$ ($[M-H]^-$) requires 416.0598, found 416.0600; λ_{max} (pH 8) < 400 nm, λ_{max} (pH 13.75) 477 nm (ε_{CB} 5880 $M^{-1}cm^{-1}$); pK_a 4.6.

4.1.18. ***N*-(3-(Furan-2''-yl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (25)**

Following *Representative Procedure 6*, using naphthoquinone **8** (200 mg, 0.58 mmol), furan-2-ylboronic acid (130 mg, 1.14 mmol), $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) and sat. aq. $NaHCO_3$ (5 mL) in THF (22 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 70:30) and subsequent recrystallisation from toluene gave 3-arylnaphthoquinone **25** as a purple solid (100 mg, 45%). mp 144-146 °C (toluene); HPLC (method B) t_R 10 min., 99%; υ_{max} (KBr) 3242 (N-H), 1668 (C=O), 1648 (C=O); δ_H (400 MHz, Acetone-d_6) 6.66-6.70 (1H, dd, *J* 3.5, 1.7, $H_{4''}$), 7.45 (1H, d, *J* 3.5, $H_{5''}$), 7.53-7.62 (2H, m, $H_{3'}$ and $H_{5'}$), 7.62-7.68 (1H, m, $H_{4'}$), 7.72 (1H, d, *J* 1.7, $H_{3''}$), 7.81-7.92 (2H, m, H_6 and H_7) 7.93-8.01 (3H, m, $H_{2'}$, $H_{6'}$ and H_5 or H_8), 8.09-8.14 (1H, m, H_5 or H_8), 8.98 (1H, s, N*H*); δ_C (100 MHz, Acetone-d_6) 114.0, 120.6, 125.5, 127.5, 127.9, 128.4, 130.2, 132.4, 133.6, 134.1, 135.5, 135.9, 137.4, 143.5, 146.2, 147.4, 181.9, 183.8; *m/z* (ESI^-) 378 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{20}H_{13}NNaO_5S^+$ ($[M+Na]^+$) requires 402.0407, found 402.0407; λ_{max} (pH 8) 458 nm (ε_N 11320 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 502 nm (ε_{CB} 7180 $M^{-1}cm^{-1}$); pK_a 5.1.

4.1.19. ***N*-(3-(Furan-3''-yl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (26)**

Following *Representative Procedure 6*, using naphthoquinone **8** (200 mg, 0.58 mmol), furan-3-ylboronic acid (130 mg, 1.14 mmol), $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) and sat. aq. $NaHCO_3$ (5 mL) in THF (22 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) and subsequent recrystallisation from toluene gave 3-arylnaphthoquinone **26** as a yellow solid (51 mg, 23%). mp 211-214 °C (toluene); HPLC (method B) t_R 9 min., 99%; υ_{max} (KBr) 3231 (N-H), 1664 (C=O), 1651 (C=O); δ_H (400 MHz, DMSO-d_6) 6.95 (1H, app. s, $H_{5''}$), 7.46-7.57 (2H, m, $H_{3'}$ and $H_{5'}$), 7.58-7.66 (1H, m, $H_{4'}$), 7.73-7.75 (1H, m, $H_{4''}$), 7.76 -7.80 (2H, m, $H_{2'}$ and $H_{6'}$), 7.82-7.93 (3H, m, H_6, H_7 and H_5 or H_8), 7.99-8.09 (1H, m, H_5 or H_8), 8.18 (1H, app. s, $H_{2''}$), 10.04 (1H, br. s, N*H*); δ_C (100 MHz, DMSO-d_6) 112.5, 116.8, 126.8, 127.2, 127.3, 129.6, 131.3, 132.6, 133.3, 134.5, 135.1, 135.3, 137.8, 142.7, 143.5, 146.8, 181.5, 184.3; *m/z* (ESI^-) 378 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{20}H_{13}NNaO_5S^+$ ($[M+Na]^+$) requires 402.0407, found

402.0403; λ_{max} (pH 8) 444 nm (ε_N 15220 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 509 nm (ε_{CB} 7840 $M^{-1}cm^{-1}$); pK_a 5.0.

4.1.20. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methanesulfonamide (31)

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (500 mg, 2.20 mmol), methanesulfonamide (209 mg, 2.20 mmol) and Cs_2CO_3 (287 mg, 0.88 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10) gave sulfonamide **31** as a yellow solid (246 mg, 39%). mp 195-200 °C; υ_{max} (neat) 3241 (N-H), 1680 (C=O), 1659 (C=O); δ_H (500 MHz, DMSO-d_6) 3.44 (3H, s, *Me*), 7.89-7.94 (2H, m, H_6 and H_7), 8.04-8.10 (2H, m, H_5 and H_8), 10.10 (1H, br. s, N*H*); δ_C (125 MHz, DMSO-d_6) 43.3, 126.6, 126.8, 130.7, 130.9, 133.7, 134.5, 134.7, 141.0, 177.5, 179.3; *m/z* (ESI$^-$) 284 ([M(^{35}Cl)-H]$^-$, 100%); HRMS (ESI$^+$) $C_{11}H_9ClNNaO_4S^+$ ([M(^{35}Cl)+Na]$^+$) requires 307.9755, found 307.9755.

4.1.21. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-cyclohexanesulfonamide (32)

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (400 mg, 1.76 mmol), cyclohexanesulfonamide **29** (287 mg, 1.76 mmol) and Cs_2CO_3 (804 mg, 2.46 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 75:25 and then pet ether:EtOAc 80:20 to 50:50) gave sulfonamide **32** as a yellow solid (396 mg, 64%). mp 178-179 °C; υ_{max} (neat) 3254 (N-H), 1679 (C=O), 1595 (C=O); δ_H (500 MHz, $CDCl_3$) 1.24-1.34 (1H, m, $H_{4'}$), 1.44 (2H, m, $H_{3'}$ and $H_{5'}$), 1.71 (2H, m, $H_{2'}$ and $H_{6'}$), 1.76-1.82 (1H, m, $H_{4'}$), 1.99 (2H, m, $H_{3'}$ and $H_{5'}$), 2.37 (2H, app. d, *J* 12.6, $H_{2'}$ and $H_{6'}$), 4.14 (1H, m, $H_{1'}$), 6.93 (1H, s, N*H*), 7.76-7.82 (2H, m, H_6 and H_7), 8.12-8.19 (2H, m, H_5 and H_8); δ_C (125 MHz, $CDCl_3$) 25.0, 25.0, 26.4, 64.0, 127.3, 127.4, 128.8, 130.4, 131.0, 134.2, 134.8, 141.1, 177.0, 178.6; *m/z* (ESI$^-$) 352 ([M(^{35}Cl)-H]$^-$, 100%), 354 ([M(^{37}Cl)-H]$^-$, 33%); HRMS (ESI$^+$) $C_{16}H_{16}ClNNaO_4S$ ([M(^{35}Cl)+Na]$^+$) requires 376.0381, found 376.0366.

4.1.22. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2'-methylbenzenesulfonamide (33)

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (500 mg, 2.20 mmol), *ortho*-toluenesulfonamide (377 mg, 2.20 mmol) and Cs_2CO_3 (1000 mg, 3.08 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) gave sulfonamide **33** as a yellow solid (555 mg, 70%). mp 157-162 °C; υ_{max} (neat) 3370 (N-H), 1666 (C=O), 1589 (C=O); δ_H (500 MHz, DMSO-d_6) 2.65 (3H, s, *Me*), 7.39 (1H, app. t, *J* 7.4, $H_{5'}$), 7.44 (1H, d, *J* 7.4, $H_{3'}$), 7.56 (1H, app. td, *J* 7.4, 1.3, $H_{4'}$), 7.84-7.93 (3H, m, H_6, H_7 and $H_{6'}$), 7.95-7.98 (1H, m, H_5 or H_8), 8.04-8.08 (1H, m, H_5 or H_8); δ_C (125 MHz, DMSO-d_6) 20.0, 125.9, 126.6, 126.7, 128.3, 130.4, 131.0, 132.2, 132.6, 134.5, 134.7, 136.2, 140.5, 141.1, 177.4, 178.8; *m/z* (ESI$^+$) 384 ([M(^{35}Cl)+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{13}ClNNaO_4S^+$ ([M(^{35}Cl)+Na]$^+$) requires 384.0068, found 384.0057.

4.1.23. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2'-nitrobenzenesulfonamide (34)

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (500 mg, 2.20 mmol), 2-nitrobenzenesulfonamide (445 mg, 2.20 mmol) and Cs_2CO_3 (287 mg, 0.88 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) gave sulfonamide **34** as a yellow solid (378 mg, 44%). mp > 280 °C; υ_{max} (neat) 3396 (N-H), 1677 (br., C=O); δ_H (500 MHz, DMSO-d_6) 7.65-7.82 (5H, m, H_6, H_7, $H_{4'}$, $H_{5'}$ and $H_{6'}$), 7.86 (1H, dd, *J* 7.9, 1.0, H_5 or H_8), 7.97 (1H, dd, *J* 7.6, 1.0, H_5 or H_8), 8.13 (1H, dd, *J* 7.9, 1.3, $H_{3'}$); δ_C (125 MHz, DMSO-d_6) 123.4, 125.7, 126.4, 128.7, 130.8, 131.5, 131.6, 131.9, 132.9, 134.1, 138.7, 146.8, 149.1, 176.7, 179.6; *m/z* (ESI$^-$) 391 ([M(^{35}Cl)-H]$^-$, 100%); HRMS (ESI$^-$) $C_{16}H_8ClN_2O_6S^-$ ([M(^{35}Cl)-H]$^-$) requires 390.9797, found 390.9797.

4.1.24. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2',6'-difluorobenzenesulfonamide (35)

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (500 mg, 2.20 mmol), 2,6-difluorobenzenesulfonamide (425 mg, 2.20 mmol) and Cs_2CO_3 (1000 mg, 3.08 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 70:30) gave sulfonamide **35** as a yellow solid (620 mg, 67%). mp 261-267 °C; υ_{max} (neat) 3444 (N-H), 1657 (C=O), 1611 (C=O); δ_H (400 MHz, DMSO-d_6) 7.32-7.40 (2H, m, $H_{3'}$ and $H_{5'}$), 7.74-7.84 (1H, m, $H_{4'}$), 7.87-7.97 (2H, m, H_6 and H_7), 7.98-8.03 (1H, m, H_5 or H_8), 8.09-8.14 (1H, m, H_5 or H_8); δ_F (470 MHz, DMSO-d_6) -108.8; δ_C (100 MHz, DMSO-d_6) 113.1 (dd, *J* 19.8, 3.0), 120.6 (m), 126.7, 126.8, 130.3, 130.9, 134.5, 134.7, 135.0 (m), 135.4, 140.8, 158.3 (dd, *J* 256.1, 3.8), 177.4, 179.0; *m/z* (ESI$^-$) 382 ([M(^{35}Cl)-H]$^-$, 100%), 384 ([M(^{37}Cl)-H]$^-$, 50%); HRMS (ESI$^-$) $C_{16}H_7ClF_2NO_4S^-$ ([M(^{35}Cl)-H]$^-$) requires 381.9758, found 381.9765.

4.1.25. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3'-nitrobenzenesulfonamide (36)

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (500 mg, 2.20 mmol), 3-nitrobenzenesulfonamide (445 mg, 2.20 mmol) and Cs_2CO_3 (1004 mg, 3.08 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20 to 50:50) gave sulfonamide **36** as a yellow solid (837 mg, quant.). mp 258-263 °C; υ_{max} (neat) 3430 (N-H), 1678 (C=O), 1588 (C=O); δ_H (500 MHz, DMSO-d_6) 7.71 (1H, app. td, *J* 7.6, 1.3, H_6 or H_7), 7.76-7.82 (2H, m, H_6 or H_7 and $H_{5'}$), 7.88 (1H, dd, *J* 7.6, 1.3, H_5 or H_8), 7.96 (1H, dd, *J* 7.6, 1.3, H_5 or H_8), 8.28 (1H, ddd, *J* 8.1, 2.0, 0.9, $H_{4'}$ or $H_{6'}$), 8.32 (1H, ddd, *J* 8.1, 2.0, 0.9, $H_{4'}$ or $H_{6'}$), 8.66 (1H, t, *J* 2.0, $H_{2'}$); δ_C (125 MHz, DMSO-d_6) 120.1, 124.5, 124.5, 125.5, 126.3, 130.1, 130.8, 131.7, 132.1, 132.5, 134.0, 147.3, 149.7, 151.0, 176.3, 179.8; *m/z* (ESI$^-$) 391 ([M(^{35}Cl)-H]$^-$, 100%), 393 ([M(^{37}Cl)-H]$^-$, 33%); HRMS (ESI$^-$) $C_{16}H_8ClN_2O_6S^-$ ([M(^{35}Cl)-H]$^-$) requires 390.9797, found 390.9801.

