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Discovery of 5-{2-[5-Chloro-2-(5-ethoxyquinoline-8-sulfonamido)phenyl]ethynyl}-4-methoxypyridine-2-carboxylic Acid, a Highly Selective in Vivo Useable Chemical Probe to Dissect MCT4 Biology

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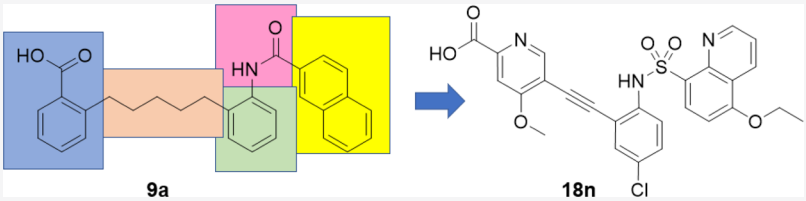

ABSTRACT: Due to increased lactate production during glucose metabolism, tumor cells heavily rely on efficient lactate transport to avoid intracellular lactate accumulation and acidification. Monocarboxylate transporter 4 (MCT4/SLC16A3) is a lactate transporter that plays a central role in tumor pH modulation. The discovery and optimization of a novel class of MCT4 inhibitors (hit **9a**), identified by a cellular screening in MDA-MB-231, is described. Direct target interaction of the optimized compound **18n** with the cytosolic domain of MCT4 was shown after solubilization of the GFP-tagged transporter by fluorescence cross-correlation spectroscopy and microscopic studies. In vitro treatment with **18n** resulted in lactate efflux inhibition and reduction of cellular viability in MCT4 high expressing cells. Moreover, pharmacokinetic properties of **18n** allowed assessment of lactate modulation and antitumor activity in a mouse tumor model. Thus, **18n** represents a valuable tool for investigating selective MCT4 inhibition and its effect on tumor biology.

INTRODUCTION

Enhanced glucose catabolism, a well-described feature of growing and dividing cancer cells often referred to as the Warburg effect, leads to the accumulation of metabolites in the tumor microenvironment. Among these metabolites, lactic acid is emerging as a key metabolic modulator responsible for different cancer cell hallmarks including sustained angiogenesis, evasion of immune surveillance, and reprogramming of energy metabolism.[1] Due to the high consumption of glucose, large quantities of lactic acid are transported out of tumor cells and this process is mediated by monocarboxylate transporters (MCTs). In mammals, a family of MCT proteins containing at least 14 members has been described so far.[2] These transporters also recognize other substrates, such as short-chain fatty acids and pyruvate and ketone bodies, but lactate is the most abundant one with up to 40 mM concentration in tumors.[3] Although many MCTs are still subject to deorphanization,[4] the physiological role for MCTs 1−4 is well characterized.[5] Overexpressions of MCT1 and MCT4 are markers for poor prognosis of several cancer indications, therefore they have been discussed as potential targets in cancer therapy.[2,6]

Several compounds have been described to non-specifically inhibit MCTs (Figure 1). 4′-Diisothiocyanatostilben-2,2′-disulfonate irreversibly binds to a lysine residue on MCT1 and MCT2 but not on MCT4, thus inactivating MCT1 and MCT2.[7] Organomercurial compounds, such as *p*-chloromercuribenzenesulfonate (*p*-CMBS), disrupt MCT interaction

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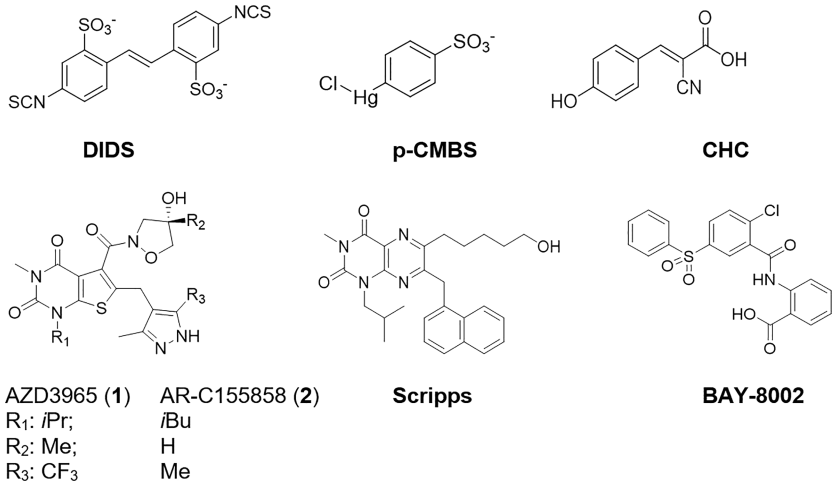

Figure 1. Selected structures of known MCT inhibitor tool compounds (upper panel) and more drug-like MCT1 inhibitors (lower panel).