4.1.26. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4'-methylbenzenesulfonamide (37)

2,3-dichloronaphthalene-1,4-dione **7** (5.00 g, 22.00 mmol), *para*-toluenesulfonamide (4.90 g, 28.60 mmol) and Cs_2CO_3 (9.30 g, 28.60 mmol) were stirred at reflux in anhydrous toluene (100 mL) for 18 h under argon. The reaction mixture was concentrated *in vacuo* and re-suspended in water (40 mL). The suspension was filtered and washed with hot water (2 x 10 mL). The precipitate was dried under vacuum to give sulfonamide **37** as a red powder (12.10 g, 14.50 mmol, 66%). mp 292-293 °C; υ_{max} (neat) 1681 (C=O), 1625 (C=O); δ_H (400 MHz, DMSO-d_6) 2.34 (3H, s, *Me*), 7.21-7.26 (2H, m, $H_{3'}$ and $H_{5'}$), 7.64-7.70 (1H, m, H_6 or H_7), 7.72-7.77 (3H, m, $H_{2'}$, $H_{6'}$ and H_6 or H_7), 7.83-7.87 (1H, m, H_5 or H_8), 7.90-7.94 (1H, m, H_5 or H_8); δ_C (100 MHz, DMSO-d_6) 21.7, 126.0, 126.3, 127.0, 129.2, 132.0, 132.7, 133.4, 134.6, 140.0, 146.6, 153.4, 176.4, 180.9; *m/z* (ESI$^-$) 360 ([M(^{35}Cl)-H]$^-$, 100%), 362 ([M(^{37}Cl)-H]$^-$, 40%); HRMS (ESI$^+$) $C_{17}H_{12}ClNO_4SNa^+$ ([M(^{35}Cl)+Na]$^+$) requires 384.0068, found 384.0061.

4.1.27. ***N*****-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4'-fluorobenzenesulfonamide (38)**

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (500 mg, 2.20 mmol), 4-fluorobenzenesulfonamide (386 mg, 2.20 mmol) and Cs_2CO_3 (1000 mg, 3.08 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) gave sulfonamide **38** as a yellow solid (515 mg, 63%). mp > 280 °C; υ_{max} (neat) 1676 (C=O), 1591 (C=O); δ_H (500 MHz, DMSO-d_6) 7.31 (2H, app. t, *J* 9.0, $H_{3'}$ and $H_{5'}$), 7.73 (1H, app. td, *J* 7.6, 1.4, H_6 or H_7), 7.79 (1H, app. td, *J* 7.6, 1.4, H_6 or H_7), 7.89 (1H, dd, *J* 7.6, 1.4, H_5 or H_8), 7.92-7.97 (3H, m, $H_{2'}$, $H_{6'}$ and H_5 or H_8); δ_F (470 MHz, DMSO-d_6) -110.4; δ_C (125 MHz, DMSO-d_6) 115.1 (d, *J* 21.9), 125.6, 126.3, 128.3 (d, *J* 8.6), 128.3, 130.9, 132.1, 132.6, 132.6, 134.0, 143.5, 162.9 (d, *J* 248.0), 176.1, 179.7; *m/z* (ESI$^-$) 364 ([M(^{35}Cl)-H]$^-$, 100%), 366 ([M(^{37}Cl)-H]$^-$, 55%); HRMS (ESI$^-$) $C_{16}H_8ClFNO_4S^-$ ([M(^{35}Cl)-H]$^-$) requires 363.9852, found 363.9853.

4.1.28. ***N*****-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)furan-2'-sulfonamide (39)**

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (500 mg, 2.20 mmol), furan-2-sulfonamide **30** (324 mg, 2.20 mmol) and Cs_2CO_3 (1004 mg, 3.08 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 50:50) gave sulfonamide **39** as a yellow solid (634 mg, 85%). mp 258-259 °C; υ_{max} (neat) 3388 (N-H), 1668 (C=O), 1652 (C=O); δ_H (500 MHz, DMSO-d_6) 6.57 (1H, dd, *J* 3.1, 1.7, $H_{4'}$), 6.91 (1H, d, *J* 3.1, $H_{5'}$), 7.75-7.84 (3H, m, H_6, H_7 and $H_{3'}$), 7.94 (1H, dd, *J* 7.6, 1.3, H_5 or H_8), 7.99 (1H, dd, *J* 7.6, 1.3, H_5 or H_8); δ_C (125 MHz, DMSO-d_6) 110.7, 111.8, 125.8, 126.4, 130.8, 132.0, 133.0, 133.0, 134.1, 144.6, 154.0, 176.5, 179.4; *m/z* (ESI$^-$) 336 ([M(^{35}Cl)-H]$^-$, 100%), 338 ([M(^{37}Cl)-H]$^-$, 33%); HRMS (ESI$^-$) $C_{14}H_7ClNO_5S^-$ ([M(^{35}Cl)-H]$^-$) requires 335.9739, found 335.9748.

4.1.29. ***N*****-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-1-phenylmethanesulfonamide (40)**

Following *Representative Procedure 1*, using 2,3-dichloronaphthalene-1,4-dione **7** (500 mg, 2.20 mmol), α-toluenesulfonamide (317 mg, 2.20 mmol) and Cs_2CO_3 (1000 mg, 3.08 mmol) in DMF (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) gave sulfonamide **40** as a yellow solid (643 mg, 81%). mp 176-179 °C; υ_{max} (neat) 3259 (N-H), 1709 (C=O), 1663 (C=O); δ_H (400 MHz, $CDCl_3$) 5.07 (2H, s, C*H*$_2$Ph), 6.78 (1H, br. s, N*H*), 7.39-7.46 (3H, m, $H_{3'}$, $H_{4'}$ and $H_{5'}$), 7.51-7.58 (2H, m, $H_{2'}$ and $H_{6'}$), 7.77-7.85 (2H, m, H_6 and H_7), 8.14-8.22 (2H, m, H_5 and H_8); δ_C (100 MHz, $CDCl_3$) 61.2, 127.4, 127.5, 128.3, 128.9, 129.2, 129.6, 130.5, 131.0, 131.2, 134.3, 134.9, 141.0, 176.9, 178.6; *m/z* (ESI$^-$) 360 ([M(^{35}Cl)-H]$^-$, 100%), 362 ([M(^{37}Cl)-H]$^-$, 33%); HRMS (ESI$^+$) $C_{17}H_{12}ClNNaO_4S^+$ ([M(^{35}Cl)+Na]$^+$) requires 384.0068, found 384.0068.

4.1.30. ***N*****-(3-(3',5'-Dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methanesulfonamide (41)**

Following *Representative Procedure 2*, using naphthoquinone **31** (100 mg, 0.35 mmol), 3,5-dimethylaniline (131 µL, 1.05 mmol) and $CeCl_3.7H_2O$ (131 mg, 0.35 mmol) in MeOH (10 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) gave 3-anilinonaphthoquinone **41** as a red solid (84 mg, 65%). mp 84-92 °C; HPLC (method A) t_R 24 min., >99%; υ_{max} (neat) 3294 (N-H), 1710 (C=O), 1673 (C=O); δ_H (500 MHz, DMSO-d_6) 2.24 (6H, s, 2 x Ar-*Me*), 2.63 (3H, s, -SO$_2$*Me*), 6.71 (3H, s, $H_{2'}$, $H_{4'}$ and $H_{6'}$), 7.81 (1H, app. td, *J* 7.6, 1.3, H_6), 7.88 (1H, app. td, *J* 7.6, 1.3, H_7), 8.01-8.06 (2H, m, H_5 and H_8), 8.54 (1H, s, sulfonamide-N*H*), 8.83 (1H, s, aniline-N*H*); δ_C (125 MHz, DMSO-d_6) 20.9, 41.1, 114.9, 121.2, 125.4, 125.9, 126.2, 130.5, 131.8, 133.1, 134.9, 136.6, 138.3, 141.4, 179.5, 182.3; *m/z* (ESI$^+$) 393 ([M(^{35}Cl)+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{19}H_{19}N_2NaO_4S^+$ ([M(^{35}Cl)+Na]$^+$) requires 393.0879, found 393.0868; λ_{max} (pH 8) 491 nm (ε_N 10060 M^{-1}cm^{-1}), λ_{max} (pH 13.75) 578 nm (ε_{CB} 6770 M^{-1}cm^{-1}); pK_a 9.5.

4.1.31. ***N*****-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)cyclohexanesulfonamide (42)**

Following *Representative Procedure 2*, using naphthoquinone **32** (100 mg, 0.28 mmol), 3,5-dimethylaniline (106 µL, 0.85 mmol) and $CeCl_3.7H_2O$ (105 mg, 0.28 mmol) in MeOH (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10) and subsequent recrystallization from toluene gave 3-anilinonaphthoquinone **42** as a red solid (40 mg, 32%). mp 192-197 °C (toluene); HPLC (method A) t_R 20 min., 97%; υ_{max} (neat) 3280 (N-H), 1711 (C=O), 1670 (C=O); δ_H (500 MHz, $CDCl_3$) 0.98-1.17 (3H, m, $H_{3'}$, $H_{4'}$ and $H_{5'}$), 1.41 (2H, m, $H_{2'}$ and $H_{6'}$), 1.62 (1H, app. d, *J* 12.6, $H_{4'}$), 1.77-1.83 (2H, m, $H_{3'}$ and $H_{5'}$), 2.02 (2H, app. d, *J* 12.1, $H_{2'}$ and $H_{6'}$), 2.14 (1H, m, $H_{1'}$), 2.32 (6H, s, 2 x Ar-*Me*), 6.53 (1H, s, sulfonamide-N*H*), 6.69 (2H, s, $H_{2''}$ and $H_{6''}$), 6.85 (1H, s, $H_{4''}$), 7.69 (1H, app. td, *J* 7.6, 1.3, H_6), 7.76 (1H, app. td, *J* 7.6, 1.3, H_7), 7.81 (1H, s, aniline-N*H*), 8.10 (1H, dd, *J* 7.6, 1.3, H_5), 8.15 (1H, dd, *J* 7.6, 1.3, H_8); δ_C (125 MHz, $CDCl_3$) 21.3, 25.0, 25.0, 25.8, 61.4, 115.0, 121.1, 126.7, 126.8, 126.9, 130.5, 131.7, 133.1, 134.8, 136.8, 137.0, 138.1, 180.1, 182.1; *m/z* (ESI$^-$) 303 (40%), 437 ([M-H]$^-$, 100%), 438 (40%); HRMS (ESI$^-$) $C_{24}H_{25}N_2O_4S^-$ ([M-H]$^-$) requires 437.1541, found 437.1536; λ_{max} (pH 8) 505 nm (ε_N 19850 M^{-1}cm^{-1}), λ_{max} (pH 13.75) 558 nm (ε_{CB} 6790 M^{-1}cm^{-1}); pK_a 10.6.

4.1.32. ***N*****-(3-(3'',5''-Dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2'-methylbenzenesulfonamide (43)**

Following *Representative Procedure 2*, using naphthoquinone **33** (100 mg, 0.28 mmol), 3,5-dimethylaniline (103 µL, 0.83 mmol) and $CeCl_3.7H_2O$ (103 mg, 0.28 mmol) in MeOH (10 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) gave 3-anilinonaphthoquinone **43** as a red solid (61 mg, 49%). mp 213-217 °C; HPLC (method A) t_R 20 min., 98%; υ_{max} (neat) 3293 (N-H), 1671 (C=O), 1613 (C=O); δ_H (500 MHz, DMSO-d_6) 2.19 (6H, s, 2 x aniline-*Me*), 2.34 (3H, s, sulfonamide-*Me*), 6.42 (2H, s, $H_{2''}$ and $H_{6''}$), 6.61 (1H, s, $H_{4''}$), 7.09 (1H, d, *J* 7.4, $H_{3'}$), 7.16 (1H, app. t, *J* 7.4, $H_{5'}$), 7.36 (1H, app. td, *J* 7.5, 0.9, $H_{4'}$), 7.53 (1H, d, *J* 7.5, $H_{6'}$), 7.78 (1H, app. td, *J* 7.6, 1.3, H_6 or H_7), 7.84 (1H, app. td, *J* 7.6, 1.3, H_6 or H_7), 7.92 (1H, m, H_5 or H_8), 8.02 (1H, m, H_5 or H_8), 8.60 (1H, s, -N*H*), 9.03 (1H, s, -N*H*); δ_C (125 MHz, DMSO-d_6) 20.1, 21.0, 114.4, 120.1, 125.0, 125.4, 125.8, 126.2, 127.8, 130.2, 131.6, 131.8, 131.8, 133.0, 135.0, 136.4, 137.0, 137.7, 139.3, 141.6, 179.4, 182.5; *m/z* (ESI$^+$) 469 ([M(^{35}Cl)+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{23}N_2NaO_4S^+$ ([M(^{35}Cl)+Na]$^+$) requires 469.1192, found 469.1179; λ_{max} (pH 8) 512 nm (ε_N 11250 M^{-1}cm^{-1}), λ_{max} (pH 13.75) 529 nm (ε_{CB} 6430 M^{-1}cm^{-1}); pK_a 9.3.

4.1.33. ***N*****-(3-(3'',5''-Dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2'-nitrobenzenesulfonamide (44)**

Following *Representative Procedure 2*, using naphthoquinone **34** (100 mg, 0.26 mmol), 3,5-dimethylaniline (95 µL, 0.76 mmol) and $CeCl_3.7H_2O$ (95 mg, 0.26 mmol) in MeOH (10 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) gave 3-

anilinonaphthoquinone **44** as a red solid (24 mg, 20%). mp 257-263 °C; HPLC (method A) t_R 20 min., 96%; υ_{max} (neat) 3297 (N-H), 1674 (C=O), 1613 (C=O); δ_H (500 MHz, $CDCl_3$) 2.32 (6H, s, 2 x Ar-*Me*), 6.74 (2H, s, $H_{2''}$ and $H_{6''}$), 6.83 (1H, s, $H_{4''}$), 7.52-7.55 (1H, m, $H_{5'}$), 7.56 (1H, s, sulfonamide-N*H*), 7.63-7.73 (3H, m, H_6, H_7 and $H_{4'}$), 7.78 (1H, dd, *J* 7.9, 1.6, $H_{6'}$), 7.90-7.93 (2H, m, H_8 and $H_{3'}$), 7.94 (1H, s, aniline–N*H*), 8.13 (1H, dd, *J* 7.4, 1.4, H_5); δ_C (125 MHz, $CDCl_3$) 21.3, 111.9, 121.9, 125.6, 126.6, 126.9, 127.7, 130.2, 130.2, 131.9, 132.7, 132.9, 133.3, 134.5, 135.1, 136.5, 138.2, 140.3, 147.3, 179.4, 182.1; *m/z* (ESI^-) 476 ($[M(^{35}Cl)-H]^-$, 100%); HRMS (ESI^-) $C_{24}H_{20}N_3NaO_6S^-$ ($[M(^{35}Cl)-H]^-$) requires 476.0922, found 476.0903; λ_{max} (pH 8) 528 nm (ε_N 12380 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 560 nm (ε_{CB} 7610 $M^{-1}cm^{-1}$); pK_a 7.9.

4.1.34. ***N*-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2',6'-difluorobenzenesulfonamide (45)**

Following *Representative Procedure 2*, using naphthoquinone **35** (200 mg, 0.52 mmol), 3,5-dimethylaniline (195 µL, 1.57 mmol) and $CeCl_3.7H_2O$ (194 mg, 0.52 mmol) in MeOH (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 85:15) and subsequent recrystallization from toluene gave 3-anilinonaphthoquninone **45** as a red solid (81 mg, 33%). mp 222-225 °C (toluene); HPLC (method A) t_R 22 min., 98%; υ_{max} (neat) 3347 (N-H), 3249 (N-H), 1679 (C=O), 1613 (C=O); δ_H (500 MHz, $CDCl_3$) 2.30 (6H, s, 2 x Ar-*Me*), 6.69 (2H, s, $H_{2''}$ and $H_{6''}$), 6.72 (1H, s, $H_{4''}$), 6.87-6.92 (2H, m, $H_{3'}$ and $H_{5'}$), 7.03 (1H, s, sulfonamide-N*H*), 7.37-7.43 (1H, m, $H_{4'}$), 7.67 (1H, app. td, *J* 7.5, 1.4, H_6), 7.71 (1H, app. td, *J* 7.5, 1.4, H_7), 7.87 (1H, s, aniline-N*H*), 8.00 (1H, dd, *J* 7.5, 1.4, H_8), 8.11 (1H, dd, *J* 7.5, 1.4, H_5); δ_F (470 MHz, $CDCl_3$) -106.6; δ_C (125 MHz, $CDCl_3$) 21.3, 112.0, 112.6 (dd, *J* 22.9, 3.9), 113.1 (dd, *J* 23.1, 3.7), 121.4, 126.6, 126.9, 127.4, 130.3, 131.6, 133.0, 134.2 (m), 135.0, 136.3, 137.9, 138.9, 159.3 (dd, *J* 258.5, 3.8), 179.4, 181.9; *m/z* (ESI^+) 491 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{24}H_{19}FN_2NaO_4S^+$ ($[M+Na]^+$) requires 491.0848, found 491.0842; λ_{max} (pH 8) 509 nm (ε_N 7420 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 548 nm (ε_{CB} 7780 $M^{-1}cm^{-1}$); pK_a 8.4.

4.1.35. ***N*-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3'-nitrobenzenesulfonamide (46)**

Following *Representative Procedure 2*, using naphthoquinone **36** (175 mg, 0.43 mmol), 3,5-dimethylaniline (162 µL, 1.23 mmol) and $CeCl_3.7H_2O$ (162 mg, 0.43 mmol) in MeOH (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 80:20) gave 3-anilinonaphthoquninone **46** as a red solid (59 mg, 26%). mp 216-221 °C; HPLC (method A) t_R 22 min., 96%; υ_{max} (neat) 3290 (N-H), 1708 (C=O), 1675 (C=O); δ_H (500 MHz, $CDCl_3$) 2.30 (6H, s, 2 x Ar-*Me*), 6.63 (2H, s, $H_{2''}$ and $H_{6''}$), 6.78 (1H, s, $H_{4'}$), 6.90 (1H, s, sulfonamide-N*H*), 7.55 (1H, app. t, *J* 8.0, $H_{5'}$), 7.68 (1H, app. td, *J* 7.6, 1.3, H_6), 7.72 (1H, app. td, *J* 7.6, 1.3, H_7), 7.89 (1H, s, aniline-N*H*), 7.93 (1H, dd, *J* 7.6, 1.3, H_8), 7.98-8.01 (1H, ddd, *J* 8.0, 1.9, 0.9, $H_{6'}$), 8.12 (1H, dd, *J* 7.6, 1.3, H_5), 8.30 (1H, ddd, *J* 8.0, 1.9, 0.9, $H_{4'}$), 8.45 (1H, app. t, *J* 1.9, $H_{2'}$); δ_C (125 MHz, $CDCl_3$) 21.3, 112.0, 121.0, 122.5, 126.5, 127.0, 127.1, 127.3, 129.7, 130.2, 131.5, 132.6, 133.2, 135.2, 136.5, 138.2, 139.2, 141.6, 147.7, 179.5, 181.8; *m/z* (ESI^-) 476 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{24}H_{18}N_3O_6S^-$ ($[M-H]^-$) requires 476.0922, found 476.0922; λ_{max} (pH 8) 509 nm (ε_N 8730 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 551 nm (ε_{CB} 5670 $M^{-1}cm^{-1}$); pK_a 8.6.

4.1.36. ***N*-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4'-methylbenzenesulfonamide (47)**

Following *Representative Procedure 2*, using naphthoquinone **37** (200 mg, 0.58 mmol), 3,5-dimethylaniline (214 µL, 1.73 mmol) and $CeCl_3.7H_2O$ (214 mg, 0.58 mmol) in toluene (10 mL) at 110 °C. Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 80:20) gave 3-anilinonaphthoquninone **47** as a red solid (64 mg, 25%). mp 172-173 °C; HPLC (method A) t_R 22 min., 96%; υ_{max} (neat) 3260 (N-H), 1672 (C=O), 1597 (C=O); δ_H (500 MHz, $CDCl_3$) 2.32 (6H, s, 2 x aniline-*Me*), 2.33 (3H, s, sulfonamide-*Me*), 6.68 (2H, s, $H_{2''}$ and $H_{6''}$), 6.80 (1H, s, sulfonamide-N*H*), 6.83 (1H, s, $H_{4''}$), 7.14 (2H, d, *J* 8.0, $H_{3'}$ and $H_{5'}$), 7.56 (2H, d, *J* 8.0, $H_{2'}$ and $H_{6'}$), 7.63-7.71 (2H, m, H_6 and H_7), 7.91 (1H, dd, *J* 7.4, 1.3, H_8), 8.04 (1H, s, aniline-N*H*), 8.06 (1H, dd, *J* 7.4, 1.3, H_5); δ_C (125 MHz, $CDCl_3$) 21.4, 21.5, 113.8, 121.0, 126.4, 126.9, 126.9, 127.3, 129.3, 130.6, 131.4, 133.0, 134.7, 135.9, 137.5, 138.0, 139.0, 143.9, 179.1, 181.9; *m/z* (ESI^-) 445 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{25}H_{22}N_2O_4SNa^+$ ($[M+Na]^+$) requires 469.1192, found 469.1190; λ_{max} (pH 8) 505 nm (ε_N 14040 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 578 nm (ε_{CB} 7170 $M^{-1}cm^{-1}$); pK_a 9.7.

4.1.37. ***N*-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4'-fluorobenzenesulfonamide (48)**

Following *Representative Procedure 2*, using naphthoquinone **38** (200 mg, 0.55 mmol), 3,5-dimethylaniline (205 µL, 1.64 mmol) and $CeCl_3.7H_2O$ (204 mg, 0.55 mmol) in MeOH (10 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) and subsequent recrystallization from toluene gave 3-anilinonaphthoquninone **48** as a red solid (146 mg, 59%). mp 211-217 °C (toluene); HPLC (method A) t_R 22 min., 96%; υ_{max} (neat) 3278 (N-H), 1674 (C=O), 1595 (C=O); δ_H (500 MHz, $CDCl_3$) 2.32 (6H, s, 2 x Ar-*Me*), 6.67 (2H, s, $H_{2''}$ and $H_{6''}$), 6.77 (1H, s, sulfonamide-N*H*), 6.84 (1H, s, $H_{4''}$), 6.98-7.03 (2H, m, $H_{3'}$ and $H_{5'}$), 7.65-7.73 (4H, m, H_6, H_7, $H_{2'}$ and $H_{6'}$), 7.91-7.94 (1H, m, H_8), 7.97 (1H, s, aniline-N*H*), 8.07-8.10 (1H, m, H_5); δ_F (470 MHz, $CDCl_3$) -104.5; δ_C (125 MHz, $CDCl_3$) 21.3, 112.8, 115.9 (d, *J* 11.5), 121.1, 126.4, 126.9, 127.0, 130.1 (d, *J* 9.5), 130.4, 131.4, 133.1, 134.9, 135.0 (d, *J* 2.8), 137.1, 138.0, 139.1, 165.2 (d, *J* 255.1), 179.2, 181.9; *m/z* (ESI^-) 449 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{24}H_{18}FN_2O_4S^-$ ($[M-H]^-$) requires 449.0977, found 449.0967; λ_{max} (pH 8) 497 nm (ε_N 6580 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 546 nm (ε_{CB} 3490 $M^{-1}cm^{-1}$); pK_a 9.9.

4.1.38. ***N*-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)furan-2'-sulfonamide (49)**

Following *Representative Procedure 2*, using naphthoquinone **39** (200 mg, 0.49 mmol), 3,5-dimethylaniline (222 µL, 1.78 mmol) and $CeCl_3.7H_2O$ (222 mg, 0.49 mmol) in MeOH (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10) and subsequent recrystallization from toluene gave 3-anilinonaphthoquninone **49** as a red solid (164 mg, 66%). mp 205-206 °C (toluene); HPLC (method A) t_R 23 min., 96%; υ_{max} (neat) 3273 (N-H), 1674 (C=O), 1596 (C=O); δ_H (500 MHz, DMSO-d_6) 2.25 (6H, s, 2 x Ar-*Me*), 6.46-6.48 (1H, dd, *J* 3.5, 1.8, $H_{4'}$), 6.69 (2H, s, $H_{2''}$ and $H_{6''}$), 6.72 (1H, s, $H_{4''}$), 6.79 (1H, dd, *J* 3.5, 0.9, $H_{5'}$), 7.75-7.81 (2H, m, H_6 and $H_{3'}$), 7.84 (1H, app. td, *J* 7.5, 1.3, H_7), 7.86-7.89 (1H, m, H_8), 8.03 (1H, dd, *J* 7.5, 1.3, H_5), 8.94 (1H, s, aniline-N*H*), 9.31 (1H, s, sulfonamide-N*H*); δ_C (125 MHz, DMSO-d_6) 21.0, 111.0, 112.2, 115.0, 121.5, 125.7, 125.7, 126.2, 130.3, 131.6, 133.0, 135.0, 136.4, 138.1, 142.8, 146.4, 149.1, 178.9, 182.3; *m/z* (ESI^+) 445 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{22}H_{18}N_2NaO_5S^+$ ($[M+Na]^+$) requires 445.0829, found 445.0819;

λ_{max} (pH 8) 509 nm (ε_N 7770 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 541 nm (ε_{CB} 8440 $M^{-1}cm^{-1}$); pK_a 8.3.