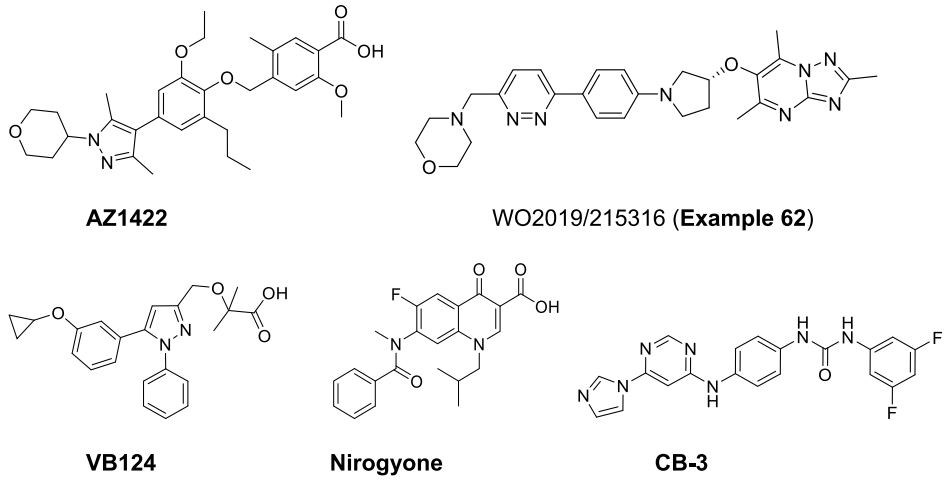

Figure 2. Selected structures of known MCT4 inhibitors.

with chaperone CD147 interfering with MCT1, MCT3, and MCT4 expression and activity, but not with MCT2.[8] CHC (α-cyano-4-hydroxy cinnamate) was described with 10-fold selectivity for MCT1 compared to other MCTs.[9] Special α-cyano-4-amino-2-methoxy cinnamates have been published as dual MCT1 and MCT4 inhibitors based on their activity in RBE4 and MDA-MB-231 cell lines.[10] Besides the mentioned dual amino-methoxy-cinnamate-based compounds, the reserpine-derived antihypertensive drug syrosingopine was published as the dual MCT1 and MCT4 inhibitor (with 60-fold higher potency on MCT4 compared to MCT1).[11] In 2019, the non-steroidal anti-inflammatory drug diclofenac was reported to inhibit MCT1 and MCT4.[12] These compounds have not yet been investigated clinically for disrupting MCT functions in cancer possibly due to polypharmacology issues.[13−15]

Currently, only a few MCT1 inhibitors (with some activity against MCT2) have been described with candidate-stage drug-like properties, such as AZD3965 (**1**) and AR-C155858 (**2**).[16] **1** was shown to inhibit lactic acid transport and cell growth in vitro and in vivo in cancer cells that preferentially express MCT1. Blocking of lactate transport led to a reduction of the glycolytic rate and to intracellular acidification resulting in the inhibition of cell proliferation or survival.[17] **1** is six times more selective for MCT1 than for MCT2 and does not inhibit MCT4. Pteridine dione compounds (**Scripps**) were described with nanomolar viability activity in the highly MCT1 expressing Raji (Burkitt's) lymphoma cell line.[18] Recently, the carboxylic acid **BAY-8002** was reported as a novel selective MCT1 inhibitor, with a 6-fold selectivity for MCT1 compared to MCT2, no activity on MCT4, and no off-target effects.[19] So far, only **1** is reported in clinical trial (NCT01791595).

In 2015, a first selective MCT4 inhibitor (AZ93) was mentioned but not disclosed.[20] Other carboxylic acids, derived from CHC, were published in 2016 as selective MCT4 inhibitors, which inhibit lactate transport in cell lines that express high levels of MCT4, such as MDA-MB-453, MDA-MB-231, or NCI-H358, but not in cells that express high levels of MCT1, such as BT20.[21] In conference reports, the carboxylic acid **AZ1422** was described as a selective MCT4 inhibitor.[22] Similar results were reported for compound **Example 62** with antitumoral activity in combination with the VEGFR inhibitor Cediranib, αCTLA4, and αPD-1 antibodies in the MC38 MCT1 KO syngeneic cell line model.[23] Phenex Pharmaceuticals compared their MCT4-selective inhibitor PX-788 of unknown structure to an undisclosed AZ MCT4 inhibitor and reported moderate monotherapy effects on tumor growth by both compounds in the NCI-H358 xenograft model.[24] Drew University reported another non-carboxylic compound **CB-3** that exhibited in vivo monotherapy effects in the MDA-MB-231 xenograft model,

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but the overall selectivity of this compound was not yet published.[25] Pyrazole-(**VB124**)[26] and quinolinone-(**Nirogyone**)-based carboxylic acids were described to inhibit MCT4-dependent lactate transport and viability using SiHa cells and claimed to interfere in lactate consumption.[27] Representative compounds are depicted in Figure 2.

This paper describes the identification and optimization of a new class of selective MCT4 inhibitors leading to compound **18n** with chemical probe characteristics.[28]

RESULTS AND DISCUSSION

Design of MCT4-Selective Inhibitors. An analysis of a panel of human cell lines for expression of MCTs yielded MDA-MB 231, NCI-H358, and NCI-H441, among others, as cells with high MCT4 and no/low MCT1 expression. The MDA-MB-231 model does not express MCT1 at all, as result of MCT1 gene promoter hypermethylation, and will henceforth be referred to as the MCT1 null cell line.[29] In a cellular screening approach on lactate efflux in MDA-MB-231 cells, compound **9a** was identified as one initial hit with submicromolar activity.[30] This compound has been published earlier in the context of hyperglycemia without a description of the mode of action.[31] The submicromolar activity in the lactate efflux assay in MDA-MB-231 prompted initial activity optimization, followed by property adjustments. For optimization attempts, different areas of the hit were considered separately (Figure 3).

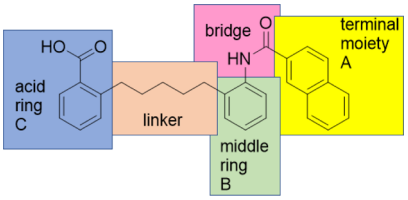
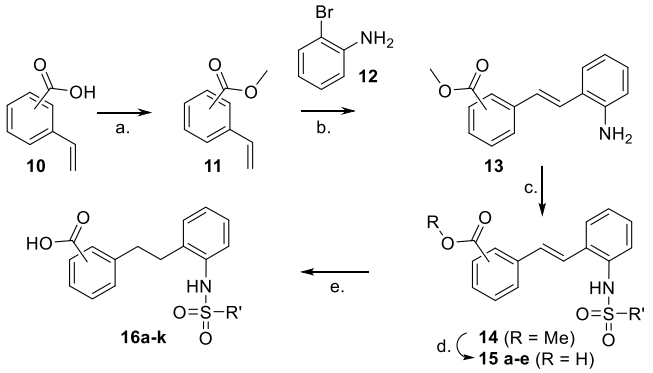


Figure 3. Screening hit **9a** and molecular sections: acid or isostere bearing ring C, linking moiety, middle ring B, oxidized bridge, and terminal moiety A.

Chemistry. The syntheses of the different scaffolds and decorations is described in the following paragraphs. Access to derivatives with elongated saturated linker moieties followed the strategy outlined in Scheme 1.

Iodo-carboxylate **3** was coupled under Sonogashira conditions with alkynol **4** to intermediate **5**, which was first hydrogenated to the saturated analog **6a**, and then converted into the corresponding triphenyl phosphine **6c** via the bromide **6b**. After Wittig condensation with nitro-benzaldehyde **7**, comprehensive reduction of the nitro group and the double bond in one step generated the amino building block **8**, which was used for amide couplings under standard conditions or sulfonamide synthesis via the corresponding sulfonylchloride, followed by ester saponification giving the target compounds **9a−m** and **10**. The preparation of compounds with shorter linking moieties is exemplified in Scheme 2.