4.1.39. *N*-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)- 1-phenylmethanesulfonamide (50)

Following *Representative Procedure 2*, using naphthoquinone **40** (100 mg, 0.28 mmol), 3,5-dimethylaniline (103 µL, 0.83 mmol) and $CeCl_3.7H_2O$ (103 mg, 0.28 mmol) in MeOH (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 85:15) and subsequent recrystallization from toluene gave 3-anilinonaphthoquninone **50** as a red solid (66 mg, 53%). mp 152-156 °C (toluene); HPLC (method A) t_R 25 min., 96%; υ_{max} (neat) 3279 (N-H), 1708 (C=O), 1671 (C=O); δ_H (500 MHz, DMSO-d_6) 2.23 (6H, s, 2 x Ar-*Me*), 4.06 (2H, s, -CH_2Ph), 6.71 (1H, s, $H_{4''}$), 6.73 (2H, s, $H_{2''}$ and $H_{6''}$), 7.24-7.27 (2H, m, $H_{2'}$ and $H_{6'}$), 7.27-7.33 (3H, m, $H_{3'}$, $H_{4'}$ and $H_{5'}$), 7.82 (1H, app. td, *J* 7.6, 1.3, H_6), 7.90 (1H, app. td, *J* 7.6, 1.3, H_7), 8.04 (1H, dd, *J* 7.6, 1.3, H_5), 8.08 (1H, dd, *J* 7.6, 1.3, H_8), 8.55 (1H, s, sulfonamide-N*H*), 8.86 (1H, s, aniline-N*H*); δ_C (125 MHz, DMSO-d_6) 20.9, 58.6, 115.1, 121.3, 125.4, 126.0, 126.2, 128.0, 128.2, 129.6, 130.5, 130.9, 131.8, 133.1, 134.9, 136.7, 138.3, 141.1, 179.6, 182.3; *m/z* (ESI^-) 445 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{25}H_{22}N_2NaO_4S^+$ ($[M+Na]^+$) requires 469.1192, found 469.1190; λ_{max} (pH 8) 507 nm (ε_N 17580 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 568 nm (ε_{CB} 6660 $M^{-1}cm^{-1}$); pK_a 10.0.

4.1.40. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)acetamide (52)[36a]

2-amino-3-chloronaphthalene-1,4-dione **51** (500 mg, 2.41 mmol) and 3 drops conc. H_2SO_4 were added to a microwave vial containing acetyl chloride (5 mL). The vial was sealed and the solution stirred at 50 °C for 1 h. The solution was then cooled to 0 °C and quenched by the dropwise addition of EtOH until gas liberation ceased, before being partitioned between 1 M HCl (30 mL) and EtOAc (30 mL). The organic layer was collected, washed with brine (3 x 20 mL), dried, filtered and concentrated *in vacuo* to give the crude reaction mixture. Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 90:10) gave amide **52** as a yellow solid (489 mg, 81%). mp 215-218 °C (lit. 219-220 °C)[36a]; δ_H (500 MHz, DMSO-d_6) 2.14 (3H, s, CO-*Me*), 7.87-7.94 (2H, m, H_6 and H_7), 8.02-8.06 (1H, m, H_8), 8.06-8.10 (1H, m, H_5), 10.17 (1H, s, N*H*); *m/z* (ESI^-) 248 ($[M(^{35}Cl)-H]^-$, 100%).

4.1.41. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzamide (53)[36a]

2-amino-3-chloronaphthalene-1,4-dione **51** (1.00 g, 4.82 mmol) was stirred in a microwave vial under N_2 with sodium hydride (as a 60% dispersion in oil, 636 mg, 15.89 mmol) in anhydrous THF (10 mL) at RT for 30 min. Benzoyl chloride (721 µL, 6.26 mmol) was added and stirring continued under N_2 at RT for 1 h. EtOH was added dropwise until gas liberation ceased, and the solution partitioned between sat. aq. NH_4Cl (30 mL) and EtOAc (30 mL). The organic layer was collected, washed with brine (3 x 20 mL), dried, filtered and concentrated *in vacuo* to give the crude product. Purification *via* column chromatography on silica gel (eluent pet ether:acetone 95:5) gave amide **53** as a beige solid (1.042 g, 69%). mp 183-185 °C (lit. 254-256 °C)[36a]; δ_H (400 MHz, DMSO-d_6) 7.55-7.60 (2H, m, $H_{3'}$ and $H_{5'}$), 7.65-7.69 (1H, m, $H_{4'}$), 7.91-7.96 (2H, m, H_6 and H_7), 8.01-8.05 (2H, m, $H_{2'}$ and $H_{6'}$), 8.07-8.10 (1H, m, H_5 or H_8), 8.12-8.15 (1H, m, H_5 or H_8), 10.49 (1H, s, N*H*); *m/z* (ESI^-) 310 ($[M(^{35}Cl)-H]^-$, 100%).

4.1.42. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phenylacetamide (54)[36a]

Method A: 2-amino-3-chloronaphthalene-1,4-dione **51** (2.50 g, 12.04 mmol) was stirred in phenyl acetyl chloride (12.5 mL). HCl gas, produced *in situ* by the dropwise addition of c.H_2SO_4 onto NaCl (20 g), was bubbled through the reaction mixture for 15 min. After the removal of the gas inlet, the reaction mixture was heated to reflux under a gaseous HCl atmosphere for 2 h. The solution was cooled to RT and EtOH was added dropwise until gas liberation ceased. The solution was then partitioned between sat. aq. NH_4Cl (100 mL) and EtOAc (100 mL). The organic layer was collected, washed with brine (3 x 50 mL), dried, filtered and concentrated *in vacuo*. Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10) gave phenylacetamide **54** as a beige solid (549 mg, 14%).

Method B: 2-amino-3-chloro-1,4-naphthoquinone **51** (100 mg, 0.44 mmol), 2-phenylacetamide (71 mg, 0.53 mmol), $Pd(OAc)_2$ (1 mg, 0.004 mmol), Xantphos (4 mg, 0.007 mmol) and KO^tBu (74 mg, 0.66 mmol) were stirred in 1,4-dioxane (1 mL) in a sealed microwave vial under argon and heated to 80 °C for 16 h. The solution was cooled to RT and partitioned between EtOAc (30 mL) and sat. aq. NH_4Cl (30 mL). The organic layer was collected, washed with brine (3 x 20 mL), dried, filtered and concentrated *in vacuo* to give the crude product. Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 75:25) gave phenylacetamide **54** as a beige solid (13 mg, 9%).

Method C: 2-amino-3-chloro-1,4-naphthoquinone **51** (2.00 g, 9.63 mmol) was stirred in toluene (25 mL) in a 2-necked round-bottomed flask at 90 °C. To a pressure-equalised dropping funnel was added phenylacetyl chloride (5.97 mL, 38.53 mmol) and boron trifluoride diethyl etherate (1.16 mL, 7.11 mmol) in toluene (15 mL). This solution was added dropwise to the reaction flask over a period of 30 min. and the reaction mixture was stirred at 90 °C for a further 4 h. The reaction mixture was cooled to RT and then concentrated *in vacuo*. Acetone (20 mL) was added to the reaction mixture and the beige precipitate that formed was collected by suction filtration. This precipitate was washed three further times with cold acetone (3 x 10 mL) to give phenylacetamide **54** as a beige solid (1.781 g, 57%). mp 205-209 °C (lit. 207-208 °C)[36a]; δ_H (400 MHz, $CDCl_3$) 3.86 (2H, s, -CH_2Ph), 7.29-7.48 (3H, m, $H_{2''}$, $H_{3''}$ and $H_{4''}$), 7.62 (1H, br. s, N*H*), 7.76 (2H, m, H_6 and H_7), 8.05-8.09 (1H, m, H_5), 8.15-8.19 (1H, m, H_8); *m/z* (ESI^+) 348 ($[M(^{35}Cl)+Na]^+$, 100%).

4.1.43. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hydrocinnanamide (55)

2-amino-3chloro-1,4-naphthoquinone **51** (1.70 g, 8.19 mmol) and hydrocinnamoyl chloride (5.0 mL, 30.13 mmol) were stirred in $BF_3.OEt_2$ (1.0 mL) in a round bottomed flask and heated to reflux for 30 min. The reaction mixture was then cooled and toluene (10 mL) was added, before being heated to reflux for a further 24 h. The crude reaction mixture was concentrated *in vacuo* and the subsequent addition of acetone (20 mL) resulted in the precipitation of a yellow solid. This solid was collected by suction filtration and recrystallized from boiling acetone to give hydrocinnanamide **55** as a yellow solid (1.39 g, 50%). mp 162-167 °C; υ_{max} (neat) 3284 (N-H), 1662 (C=O); δ_H (500 MHz, $CDCl_3$) 2.84 (2H, t, *J* 7.6, CO-CH_2-CH_2Ph), 3.09 (2H, t, *J* 7.6, Ph-CH_2-CH_2CO), 7.21-7.28 (3H, m, $H_{2'}$, $H_{4'}$ and $H_{6'}$), 7.30-7.34 (2H, m, $H_{3'}$ and $H_{5'}$), 7.61 (1H, s, N*H*), 7.74-7.81 (2H, m, H_6 and H_7), 8.10 (1H, dd, *J* 7.3, 1.5, H_8), 8.19 (1H, dd, *J* 7.3, 1.5, H_5); δ_C (125 MHz, $CDCl_3$) 31.1, 38.9, 126.5, 127.0, 127.5, 128.4, 128.7, 130.2, 131.5, 133.2, 134.1, 134.8, 138.9, 139.9, 168.8, 177.6, 179.8; *m/z* (ESI^-) 113 (30%), 249 (40%), 338 ($[M(^{35}Cl)-H]^-$, 100%); HRMS $C_{19}H_{14}ClNNaO_3^+$ ([M(^{35}Cl)+Na]$^+$) requires 362.0554, found 362.0554.

4.1.44. *N*-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-1-phenylcyclopropanecarboxamide (58)

1-phenyl-1cyclopropanecarboxylic acid **56** (3.12 g, 19.27 mmol) and DMF (3 drops) were stirred in anhydrous CH_2Cl_2 (10 mL) at RT. Oxalyl chloride (1.83 mL, 19.27 mmol) was added to this solution and stirring continued at RT for 3 h. The reaction mixture was then concentrated *in vacuo*. 2-amino-3-chloro-1,4-naphthoquinone **51** (1.00 g, 4.82 mmol), boron trifluoride diethyl etherate (0.81 mL, 4.82 mmol) and toluene (15 mL) were added and the mixture was stirred at 90 °C for 30 min. and subsequently at 110 °C for 1 h. The crude reaction mixture was concentrated *in vacuo* and the subsequent addition of acetone (20 mL) resulted in the precipitation of a yellow solid. This solid was collected by suction filtration and washed with cold acetone (2 x 10 mL) to give phenylcyclopropane-carboxamide **58** as a yellow solid (1.354 g, 80%). mp 214-215 °C; υ_{max} (neat) 3338 (N-H), 1739 (C=O), 1713 (C=O), 1665 (C=O); δ_H (500 MHz, DMSO-d_6) 1.22-1.25 (2H, m, 2 x cyclopropyl-C*H*), 1.51-1.54 (2H, m, 2 x cyclopropyl-C*H*), 7.33-7.38 (1H, m, $H_{4'}$), 7.40-7.45 (2H, m, $H_{3'}$ and $H_{5'}$), 7.49-7.53 (2H, m, $H_{2'}$ and $H_{6'}$), 7.87-7.92 (2H, m, H_6 and H_7), 7.99-8.03 (1H, m, H_8), 8.05-8.08 (1H, m, H_5), 8.55 (1H, s, N*H*); δ_C (125 MHz, DMSO-d_6) 16.0, 31.0, 126.6, 126.8, 127.8, 128.9, 130.1, 130.5, 130.9, 134.4, 134.6, 134.7, 138.9, 140.9, 170.9, 177.6, 178.8; *m/z* (ESI$^+$) 199 (100%), 352 ([M(^{35}Cl)+H]$^+$, 40%), 374 ([M(^{35}Cl)+Na]$^+$, 60%), 376 ([M(^{37}Cl)+Na]$^+$, 20%); HRMS (ESI$^+$) $C_{20}H_{14}ClNNaO_3^+$ ([M(^{35}Cl)+Na]$^+$) requires 374.0554, found 374.0549.

4.1.45. *N*-(3-(3'',5''-Dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)acetamide (59)

Following *Representative Procedure 2*, using naphthoquinone **52** (101 mg, 0.41 mmol), 3,5-dimethylaniline (151 µL, 1.21 mmol) and $CeCl_3.7H_2O$ (151 mg, 0.41 mmol) in MeOH (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) gave 3-anilinonaphthoquinone **59** as a dark red solid (57 mg, 42%). mp 231-234 °C; HPLC (method A) t_R 23 min., 97%; υ_{max} (neat) 3291 (N-H), 1667 (C=O), 1638 (C=O); δ_H (400 MHz, DMSO-d_6) 1.36 (3H, s, CO-*Me*), 2.22 (6H, s, 2 x Ar-*Me*), 6.56 (2H, s, $H_{2'}$ and $H_{6'}$), 6.69 (1H, s, $H_{4'}$), 7.78 (1H, app. td, *J* 7.5, 1.4, H_6), 7.85 (1H, app. td, *J* 7.3, 1.3, H_7), 8.00 (1H, dd, *J* 7.6, 1.0, H_8), 8.03 (1H, dd, *J* 7.6, 1.3, H_5), 8.84 (1H, s, aniline-N*H*), 9.19 (1H, br. s, amide-N*H*); δ_C (100 MHz, DMSO-d_6) 20.9, 21.7, 115.4, 120.9, 124.9, 125.7, 126.1, 130.2, 131.9, 133.0, 134.9, 136.2, 137.3, 165.9, 179.1, 182.5; *m/z* (ESI$^+$) 357 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{18}N_2NaO_3^+$ ([M+Na]$^+$) requires 357.1210, found 357.1198.

4.1.46. *N*-(3-(3'',5''-Dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzamide (60)

^{t}BuOH (0.63 mL) and H_2O (0.2 µL, 0.013 mmol) were sealed in a vessel under argon. $Pd(OAc)_2$ (0.9 mg, 0.003 mmol) and XPhos (5.5 mg, 0.01 mmol) were added and the resulting light yellow mixture was heated under argon to 110 °C for 3 min. until it became dark brown. This solution was then syringed into a vessel containing naphthoquinone **53** (100 mg, 0.32 mmol), 3,5-dimethylaniline (48 µL, 0.39 mmol) and K_2CO_3 (62 mg, 0.45 mmol) under argon; this vessel was sealed and heated to 110 °C for 16 h. The solution was cooled to RT, diluted with EtOAc (10 mL) and filtered through Celite®. The filtrate was then partitioned between EtOAc (30 mL) and sat. aq. NH_4Cl (20 mL); the organic layer was collected, washed with brine (3 x 20 mL), dried, filtered and concentrated *in vacuo*. Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 80:20) gave 3-anilinonaphthoquinone **60** as a purple solid (104 mg, 82%). mp 198-204 °C; HPLC (method A) t_R 23 min., 95%; υ_{max} (neat) 3303 (N-H), 1667 (C=O), 1595 (C=O); δ_H (400 MHz, $CDCl_3$) 2.16 (6H, s, 2 x Ar-*Me*), 6.53 (1H, s, $H_{4''}$), 6.59 (2H, s, $H_{2''}$ and $H_{6''}$), 7.30-7.36 (2H, m, $H_{3'}$ and $H_{5'}$), 7.42-7.47 (1H, m, $H_{4'}$), 7.50-7.55 (2H, m, $H_{2'}$ and $H_{6'}$), 7.68 (1H, app. td, *J* 7.3, 1.5, H_6 or H_7), 7.74 (1H, app. td, *J* 7.8, 1.3, H_6 or H_7), 7.94 (1H, s, aniline-N*H*), 8.10-8.15 (2H, m, H_5 and H_8), 8.34 (1H, br. s, amide-N*H*); δ_C (100 MHz, $CDCl_3$) 21.1, 115.4, 120.0, 126.0, 126.4, 126.8, 127.3, 128.2, 130.7, 131.7, 131.9, 133.0, 133.5, 134.2, 134.6, 137.0, 137.6, 163.8, 180.1, 182.1; *m/z* (ESI$^-$) 395 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{25}H_{20}N_2NaO_3^+$ ([M+Na]$^+$) requires 419.1366, found 419.1347.

4.1.47. *N*-(3-(3'',5''-Dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phenylacetamide (61)

Following *Representative Procedure 2*, using naphthoquinone **54** (80 mg, 0.25 mmol), 3,5-dimethylaniline (92 µL, 0.74 mmol) and $CeCl_3.7H_2O$ (92 mg, 0.25 mmol) in MeOH (3 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 70:30) and subsequent recrystallisation from boiling toluene gave 3-anilinonaphthoquinone **61** as a purple solid (49 mg, 49%). mp 216-217 °C; HPLC (method A) t_R 24 min., 98%; υ_{max} (neat) 3299 (N-H), 1670 (C=O), 1612 (C=O); δ_H (500 MHz, $CDCl_3$) 2.31 (6H, s, 2 x Ar-*Me*), 3.20 (2H, s, -C*H*$_2$Ph), 6.53 (2H, s, $H_{2''}$ and $H_{6''}$), 6.81 (1H, s, $H_{4''}$), 7.03 (2H, d, *J* 6.9, $H_{2'}$ and $H_{6'}$), 7.28-7.36 (3H, m, $H_{3'}$, $H_{4'}$ and $H_{5'}$), 7.45 (1H, s, amide-N*H*), 7.65 (1H, app. td, *J* 7.5, 1.1, H_6), 7.71 (1H, app. td, *J* 7.5, 1.1, H_7), 7.75 (1H, s, aniline-N*H*), 8.05 (1H, dd, *J* 7.5, 1.1, H_8), 8.08 (1H, dd, *J* 7.5, 1.1, H_5); δ_C (125 MHz, $CDCl_3$) 21.3, 43.1, 114.8, 120.4, 126.2, 126.2, 126.7, 127.5, 129.0, 129.6, 130.5, 131.8, 132.9, 133.8, 134.6, 134.7, 136.9, 137.6, 167.3, 179.9, 182.1; *m/z* (ESI$^-$) 409 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{26}H_{22}N_2NaO_3^+$ ([M+Na]$^+$) requires 433.1523, found 433.1502.

4.1.48. *N*-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hydrocinnanamide (62)

Following *Representative Procedure 2*, using naphthoquinone **55** (100 mg, 0.30 mmol), 3,5-dimethylaniline (110 µL, 0.89 mmol) and $CeCl_3.7H_2O$ (110 mg, 0.30 mmol) in methanol (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 75:25) gave 3-anilinonaphthoquinone **62** as a red solid (67 mg, 54%). mp 186-190 °C; HPLC (method A) t_R 23 min., 95%; υ_{max} (neat) 3305 (N-H), 1669 (C=O), 1596 (C=O); δ_H (500 MHz, $CDCl_3$) 2.19-2.24 (2H, m, -C*H*$_2$CH$_2$Ph), 2.30 (6H, s, 2 x Ar-*Me*), 2.46-2.51 (2H, m, -CH$_2$C*H*$_2$Ph), 6.57 (2H, s, $H_{2''}$ and $H_{6''}$), 6.78 (1H, s, $H_{4''}$), 7.06 (2H, app. d, *J* 7.3, $H_{2'}$ and $H_{6'}$), 7.19 (1H, app. t, *J* 7.3, $H_{4'}$), 7.24-7.29 (2H, m, $H_{3'}$ and $H_{5'}$), 7.60 (1H, s, amide-N*H*), 7.67 (1H, app. td, *J* 7.6, 1.3, H_6 or H_7), 7.73 (1H, app. td, *J* 7.6, 1.3, H_6 or H_7), 7.84 (1H, s, aniline-N*H*), 8.09-8.12 (2H, m, H_5 and H_8); δ_C (125 MHz, $CDCl_3$) 21.3, 30.8, 37.7, 114.9, 120.1, 125.9, 126.2, 126.3, 126.7, 128.1, 128.5, 130.6, 131.8, 133.0, 134.2, 134.6, 136.9, 137.6, 140.6, 168.4, 180.1, 182.1; *m/z* (ESI$^+$) 425 ([M+H]$^+$, 45%), 447 ([M+Na]$^+$, 80%), 871 ([2M+H]$^+$, 100%); HRMS $C_{27}H_{24}N_2NaO_3S^+$ ([M+Na]$^+$) requires 447.1679, found 447.1672.

4.1.49. *N*-(3-((3'',5''-Dimethylphenyl)amino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-1-phenylcyclopropanecarboxamide (63)

Following *Representative Procedure 2,* using naphthoquinone **58** (150 mg, 0.43 mmol), 3,5-dimethylaniline

(160 µL, 1.28 mmol) and $CeCl_3.7H_2O$ (159 mg, 0.43 mmol) in MeOH (5 mL). Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 95:5 to 75:25) gave 3-anilinonaphthoquinone **63** as a red solid (107 mg, 58%). mp 241-242 °C; HPLC (method A) t_R 23 min., >99%; $υ_{max}$ (neat) 3356 (N-H), 1734 (C=O), 1669 (C=O); $δ_H$ (500 MHz, $CDCl_3$) 0.83-0.86 (2H, m, 2 x cyclopropyl-C*H*), 1.07-1.10 (2H, m, 2 x cyclopropyl-C*H*), 2.35 (6H, s, 2 x Ar-*Me*), 6.52 (2H, s, $H_{2''}$ and $H_{6''}$), 6.87 (1H, s, $H_{4''}$), 7.25-7.29 (2H, m, $H_{2'}$ and $H_{6'}$), 7.35-7.38 (1H, m, $H_{4'}$), 7.39-7.44 (2H, m, $H_{3'}$ and $H_{5'}$), 7.46 (1H, s, amide-N*H*), 7.62 (1H, app. td, *J* 7.5, 1.3, H_6), 7.65-7.70 (2H, m, aniline-N*H* and H_7), 8.00 (1H, dd, *J* 7.5, 1.3, H_8), 8.05 (1H, dd, *J* 7.5, 1.3, H_5); $δ_C$ (125 MHz, $CDCl_3$) 16.3, 21.4, 30.4, 115.5, 120.4, 126.0, 126.1, 126.6, 128.3, 129.1, 130.5, 131.0, 131.8, 132.8, 134.3, 134.5, 137.2, 137.6, 139.0, 170.8, 179.9, 182.2; *m/z* (ESI^+) 437 ($[M+H]^+$, 60%), 459 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{28}H_{24}N_2NaO_3^+$ ($[M+Na]^+$) requires 459.1679, found 459.1668.

4.1.50. *N*-(3-(3''-Formylphenyl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzamide (64)

Following *Representative Procedure 6*, using naphthoquinone **53** (100 mg, 0.32 mmol), 3-formylphenylboronic acid (96 mg, 0.64 mmol), $Pd(PPh_3)_2Cl_2$ (22 mg, 0.03 mmol) and sat. aq. $NaHCO_3$ (2.5 mL) in THF (11 mL). Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 85:15) gave 3-arylnaphthoquinone **64** as a yellow-orange solid (19 mg, 15%). mp 63-69 °C; HPLC (method A) t_R 24 min., >99%; $υ_{max}$ (neat) 3307 (N-H), 1694 (C=O), 1663 (C=O); $δ_H$ (500 MHz, $CDCl_3$) 7.41-7.46 (2H, m, $H_{3'}$ and $H_{5'}$), 7.52-7.61 (2H, m, $H_{4'}$ and $H_{5''}$), 7.70-7.74 (2H, m, $H_{2'}$ and $H_{6'}$), 7.74-7.78 (1H, m, $H_{6''}$), 7.78-7.87 (3H, m, H_6, H_7 and $H_{4''}$), 7.94-7.97 (1H, m, $H_{2''}$), 8.18-8.25 (2H, m, H_5 and H_8), 8.62 (1H, s, N*H*), 9.98 (1H, s, C*H*O); $δ_C$ (500 MHz, $CDCl_3$) 126.5, 127.2, 127.6, 128.8, 128.9, 129.7, 130.1, 130.3, 132.1, 132.2, 132.8, 133.2, 133.7, 134.8, 135.1, 135.4, 136.1, 137.9, 163.7, 182.3, 183.0, 191.9; *m/z* (ESI^-) 380 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{24}H_{15}NNaO_4^+$ ($[M+Na]^+$) requires 404.0893, found 404.0890; $λ_{max}$ (pH 8) < 400 nm, $λ_{max}$ (pH 13.75) 485 nm ($ε_{CB}$ 2670 $M^{-1}cm^{-1}$); pK_a 10.9.