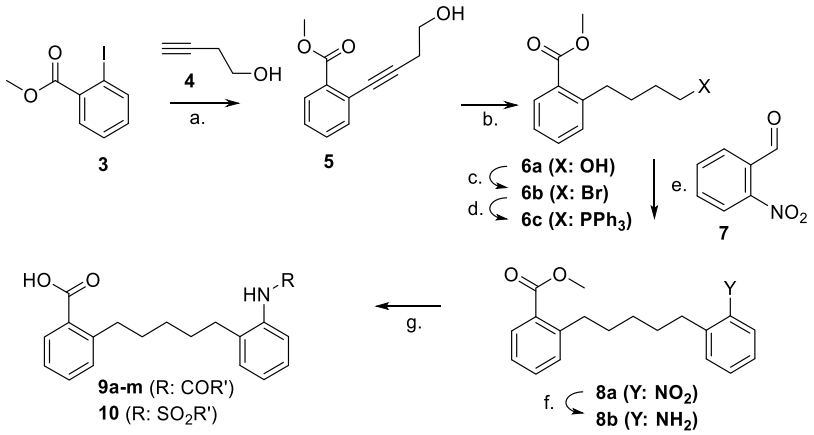
Scheme 2. Synthesis of Ethyl- and Ethenyl-Linked Sulfonamides Described in Tables 2−4[a]



[a]Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C—RT, 82%; (b) $Pd(Ac)_2$, P(*o*-tolyl)$_3$, TEA, 125 °C, 75%; (c) sulfonyl chloride, TEA, DCM; (d) NaOH, MeOH, 65−85%; and (e) i. Pd/C, H2, MeOH, 95%.

Acid **10** was converted into the corresponding ester **11** and coupled either under Heck or Herrmann−Beller's phosphapalladacycle conditions with bromo-aniline **12** to building block **13**.[32] After sulfonamide formation (**14**), either the ester was saponified (**15**) or the double bond was hydrogenated concomitant with ester saponification, giving the free acid **16**

Scheme 1. Synthesis of Pentyl-Linked Carboxamides Described in Table 1 and Sulfonamide in Table 2[a]



[a]Reagents and conditions: (a) CuI, $Pd(PPh_3)_2Cl_2$, diethylamine, RT, 90%; (b) Noblyst P1086, THF, RT, 73%; (c) PPh_3, NBS, THF, 0 °C—RT, 62%; (d) PPh_3, AcCN, 82%, 65 °C; (e) 7, K_2CO_3, dioxane/water, reflux, 93%; (f) Noblyst P1086, H_2, THF, RT, 92%; and (g) i. acid chloride, TEA, DCM, RT (48−92%); ii. NaOH, MeOH, RT (27−89%).

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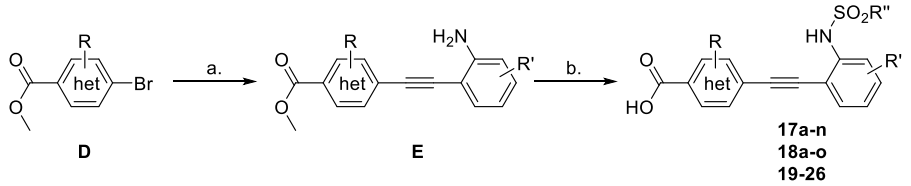
Scheme 3. Synthesis of Ethynyl-Linked Sulfonamides Described in Tables 5−7[a]

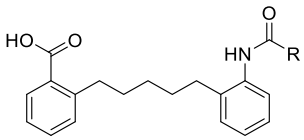


[a]Reagents and conditions: (a) 2-ethynyl-R′-phenylamine, CuI, diisoproylamine, $Pd(PPh_3)_4$, AcCN; (b) i: i. R″-sulfonyl chloride, $NaHCO_3$, AcCN, RT or R″-sulfonyl chloride, pyridine, RT ii. NaOH or LiOH, MeOH, RT.

in one step. The synthesis of the alkynyl-linked compounds is depicted in Scheme 3.

Arylbromides **D** were coupled under Sonogashira conditions with 2-ethynyl-R′-phenylamines to intermediates **E**. After sulfonamide formation with the respective sulfonylchlorides, the corresponding ester was saponified under basic conditions to give the products shown in Tables 5−7. Derivative **18n** required a slight adaptation of the first step of this sequence as the respective middle ring building block was not available. Arylbromide **D** (methyl-5-Br-4-methoxypicolinate) was coupled with trimethylsilylacetylene under Sonogashira conditions, followed by desilylation with potassium carbonate in methanol. The obtained ethynyl-intermediate was coupled

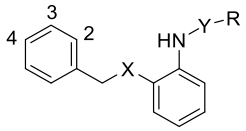
Table 1. Lactate Efflux Inhibition of Analogs of Screening Hit 9a



Entry	R	IC_{50} [μM][a]
9a		0.6
9b		0.5
9c		2.1
9d		>50
9e		16
9f		24
9g		4.1
9h		>50
9i		>50
9j		15
9k		7.4
9l		1.8
9m		1.1

[a]Efflux inhibition assay in MDA-MB-231 cells and geometric means of three replicates.

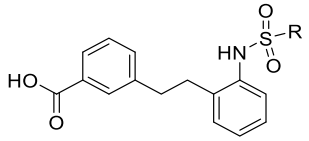
Table 2. Lactate Efflux Inhibition of Derivatives from Geometry Investigations: Ring C, Bridge Y Alternatives, and Linker X

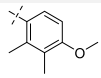


entry	COOH	R	Y	X	IC_{50} [μM][a]
16a	3	1-naphthyl	CO	CH_2	>50
16b	3	1-naphthyl	SO_2	CH_2	3
16c	4	1-naphthyl	SO_2	CH_2	3.4
16d	4	2-naphthyl	SO_2	CH_2	1.4
16e	2	1-naphthyl	SO_2	CH_2	>50
10	4	1-naphthyl	SO_2	$(CH_2)_4$	>100

[a]Efflux inhibition assay in MDA-MB-231 cells and geometric means of three replicates.

Table 3. Lactate Efflux Inhibition of Ethyl Sulfonamides



Entry	R	IC_{50} [μM][a]
16f		1.2
16g		>50
16h		8.7
16i		0.1
16j		0.8
16k		0.3

[a]Efflux inhibition assay in MDA-MB-231 cells and geometric means of three replicates.

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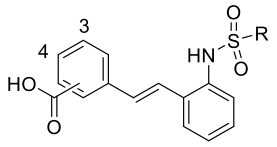
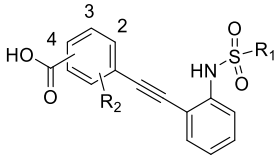
Table 4. Lactate Efflux Inhibition of Ethenyl Sulfonamides



entry	R	COOH	DB geometry	IC_{50} [μM][a]
15a	2,3-Me_2 4-OMe	3	trans	2.9
15b	1-naphthyl	3	trans	1.2
15c	2-naphthyl	3	trans	0.9
15d	2-naphthyl	3	cis	2.7
15e	2-naphthyl	4	trans	1.8

[a]Efflux inhibition assay in MDA-MB-231 cells and geometric means of three replicates.

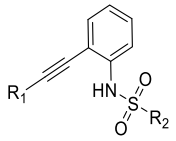
Table 5. Lactate Efflux Inhibition of Ethynyl Sulfonamides on Benzoic Acid



Entry	R_1	R_2	COOH	Clint [µL/min] (mouse)	IC_{50} [µM][a]
17a		H	4	<10	2.7
17b		H	4	30	4.9
17c		H	4	10	23
17d		H	4	54	0.6
17e		H	4	<10	0.6
17f	1-naphthyl	H	4	<10	0.4
17g	1-naphthyl	2-F	4	14	0.2
17h	2-naphthyl	H	4	41	0.8
17i	2-naphthyl	H	3		0.7
17j		H	4	<10	0.2
17k		3-NHMe	4	416	0.05
17l		H	4	<10	0.1
17m	7-Me-8-quinolinyl	H	4	54	0.1
17n	7-Me-8-quinolinyl	3-OMe	4	54	0.3

[a]Efflux inhibition assay in MDA-MB-231 cells and geometric means of three replicates.