4.1.51. *N*-(3-(Furan-3''-yl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzamide (65)

Following *Representative Procedure 6*, using naphthoquinone **54** (100 mg, 0.32 mmol), 3-furanylboronic acid (72 mg, 0.64 mmol), $Pd(PPh_3)_2Cl_2$ (22 mg, 0.3 mmol) and sat. aq. $NaHCO_3$ (2.5 mL) in THF (11 mL). Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 80:20) gave 3-arylnaphthoquinone **65** as a copper brown solid (65 mg, 59%). mp 202-205 °C; HPLC (method A) t_R 24 min., 97%; $υ_{max}$ (neat) 3232 (N-H), 1675 (C=O), 1647 (C=O); $δ_H$ (400 MHz, $CDCl_3$) 6.62-6.65 (1H, m, $H_{5''}$), 7.35-7.38 (1H, m, $H_{4''}$), 7.50-7.55 (2H, m, $H_{3'}$ and $H_{5'}$), 7.59-7.64 (1H, m, $H_{4'}$), 7.75 (1H, app. td, *J* 7.6, 1.3, H_7), 7.79 (1H, app. td, *J* 7.3, 1.6, H_6), 7.90-7.94 (2H, m, $H_{2'}$ and $H_{6'}$), 8.13 (1H, dd, *J* 7.4, 1.1, H_8), 8.19 (1H, dd, *J* 7.6, 1.0, H_5), 8.25 (1H, app. s, $H_{2''}$), 8.66 (1H, br. s, N*H*); $δ_C$ (400 MHz, $CDCl_3$) 109.3, 117.3, 126.3, 127.1, 127.6, 127.8, 129.0, 130.2, 132.6, 132.8, 133.3, 133.6, 134.6, 135.9, 142.5, 145.8, 164.2, 182.1, 183.2; *m/z* (ESI^-) 342 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{21}H_{12}NO_4^-$ ($[M-H]^-$) requires 342.0772, found 342.0782; $λ_{max}$ (pH 8) < 400 nm, $λ_{max}$ (pH 13.75) 513 nm ($ε_{CB}$ 4820 $M^{-1}cm^{-1}$); pK_a 12.1.

4.1.52. *N*-(3-(3''-Formylphenyl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phenylacetamide (66)

Following *Representative Procedure 6*, using naphthoquinone **53** (200 mg, 0.61 mmol), 3-formylphenylboronic acid (184 mg, 1.23 mmol), $Pd(PPh_3)_2Cl_2$ (43 mg, 0.06 mmol) and Na_2CO_3 (194 mg, 1.84 mmol) in toluene (10 mL). Purification *via* column chromatography on silica gel (eluent pet ether:EtOAc 90:10 to 70:30) gave 3-arylnaphthoquinone **66** as a yellow solid (107 mg, 44%). mp 158-162 °C; HPLC (method A) t_R 23 min., >99%; $υ_{max}$ (neat) 3272 (N-H), 1695 (C=O), 1665 (C=O); $δ_H$ (500 MHz, $CDCl_3$) 3.51 (2H, s, -C*H*$_2$Ph), 7.12 (2H, dd, *J* 7.4, 1.7, $H_{2'}$ and $H_{6'}$), 7.31-7.37 (3H, m, $H_{3'}$, $H_{4'}$ and $H_{5'}$), 7.53 (1H, app. t, *J* 7.7, $H_{5''}$), 7.58 (1H, app. dt, *J* 7.7, 1.5, $H_{6''}$), 7.72 (1H, app. t, *J* 1.5, $H_{2''}$), 7.75 (1H, app. td, *J* 7.6, 1.3, H_7), 7.79 (1H, app. td, *J* 7.6, 1.3, H_6), 7.85 (1H, app. dt, *J* 7.7, 1.5, $H_{4''}$), 7.94 (1H, s, N*H*), 8.11 (1H, dd, *J* 7.6, 1.3, H_8), 8.14 (1H, dd, *J* 7.6, 1.3, H_5), 9.93 (1H, s, C*H*O); $δ_C$ (125 MHz, $CDCl_3$) 44.6, 126.4, 127.1, 127.8, 128.6, 129.1, 129.2, 129.3, 130.2, 130.2, 132.1, 133.0, 133.2, 133.8, 134.4, 134.9, 135.6, 136.0, 137.3, 166.9, 182.1, 183.0, 191.9; *m/z* (ESI^+) 111 (30%), 418 ($[M+Na]^+$, 100%), 434 (25%); HRMS (ESI^-) $C_{25}H_{16}NO_4^-$ ($[M-H]^-$) requires 394.1085, found 394.1076; $λ_{max}$ (pH 8) < 400 nm, $λ_{max}$ (pH 13.75) 451 nm ($ε_{CB}$ 8490 $M^{-1}cm^{-1}$); pK_a 11.4.

4.1.53. *N*-(3-(Furan-3''-yl)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phenylacetamide (67)

Following *Representative Procedure 6*, using naphthoquinone **54** (80 mg, 0.25 mmol), 3-furanylboronic acid (55 mg, 0.49 mmol), $Pd(PPh_3)_2Cl_2$ (18 mg, 0.03 mmol) and sat. aq. $NaHCO_3$ (2 mL) in THF (8.8 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 then pet ether:EtOAc 80:20) and recrystallisation from boiling toluene gave 3-arylnaphthoquinone **67** as a copper brown solid (8 mg, 9%). mp 120-124 °C; HPLC (method A) t_R 25 min., 97%; $υ_{max}$(neat) 3306 (N-H), 1665 (br., C=O); $δ_H$ (400 MHz, $CDCl_3$) 3.71 (2H, s, -C*H*$_2$Ph), 6.38-6.41 (1H, m, $H_{5''}$), 7.33-7.47 (6H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$ and $H_{4''}$), 7.69-7.79 (2H, m, H_6 and H_7), 7.86 (1H, s, N*H*), 8.05-8.09 (2H, m, $H_{2''}$ and H_5 or H_8), 8.13 (1H, dd, *J* 7.5, 1.4, H_5 or H_8); $δ_C$ (500 MHz, $CDCl_3$) 44.8, 109.5, 117.0, 126.2, 127.0, 127.9, 128.4, 129.3, 129.5, 130.3, 132.4, 133.5, 133.6, 134.5, 135.6, 142.3, 145.6, 167.6, 181.8, 183.2; *m/z* (ESI^-) 356 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{22}H_{15}NNaO_4^+$ ($[M+Na]^+$) requires 380.0893, found 380.0883; $λ_{max}$ (pH 8) < 400 nm, $λ_{max}$ (pH 13.75) 512 nm ($ε_{CB}$ 2450 $M^{-1}cm^{-1}$); pK_a 12.5.

4.1.54. *N*-(3-Chloro-5-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (70)

Following *Representative Procedure 1*, using 2,3-dichloro-5-nitronaphthalene-1,4-dione **68** (1.00 g, 3.68 mmol), benzenesulfonamide (577 mg, 3.68 mmol) and Cs_2CO_3 (1.68 g, 5.15 mmol) in DMF (15 mL). Purification and separation of regioisomers *via* column chromatography on silica gel (eluent pet ether:acetone 50:50) gave sulfonamide **70** as a yellow solid (556 mg, 38%). mp 220-223 °C; $υ_{max}$ (KBr) 3201 (N-H), 1687 (br., C=O); $δ_H$ (400 MHz, DMSO-d_6) 7.57-7.65 (2H, m, $H_{2'}$ and $H_{6'}$ or $H_{3'}$ and $H_{5'}$), 7.65-7.71 (1H, m, $H_{4'}$), 7.94-7.99 (2H, m, $H_{2'}$ and $H_{6'}$ or $H_{3'}$ and $H_{5'}$), 7.99-8.06 (1H, m, H_7), 8.14-8.20 (2H, m, H_6 and H_8); $δ_C$ (100 MHz, DMSO-d_6) 122.0, 126.7, 128.1, 129.1, 129.3, 131.9, 132.9, 135.7, 142.6, 147.9, 174.7, 177.5; *m/z* (ESI^-) 391 ($[M(^{35}Cl)-H]^-$, 100%); HRMS (ESI^-) $C_{16}H_8ClN_2O_6S^-$ ($[M-H]^-$) requires 390.9797, found 390.9797.

4.1.55. *N*-(3-Chloro-8-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (73)

Following *Representative Procedure 1*, using 2,3-dichloro-5-nitronaphthalene-1,4-dione **68** (1.00 g, 3.68 mmol), benzenesulfonamide (577 mg, 3.68 mmol) and Cs_2CO_3 (1.68 g, 5.15 mmol) in DMF (15 mL). Purification and separation of regioisomers *via* column chromatography on silica gel (eluent pet ether:acetone 50:50) gave sulfonamide **73** as a yellow solid (405 mg, 28%). mp 222-225 °C; $υ_{max}$ (KBr) 3223 (N-H), 1694

(C=O), 1675 (C=O); δ_H (400 MHz, DMSO-d_6) 7.57-7.65 (2H, m, $H_{2'}$ and $H_{6'}$ or $H_{3'}$ and $H_{5'}$), 7.65-7.71 (1H, m, $H_{4'}$), 7.87-7.92 (2H, m, $H_{2'}$ and $H_{6'}$ or $H_{3'}$ and $H_{5'}$), 8.05 (1H, app. t, *J* 8.0, H_6), 8.17 (1H, d, *J* 8.0, H_5 or H_7), 8.26 (1H, d, *J* 8.0, H_5 or H_7); δ_C (100 MHz, DMSO-d_6) 122.3, 126.6, 127.9, 129.1, 129.3, 132.7, 132.9, 135.9, 142.6, 148.1, 175.9, 176.6; *m/z* (ESI$^-$) 391 ([M(^{35}Cl)-H]$^-$, 100%); HRMS (ESI$^-$) $C_{16}H_8ClN_2O_6S^-$ ([M(^{35}Cl)-H]$^-$) requires 390.9797, found 390.9796.

4.1.56. *N*-(3-(3'',5''-Dimethylphenylamino)-5-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (74)

Following *General Procedure 2*, using naphthoquinone **70** (100 mg, 0.26 mmol), 3,5-dimethylaniline (95 μL, 0.77 mmol) and $CeCl_3.7H_2O$ (95 mg, 0.26 mmol) in toluene (10 mL) at 110 °C. Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) and subsequent trituration using hexane gave 3-anilinonaphthoquinone **74** as a purple solid (86 mg, 70%). mp 242-244 °C; HPLC (method B) t_R 9 min., 95%; υ_{max} (KBr) 3272 (N-H), 1687 (C=O), 1595 (C=O); δ_H (500 MHz, DMSO-d_6) 2.24 (6H, s, 2 x Ar-*Me*) 6.60 (2H, s, $H_{2''}$ and $H_{6''}$), 6.69 (1H, s, $H_{4''}$), 7.37-7.43 (2H, m, $H_{3'}$ and $H_{5'}$), 7.49-7.54 (1H, m, $H_{4'}$), 7.60-7.65 (2H, m, $H_{2'}$ and $H_{6'}$), 7.97 (1H, app. t, *J* 7.9, H_7), 8.02 (1H, dd, *J* 7.9, 1.3, H_6 or H_8), 8.08 (1H, dd, *J* 7.9, 1.3, H_6 or H_8), 9.05 (1H, s, N*H*), 9.24 (1H, s, N*H*); δ_C (100 MHz, DMSO-d_6) 21.8, 115.5, 121.7, 123.1, 126.4, 127.4, 127.6, 129.1, 129.4, 133.0, 133.3, 136.6, 137.5, 139.2, 141.8, 144.4, 148.5, 177.5, 180.5; *m/z* (ESI$^-$) 476 ([M-H]$^-$, 100%); HRMS (ESI$^-$) $C_{24}H_{18}N_3O_6S^-$ ([M-H]$^-$) requires 476.0922, found 476.0917; λ_{max} (pH 8) 555 nm (ε_N 5360 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 578 nm (ε_{CB} 4520 $M^{-1}cm^{-1}$); pK_a 8.2.