Table 6. Screening of Pyridine, Pyrimidine, Pyrazine, and Pyridazine Carboxylic Acids on Ethynyl Sulfonamides



Entry	R_1	R_2	IC_{50} [µM][a]
18a		2-naphthyl	1
19		2-naphthyl	3.8
20		2-naphthyl	>50
21		2-naphthyl	3.7
22		2-naphthyl	9.2
23		2-naphthyl	30
24		4-OMe-1-naphthyl	3.7
25		2-naphthyl	21
26		5-MeO-8-quinolinyl	16

[a]Efflux inhibition assay in MDA-MB-231 cells and geometric means of three replicates.

again under Sonogashira conditions with 4-Cl-2-I-aniline to intermediate of type **E**. The synthesis of derivatives from Table 8 (**18p−u**) followed the same strategy as outlined in Scheme 3. Intermediate **E** was reacted with commercially available 4-chloroquinoline-8-sulfonyl chloride, followed by substitution of the chlorine with the respective alcohol function of the different residues in the presence of potassium *tert*-butylate in DMF at RT. Ester saponification occurred under these conditions.

SAR Studies of Terminal Moiety A. From cellular MCT expression profiles and literature evidence,[33] the high MCT4/no MCT1-expressing human breast cancer cell line MDA-MB-231 was chosen to test compounds on their MCT4 transporter inhibition potential. In addition, the effect of selected MCT4 inhibitors on MDA-MB-231 viability was tested to prove the hypothesis that intracellular lactate accumulation might be associated with reduced viability. The terminal moiety A was systematically optimized first and a focused selection of compounds is displayed in Table 1.

The submicromolar activity of the 2-naphthyl-screening hit **9a** could be confirmed with the 1-naphthyl isomer **9b**. A methyl extension para to the amide bridge like in **9c** reduced activity 4-fold. Being cognizant of the resulting compound properties, the naphthyl group was replaced with an array of substituted phenylamides. The unsubstituted phenyl **9d** turned out to be inactive while decoration with electron-donating

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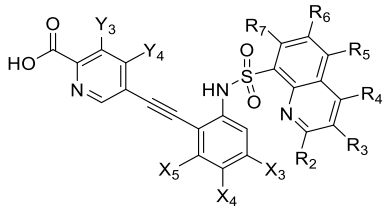
Table 7. Lactate Efflux Inhibition, Binding Constants, and PK Data of Ethynyl Sulfonamides on Picolinic Acid



entry	R_2–R_7	Y	X	IC_{50} [nM][a]	FCCS IC_{50} Ki [nM][b]	pred mouse CL microsomes/hepatocytes [L/kg/h]	mouse CL [L/kg/h]	mouse V_D [L/kg]
18b	H	H	H	177	215			
					37			
18c	2-Me	H	H	245	579			
					99			
18d	3-Me	H	H	153	139			
					29			
18e	4-Me	H	H	209	154			
					32			
18f	5-Me	H	H	283	1020			
					222			
18g	6-Me	H	H	234	243			
					51			
18h	7-Me	H	H	34	79	0.2/0.4	1.9	1.8
					17			
18i	7-Me	3-Me	H	34	490		1.9	1.9
					56			
18j	5-OMe	H	H	91		≪0.03/0.1	0.4	0.6
18k	5-OMe	3-Me	H	29	99		0.6	0.6
					11			
18l	5-OEt	H	H	17	92	0.1/0.1	0.6	0.4
					16			
18m	5-OEt	4-OMe	H	3	80	0.1/0.1	0.4	0.4
					17			
18n	5-OEt	4-OMe	4-Cl	1	77	≪0.04/0.1	0.3	0.4
					11			
18o	5-OEt	3-NHMe	H	3	62	≪0.03/0.1	1.6	1.6
					17			

[a]Efflux inhibition assay in MDA-MB-231 cells and geometric means of three replicates. [b]Biophysical characterization via fluorescence cross-correlation spectroscopy, mean of three replicates, and discussion of FCCS data vide infra.

(**9e**–**9g**) or -withdrawing moieties (**9h**–**9m**) with different steric demands only allowed a re-establishment of micromolar activity. Screening hit **9a** reduced MDA-MB-231 viability in the micromolar range (GI_{50}: 5.7 μM; IC_{50}: 7.9 μM) emphasizing the need to increase lactate transporter inhibition potency. Microsomal turnover of compounds from Table 1 was in the high to very high range (data not shown), indicating that optimization of metabolic stability was necessary as well.

SAR of Linker and Bridge. It was anticipated that a reduced linker length would be beneficial for activity as entropic penalties would be reduced. The relative positions of the carboxylate to the amide and its residue were considered to be important. An ortho-acid ring C together with a shorter linker moiety would not match geometry requirements. In silico analysis of preferred conformations (Figure 4A) and field points (Figure 4B) confirmed the expectation that a carboxylate in the meta position with an ethyl linker would superimpose with the ortho-acid and pentyl linker quite well. Specifically, field analysis showed that, despite a shorter linker in ethyl compared to pentyl, the compounds' negative field points from carboxyl groups overlap very well, thus likely retaining the same set of interactions.

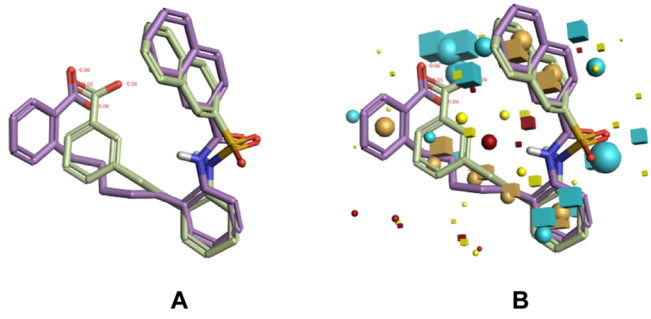


Figure 4. Superimposition of preferred conformations (A) and field points (B) of ortho-acid with pentyl linker **9a** (magenta) and meta-acid with an ethyl linker (olive). Square field points are derived from pentyl linker compound, sphere field points are derived from the ethyl linker compound. Cyan and red points indicate the maxima of negative and positive electrostatic potentials, respectively; orange and yellow points indicate the maxima of aromatic and hydrophobic potentials, respectively.

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Moving the acid-moiety into the meta position in ring C together with 1-naphthyl amide ring A and ethyl linker resulted in the inactive compound **16a** (Table 2).

This inactivity was not anticipated from the analyses depicted in Figure 4 but initiated optimization of the bridging moiety. Changing the carboxamide bridge into sulfonamide (**16b**) reintroduced micromolar activity. Ring C geometry was further investigated leading to the finding that the para-acid isomers **16c** and **16d** resulted in equipotent micromolar compounds. As expected, the ortho-benzoic acid **16e** with an ethyl linker and a sulfonamide bridge was inactive, in contrast to the pentyl-linked carboxamide **9a**, confirming the initial considerations of the linker length and carboxylate position. The pentyl-linked sulfonamide **10** was completely inactive as well (Table 2). Accordingly, it was concluded that the carboxamide bridge is required for the extended pentyl linker activity, whereas the 2-atom-linker only yielded active compounds when combined with the sulfonamide bridge. Continued optimization of the ethyl-linked sulfonamide core is shown in Table 3.

The micromolar activity of the 1-naphthyl derivative **16b** from Table 2 could be confirmed with the 2-naphthyl isomer **16f**. Similar to the pentyl-carboxamide compound with an unsubstituted phenyl ring A **9d** (Table 1), also the ethyl sulfonamide **16g** (Table 3) was inactive. Notably, the ethyl-linked 4-*F*-phenyl sulfonamide isomer **16h** showed micromolar activity, despite the analogues pentyl-linked carboxamide **9h** (Table 1) being inactive. Remarkably, the addition of a methyl group ortho to the sulfonamide (**16i**) further increased potency into the 100 nM range. Identifying the first analog in this lead series with submicromolar activity underscored the importance of having redesigned the central core of the inhibitor series toward higher rigidity. Even though this halogen alkyl substitution improved activity, the stability in mouse liver microsome incubations was unfavorable (**16i** mouse Clint: 167 μL/min/mg prot.). This was overcome by the 2,3-Me_2 4-OMe-phenyl residue (**16j**), which was submicromolar active and showed acceptable metabolic stability (**16j** mouse Clint: <10 μL/min/mg prot.). This suggested that compound **16j** would be an attractive starting point for a further cycle of optimization (vide infra). The bis-ortho chlorine substitution (**16k**) slightly increased the activity but compromised the microsomal stability in mouse (89 μL/min/mg prot.). More rigid ethenyl-linked sulfonamides were investigated then (Table 4).

In **16j** (Table 3), the dimethyl-methoxy-phenyl residue resulted in submicromolar activity, but the more rigid ethenyl compound **15a** was less active (Table 4). The naphthyl isomers **16b** (Table 2) and **16f** (Table 3) had micromolar activity, which could be confirmed with the ethenyl bridge in **15b** and **15c** (Table 4). The latter core was also investigated with the acid in the para position to the double bond. In **15e**, activity dropped 2-fold. In addition, the **15d** cis isomer of **15c** showed a 3-fold activity drop. Based on these observations, the scaffold with an ethynyl bridge and 4-carboxylate was chosen for further investigations (Table 5).