4.1.57. *N*-(3-(3'',5''-Dimethylphenylamino)-6-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (75)

Following *Representative Procedure 1*, using 2,3-dichloro-6-nitronaphthalene-1,4-dione **69** (1.00 g, 3.68 mmol), benzenesulfonamide (577 mg, 3.68 mmol) and Cs_2CO_3 (1.68 g, 5.15 mmol) in DMF (15 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 95:5 to 80:20) gave a mixture of regioisomeric 6-nitro and 7-nitro products, **71** and **72** (788 mg, 55%). Subsequently, following *Representative Procedure 2*, using this regioisomeric mixture (788 mg, 2.01 mmol), 3,5-dimethylaniline (751 μL, 6.02 mmol) and $CeCl_3.7H_2O$ (748 mg, 2.01 mmol) in MeOH (5 mL). Purification and separation of regioisomers *via* column chromatography (eluent pet ether:EtOAc 95:5 to 85:15) gave 3-anilinonaphthoquinone **75** as a purple solid (115 mg, 12% over 2 steps). mp 250-253 °C; HPLC (method A) t_R 23 min., >99%; υ_{max} (neat) 3460 (N-H), 1653 (br., C=O); δ_H (500 MHz, $CDCl_3$) 2.27 (6H, s, 2 x Ar-*Me*), 6.63 (2H, s, $H_{2''}$ and $H_{6''}$), 6.67 (1H, s, N*H*), 6.81 (1H, s, $H_{4''}$), 7.26-7.32 (2H, m, $H_{3'}$ and $H_{5'}$), 7.39-7.46 (1H, m, $H_{4'}$), 7.58-7.62 (2H, m, $H_{2'}$ and $H_{6'}$), 8.03 (1H, d, *J* 8.4, H_8), 8.06 (1H, s, N*H*), 8.42 (1H, dd, *J* 8.4, 2.3, H_7), 8.80 (1H, d, *J* 2.3, H_5); δ_C (125 MHz, $CDCl_3$) 20.3, 113.1, 120.4, 121.0, 126.3, 126.8, 127.1, 127.8, 127.9, 130.5, 132.2, 134.3, 135.7, 137.2, 137.6, 138.6, 149.5, 175.9, 179.1; *m/z* (ESI$^-$) 476 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{24}H_{19}N_3NaO_6S$ ([M+Na]$^+$) requires 500.0887, found 500.0876; λ_{max} (pH 8) 527 nm (ε_N 4500 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 600 nm (ε_{CB} 2240 $M^{-1}cm^{-1}$); pK_a 7.5.

4.1.58. *N*-(3-(3'',5''-Dimethylphenylamino)-7-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (76)

Following an identical procedure to the synthesis of **75** above, the other regioisomeric product, 3-anilinonaphthoquinone **76**, was obtained by column chromatography as a purple solid (121 mg, 13% over 2 steps). mp 253-256 °C; HPLC (method A) t_R 24 min., 97%; υ_{max} (neat) 3390 (N-H), 1656 (br., C=O); δ_H (500 MHz, DMSO-d_6) 2.23 (6H, s, 2 x Ar-*Me*), 6.63 (2H, s, $H_{2''}$ and $H_{6''}$), 6.70 (1H, s, $H_{4''}$), 7.36-7.41 (2H, m, $H_{3'}$ and $H_{5'}$), 7.49-7.54 (1H, m, $H_{4'}$), 7.57 (2H, dd, *J* 8.4, 1.1, $H_{2'}$ and $H_{6'}$), 8.25 (1H, d, *J* 8.5, H_5), 8.40 (1H, d, *J* 2.2, H_8), 8.53 (1H, dd, *J* 8.5, 2.2, H_6), 9.05 (1H, s, N*H*), 9.18 (1H, br. s, N*H*); δ_C (125 MHz, DMSO-d_6) 21.0, 114.2, 120.0, 121.1, 125.6, 126.4, 127.2, 128.2, 128.4, 131.9, 132.8, 134.4, 136.4, 138.0, 141.4, 142.7, 150.9, 176.7, 181.0; *m/z* (ESI$^-$) 476 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{24}H_{19}N_3NaO_6S$ ([M+Na]$^+$) requires 500.0887, found 500.0863; λ_{max} (pH 8) 529 nm (ε_N 7720 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 616 nm (ε_{CB} 3880 $M^{-1}cm^{-1}$); pK_a 8.0.

4.1.59. *N*-(3-(3'',5''-Dimethylphenylamino)-8-nitro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (77)

Following *Representative Procedure 2*, using naphthoquinone **73** (60 mg, 0.15 mmol), 3,5-dimethylaniline (57 μL, 0.46 mmol) and $CeCl_3.7H_2O$ (58 mg, 0.15 mmol) in toluene (10 mL) at 110 °C. Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) and subsequent recrystallisation from toluene gave 3-anilinonaphthoquinone **77** as a purple solid (8 mg, 11%). mp 218-220 °C (toluene); HPLC (method B) t_R 9 min., 99%; υ_{max} (KBr) 3298 (N-H), 3219 (N-H), 1678 (C=O), 1641 (C=O); δ_H (400 MHz, DMSO-d_6) 2.22 (6H, s, 2 x Ar-*Me*), 6.60 (2H, s, $H_{2''}$ and $H_{6''}$), 6.68 (1H, s, $H_{4''}$), 7.32-7.40 (2H, m, $H_{3'}$ and $H_{5'}$), 7.46-7.55 (3H, m, $H_{2'}$, $H_{4'}$ and $H_{6'}$), 7.94 (1H, app. t, *J* 7.9, H_6), 8.05 (1H, dd, *J* 7.9, 1.0, H_5 or H_7), 8.22 (1H, dd, *J* 7.9, 1.0, H_5 or H_7), 9.07 (1H, s, N*H*), 9.08 (1H, s, N*H*); δ_C (100 MHz, DMSO-d_6) 21.8, 114.1, 122.2, 123.0, 126.6, 127.2, 129.0, 129.3, 129.4, 132.4, 132.8, 135.0, 137.2, 138.6, 141.8, 143.4, 148.6, 176.2, 181.6; *m/z* (ESI$^-$) 476 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{24}H_{19}N_3NaO_6S^+$ ([M+Na]$^+$) requires 500.0887, found 500.0882; λ_{max} (pH 8) 515 nm (ε_N 5500 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 573 nm (ε_{CB} 2740 $M^{-1}cm^{-1}$); pK_a 8.6.

4.1.60. *N*-(8-Nitro-1,4-dioxo-3-(phenylamino)-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (78)

Following *Representative Procedure 2*, using naphthoquinone **73** (100 mg, 0.26 mmol), aniline (70 μL, 0.77 mmol) and $CeCl_3.7H_2O$ (95 mg, 0.26 mmol) in toluene (10 mL) at 110 °C. Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25) and subsequent recrystallisation from toluene gave 3-anilinonaphthoquinone **78** as a purple solid (30 mg, 26%). mp 231-233 °C (toluene); HPLC (method A) t_R 24 min., 98%; υ_{max} (KBr) 3330 (N-H), 1673 (C=O), 1649 (C=O); δ_H (500 MHz, DMSO-d_6) 7.03-7.12 (3H, m, $H_{2''}$, $H_{4''}$ and $H_{6''}$), 7.22-7.29 (2H, m, $H_{3''}$ and $H_{5''}$), 7.34-7.41 (2H, m, $H_{3'}$ and $H_{5'}$), 7.48-7.57 (3H, m, H_2, $H_{4'}$ and $H_{6'}$), 7.91-7.96 (1H, m, H_6), 8.03 (1H, dd, *J* 7.8, 1.0, H_7), 8.23 (1H, dd, *J* 7.8, 1.0, H_5), 9.04 (1H, s, sulfonamide-N*H*), 9.28 (1H, s, aniline-N*H*); δ_C (125 MHz, DMSO-d_6) 113.1, 122.0, 123.9, 124.1, 126.5, 127.4, 128.1, 128.5, 128.5, 131.6, 132.2, 134.2, 138.1, 140.8, 142.6, 147.7, 175.0, 180.7; *m/z* (ESI$^-$) 448 ([M-H]$^-$, 100%); HRMS (ESI$^-$) $C_{22}H_{14}N_3O_6S^-$ ([M-H]$^-$) requires 448.0609, found 448.0591; λ_{max} (pH 8) 504 nm (ε_N 3510 $M^{-1}cm^{-1}$), λ_{max} (pH 13.75) 578 nm (ε_{CB} 3010 $M^{-1}cm^{-1}$); pK_a 8.4.

4.1.61. *N*-(5-Amino-3-(3'',5''-dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (79)

Following *Representative Procedure 7*, using naphthoquinone **74** (22 mg, 0.05 mmol) and 10% Pd/C (1.0 mg, 0.01 mmol) in MeOH (2 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 90:10 to 75:25) gave 5-aminonaphthoquinone **79** as an orange solid (20mg, quant.). mp 232-234 °C; HPLC (method B) t_R 9 min., 95%; υ_{max} (KBr) 3464 (N-H), 3300 (N-H), 1573 (br., C=O); δ_H (400 Hz, DMSO-d_6) 2.21 (6H, s, 2 x Ar-*Me*), 6.58 (2H, s, $H_{2''}$ and $H_{6''}$), 6.64 (1H, s, $H_{4''}$), 7.01-7.08 (2H, m, H_6 and H_8), 7.32-7.43 (3H, m, H_7, $H_{3'}$ and $H_{5'}$), 7.46-7.52 (3H, m, $H_{2'}$, $H_{4'}$ and $H_{6'}$), 7.88 (2H, br. s, N*H*₂), 8.60 (1H, s, aniline-N*H*), 8.86 (1H, s, sulfonamide-N*H*); δ_C (100 MHz, DMSO-d_6) 21.9, 109.0, 113.3, 115.9, 121.7, 123.0, 126.0, 127.2, 129.2, 132.6, 133.3. 136.0, 137.2, 138.8, 142.2, 143.1, 152.5, 179.6, 182.9; *m/z* (ESI$^+$) 470 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{21}N_3NaO_4S^+$, ([M+H]$^+$) requires 448.1326; found 448.1326; λ_{max} (pH 8) 474 nm (ε_N 22420 M^{-1}cm^{-1}), λ_{max} (pH 13.75) 490 nm (ε_{CB} 15800 M^{-1}cm^{-1}).

4.1.62. *N*-(6-Amino-3-(3'',5''-dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (80)

Following *Representative Procedure 7*, using naphthoquinone **75** (26 mg, 0.06 mmol) and 10% Pd/C (1.2 mg, 0.01 mmol) in MeOH (2 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) gave 6-aminonaphthoquinone **80** as a brown solid (24 mg, quant.). mp 282-288 °C; HPLC (method A) t_R 24 min., >99%; υ_{max} (neat) 3475 (N-H), 3372 (N-H), 1589 (br., C=O); δ_H (500 MHz, DMSO-d_6) 2.21 (6H, s, 2 x Ar-*Me*), 6.36 (2H, s, N*H*₂), 6.51 (2H, s, $H_{2''}$ and $H_{6''}$), 6.59 (1H, s, $H_{4''}$), 6.81 (1H, dd, *J* 8.5, 2.5, H_7), 7.12 (1H, d, *J* 2.5, H_5), 7.34-7.39 (2H, m, $H_{3'}$ and $H_{5'}$), 7.47-7.55 (4H, m, H_8, $H_{2'}$, $H_{4'}$ and $H_{6'}$), 8.32 (1H, s, aniline-N*H*), 8.94 (1H, br. s, sulfonamide-N*H*); δ_C (125 MHz, DMSO-d_6) 21.0, 109.7, 115.5, 117.8, 119.3, 119.8, 124.3, 126.3, 128.1, 128.3, 131.9, 132.0, 136.4, 138.7, 140.0, 141.2, 153.4, 178.2, 183.2; *m/z* (ESI$^+$) 470 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{21}N_3NaO_4S^+$ ([M+Na]$^+$) requires 470.1145, found 470.1142; λ_{max} (pH 8) 398 nm (ε_N 9830 M^{-1}cm^{-1}), λ_{max} (pH 13.75) 389 nm (ε_{CB} 12540 M^{-1}cm^{-1}).

4.1.63. *N*-(7-Amino-3-(3'',5''-dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (81)

Following *Representative Procedure 7*, using naphthoquinone **76** (40 mg, 0.08 mmol) and 10% Pd/C (1.8 mg, 0.02 mmol) in MeOH (2 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 85:15) gave 7-aminonaphthoquinone **81** as an orange solid (29 mg, 77%). mp 255-258 °C; HPLC (method A) t_R 24 min., 97%; υ_{max} (neat) 3364 (N-H), 1579 (br., C=O); δ_H (500 MHz, DMSO-d_6) 2.22 (6H, s, 2 x Ar-*Me*), 6.58 (2H, s, $H_{2''}$ and $H_{6''}$), 6.64 (1H, s, $H_{4''}$), 6.71 (2H, s, N*H*₂), 6.74 (1H, dd, *J* 8.5, 2.2, H_6), 6.96 (1H, d, *J* 2.2, H_8), 7.33-7.39 (2H, m, $H_{3'}$ and $H_{5'}$), 7.46-7.53 (3H, m, H_2, $H_{4'}$ and $H_{6'}$), 7.73 (1H, d, *J* 8.5, H_5), 8.60 (1H, s, aniline-N*H*), 8.80 (1H, s, sulfonamide-N*H*); δ_C (125 MHz, DMSO-d_6) 21.0, 109.8, 111.8, 115.7, 117.6, 121.0, 125.2, 126.3, 128.3, 129.4, 131.8, 134.2, 136.3, 137.9, 141.4, 142.2, 155.4, 179.1, 179.3; *m/z* (ESI$^-$) 446 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{24}H_{21}N_3NaO_4S^+$ ([M+Na]$^+$) requires 470.1145, found 470.1139; λ_{max} (pH 8) 410 nm (ε_N 37310 M^{-1}cm^{-1}), λ_{max} (pH 13.75) 420 nm (ε_{CB} 21890 M^{-1}cm^{-1}).

4.1.64. *N*-(8-Amino-3-(3'',5''-dimethylphenylamino)-1,4-dioxo-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (82)

Following *Representative Procedure 7*, using naphthoquinone **77** (60 mg, 0.13 mmol) and 10% Pd/C (2.7 mg, 0.03 mmol) in MeOH (2 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 75:25 to 60:40) gave 8-aminonaphthoquinone **82** as a magenta solid (56 mg, quant.). mp 223-225 °C; HPLC (method B) t_R 9 min., 95%; υ_{max} (KBr) 3337 (N-H), 3295 (N-H), 1661 (C=O), 1618 (C=O); δ_H (400 MHz, DMSO-d_6) 2.20 (6H, s, 2 x Ar-*Me*), 6.50 (2H, s, $H_{2''}$ and $H_{6''}$), 6.59 (1H, s, $H_{4''}$), 7.09 (1H, d, *J* 8.4, H_7), 7.25 (1H, d, *J* 7.0, H_5), 7.33-7.42 (5H, m, H_6, $H_{3'}$, $H_{5'}$ and N*H*₂), 7.47-7.55 (3H, m, $H_{2'}$, $H_{4'}$ and $H_{6'}$), 8.35 (1H, s, aniline-N*H*), 8.96 (1H, s, sulfonamide-N*H*); δ_C (100 MHz, DMSO-d_6) 21.9, 110.1, 116.4, 116.5, 120.9, 125.2, 125.4, 127.2, 129.2, 132.2, 132.8, 134.1, 137.2, 139.4, 140.8, 141.9, 151.3, 182.7, 183.4; *m/z* (ESI$^-$) 470 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{21}N_3NaO_4S^+$ ([M+Na]$^+$) requires 470.1145, found 470.1147; λ_{max} (pH 8) 542 nm (ε_N 15090 M^{-1}cm^{-1}), λ_{max} (pH 13.75) 479 nm (ε_{CB} 12380 M^{-1}cm^{-1}); pK_a 8.4.

4.1.65. *N*-(8-Amino-1,4-dioxo-3-(phenylamino)-1,4-dihydronaphthalen-2-yl)benzenesulfonamide (83)

Following *Representative Procedure 7*, using naphthoquinone **78** (25 mg, 0.06 mmol) and 10% Pd/C (1.2 mg, 0.01 mmol) in MeOH (2 mL). Purification *via* column chromatography on silica gel (eluent pet ether:acetone 80:20) gave 8-aminonaphthoquinone **83** as a magenta solid (22 mg, quant.). mp 242-244 °C; HPLC (method A) t_R 25 min., >99%; υ_{max} (neat) 3459 (N-H), 3335 (N-H), 1618 (br., C=O); δ_H (500 MHz, DMSO-d_6) 6.93-6.97 (2H, m, $H_{2''}$ and $H_{6''}$), 6.98-7.02 (1H, m, $H_{4''}$), 7.08 (1H, dd, *J* 8.5, 1.1, H_7), 7.18-7.23 (2H, m, $H_{3''}$ and $H_{5''}$), 7.26 (1H, dd, *J* 7.3, 1.1, H_5), 7.36-7.40 (3H, m, H_6, $H_{3'}$ and $H_{5'}$), 7.48-7.52 (1H, m, $H_{4'}$), 7.54-7.58 (2H, m, $H_{2'}$ and $H_{6'}$), 8.57 (1H, s, N*H*), 8.90 (1H, s, N*H*); δ_C (125 MHz, DMSO-d_6) 109.2, 115.1, 115.6, 122.5, 122.7, 124.2, 126.5, 127.5, 128.5, 131.4, 132.1, 133.2, 138.9, 140.0, 140.9, 150.3, 181.6, 182.5; *m/z* (ESI$^+$) 442 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{22}H_{17}N_3NaO_4S^+$ ([M+Na]$^+$) requires 442.0832, found 442.0821; λ_{max} (pH 8) 514 nm (ε_N 16660 M^{-1}cm^{-1}), λ_{max} (pH 13.75) 486 nm (ε_{CB} 15840 M^{-1}cm^{-1}); pK_a 8.2.

4.2 Biology

4.2.1. General Experimental

Molecular biology reagents and competent *E. coli* cells were purchased from Promega (Southampton, UK).

4.2.2. Expression and Purification of mNat2, hNAT1 and shNat2

Recombinant mNat2 with an *N*-terminal hexa-His tag was expressed from *E. coli* Rosetta®(-DE3)pLysS cells containing pET28b(+), into which the *mNat2* open-reading frame had been sub-cloned.[39] The protein was purified *via* Ni-NTA affinity chromatography (Qiagen) and thrombin cleavage of the His tag, as previously described.[22] Both pure recombinant hNAT1[28, 40] and shNat2[32] were produced as previously described.

The concentrations of solutions containing purified proteins were determined by measuring their absorption at 280 nm and using the molar extinction coefficients calculated for each enzyme. The purified NATs were stored at -80 °C in 20 mM Tris.HCl (pH 8.0) buffer solution containing 5 mM dithiothreitol and 5% (v/v) glycerol at the following concentrations: mNat2 8 mg/mL; hNAT1 1.5 mg/mL; shNat2 20 mg/mL. These solutions were diluted as required on the day of use.

4.2.3. ZR-75-1 Breast Cancer Cell Line Studies

4.2.3.1. Growth of ZR-75-1 Cells. Cells from the human breast cancer cell line ZR-75-1, which had previously been stored in liquid N_2, were grown in RMPI 1640 L-glutamine-containing growth medium, with 10% (v/v) foetal calf serum and 1% (v/v) Pen/Strep/Glutamine added, at 37 °C in a 5% CO_2 environment. Cell growth was monitored at regular intervals and once almost confluent (every 5-7 days) the cells were split in a 1:2 fashion, using Trypsin-EDTA solution (0.25% (w/v)) to detach the cell monolayer from the culture flask. After 5 passages, the cells were harvested to prepare a lysate, by resuspending the cell pellet in complete lysis buffer (100 µL frozen protease inhibitors and

10 µL 100 mM dithiothreitol added per 900 µL of lysis buffer (20 mM Tris, 20 mM NaCl, 0.5% (w/v) Nonidet P40 containing one EDTA-free Complete Protease Inhibitor per 2.5 mL)) to a final cell concentration of 5 x 10^7 cells/mL. Cell debris was removed by centrifugation (10 min., 4 °C, 14000 rpm, microfuge) and the supernatant retained. The cell lysate was kept on ice and all studies with inhibitors were carried out on the same day.

4.2.3.2. Quantification of hNAT1 in ZR-75-1 Cell Extracts by Western and Dot Blotting. For Western Blotting, ZR-75-1 cell extract samples were run on a 12% SDS-PAGE gel, using 8 µL sample and 2 µL reduced gel loading buffer (10 mM Tris.HCl (pH 6.8), 8 M urea, 2% (w/v) SDS, 5 mg/mL DTT) per well. Following transfer to PVDF micro-porous membranes in a suitable buffer (48 mM Tris.HCl (pH 8.3), 39 mM glycine, 20% MeOH) for 4 h using a semi-dry blotter, membranes were blocked with 10% milk in Tris-Buffered Saline Tween-20 (9 g/L NaCl, 6 g/L Tris.HCl, 0.05% (v/v) Tween-20), washed thrice with 3% milk and incubated with the polyclonal antibody 195[25] at a dilution of 1:2000 for 1 h. Membranes were subsequently incubated with horseradish peroxidise-conjugated mouse anti-rabbit IgG at a dilution of 1:10000 for 1 h as a secondary antibody. Bound mNat2 and hNAT1 were detected using a chemiluminescent kit (ECL Reagent Kit, Amersham). For Dot Blotting, the samples (200 µL/well containing 100 µL of recombinant mNat2 or ZR-75-1 cell lysate) were directly transferred onto a PVDF membrane using the Bio-Dot® Microfiltration Apparatus and a procedure similar to Western Blotting was used to detect the relevant protein.

4.2.4. Acetyl CoA Hydrolysis Assay

The rate of production of free thiol Coenzyme A (CoA-SH) by NATs in the presence of both an arylamine and AcCoA was measured in end-point assays using Ellman's reagent, 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), as previously described.[29] The final NAT concentration in each assay well was 1.5 µg/mL.

For a preliminary test of inhibitory potency of a compound at 30 µM, assays were conducted in triplicate and the specific NAT activity was calculated from a graph of the OD_{405}, measured on a plate reader (Tecan Sunrise), against time. The % retained specific activity was calculated against a control assay without inhibitor. In IC_{50} tests, a range of ten concentrations was selected for each compound, most often beginning at 10, 30 or 50 µM and diluting by a factor of two in each subsequent well. Assays were conducted in duplicate and % specific activity for each assay was determined relative to a control without inhibitor. KyPlot® software was used to determine the IC_{50} value from a plot of % retained specific activity against inhibitor concentration using the IC50%FUN regression model.

4.2.5. Colorimetric Evaluation of Inhibitors

4.2.5.1. Spectrophotometry. Visible spectra of each compound were recorded using a U-2001 spectrophotometer (Hitachi) and 50 µL UVettes® (Eppendorf). The compounds were tested at 15 µM in buffer solutions containing 5% (v/v) DMSO at pH 8 (20 mM Tris.HCl), pH 13.75 (4 M NaOH) and, where relevant, in the presence of mNat2 and/or hNAT1 (30 µM in 20 mM Tris.HCl). All spectra were blank-corrected and normalised.

4.2.5.2. Determination of pK_a Values. Full absorbance spectra of selected compounds at 100 µM in 14 appropriately buffered solutions containing 5% (v/v) DMSO in a flat-bottomed 96-well plate (Corning) were recorded on a plate reader (Omega). Recorded spectra were blank-corrected and normalised before λ_{max} was determined. A graph of λ_{max} against pH was then plotted for each inhibitor using GraphPad® software. A sigmoidal dose-response (variable slope) regression model was used to determine the pK_a by the method of least squares.

4.2.5.3. Determination of Absorption Coefficients. Full absorbance spectra of selected compounds at 200 µM, 100 µM, 50 µM and 25 µM in buffer solutions at pH 8 or 13 containing 5% (v/v) DMSO in a 96-well flat-bottomed plate (Corning) were recorded on a plate reader (Omega). Spectra were blank-corrected and normalised and experiments performed in duplicate. A plot of absorbance at λ_{max} against concentration then yielded the absorption coefficient, at either pH 8 (ε_N) or pH 13 (ε_{CB}), as the gradient divided by the path length.

4.2.6. Docking Studies

All images showing protein structures were generated using the software PyMOL (W. L. DeLano (2002) PyMOL, DeLano Scientific, San Carlos, CA). Prior to docking a ligand into the hNAT1 active site (pdb: 2PQT)[12e], the ground state conformation of the ligand was predicted using the molecular editor Avogadro,[41] and the protein was protonated to be consistent with the assay conditions of pH 8. The docking simulations and the analysis of the possible interactions between the protein and the ligand were performed using GOLD®.[30] The docking site was defined as a region of 10 Å within the active pocket of the enzyme and the generated solutions were ranked using the GOLD® Score Fitness function.[30] Each docking simulation was repeated ten times to ensure the observed solutions were consistent.

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