The fully unsubstituted starting point **17a** showed only micromolar activity. The meta methyl ether **17b** did not much alter activity but the 4-anisole analog **17c** was 5−10-fold less potent. The addition of two methyl groups in ortho and meta positions to the 4-OMe moiety (**17d**) on the other hand contributed to submicromolar activity, which was confirmed with the bis-ortho chlorine substitution in **17e**. Submicromolar activity could also be achieved with 1- and 2-naphthyl derivatives **17f**, **17g**, and **17h**. Surprisingly, the meta-isomer **17i** and *para*-regio isomer **17h** showed equal potency, even though the carboxylate is presented in different geometry. The 8-quinolinyl analog **17j** and 1-naphthyl isomer **17f** showed comparable IC_{50} values of 0.7 and 0.8 μM, respectively. Appropriate substitution of the 8-quinolinyl residue improved potency even further. For the 7-methyl derivative **17k**, an IC_{50} value of 50 nM was achieved for the first time. This analogue showed high turnover in mouse liver microsomes (**17k** mouse Clint: 416 μL/min/mg prot.), possibly caused by the elevated lipophilicity and/or by the electron-rich, activated amino alkyl substituent. The two derivatives **17j** and **17l** inhibited lactate efflux with IC_{50} values of 200 and 100 nM, respectively, and were stable in microsomes (**17j** and **17l** mouse Clint: <10 μL/min). Both were tested in the MDA-MB-231 viability assay and were active with IC_{50}s of 7.2 μM and 1.7 μM and GI_{50}s of 4.4 μM and 0.9 μM, respectively.

Nevertheless, combining suitable potency with high liver microsomal stability was generally challenging for this series. Under the assumption that heterocycles may be beneficial, phenyl ring C replacements were studied (Table 6). A series of the pyridine and diazines carboxylic acids bearing the carboxylate in meta and para positions relative to the acetylene linker were synthesized. The picolinic acid derivative **18a** was identified as the most active (1 μM) together with an acceptable mouse microsomal turnover of 14 μL/min/mg prot. and set the basis for further investigations (Tables 6 and 7).

The four derivatives, **19**−**22**, contained a meta orientation of the acid and the ethynyl linker. All derivatives were only active in the upper micromolar range and confirmed that the geometry is critical to maintain the activity. The remaining four derivatives, **23**−**26**, from Table 6 in addition to **18a** with a para orientation of the acid and the ethynyl linker demonstrated that the position of the ring-nitrogen(s) was also of importance. In all cases, where the nitrogen is positioned meta to the acid, activity was reduced (**23**, **25**, and **26**). Ortho orientation gave an IC_{50} value of 3.7 μM again (**24**). Consequently, further optimization was based on these ring A derivatives together with the optimized picolinic ring C moiety (Table 7).

Compared to the unsubstituted analog **18b**, permutation of a methyl group on various positions on the quinoline (**18c**−**18h**) only improved activity for the 7-yl isomer **18h**. Compound **18h** was characterized further. It showed acceptable solubility in PBS buffer (97 μg/mL) and turnover in mouse microsomes (18 μL/min/mg prot.). The turnover was much higher in human microsomes (82 μL/min/mg prot.), further an unfavorable efflux ratio of 12 in the Caco-2 assay was observed. Introduction of a methyl group on the picolinate (**18i**) had no impact on the potency, but PBS solubility (384 μg/mL), as well as the mentioned liabilities of **18h**, was addressed: human Clint: 18 mg/min/mg prot.; efflux ratio: 3. Ether substituents on position 5 of the quinoline were also investigated. The methyl ether **18j** was 3-fold more potent than the aliphatic methyl analog **18f**. Properties of **18j** showed a mixed profile with acceptable solubility and mouse microsomal stability (PBS: 445 μg/mL; mouse Clint: <10 μL/min/mg prot.), but again turnover in human microsomes was high (63 μL/min/mg prot.) as well as the efflux ratio (16). Also, for the 5-yl methyl ether, the introduction of a methyl group on the picolinate (**18k**) improved properties: PBS 876 μg/mL; human clint: 12 μL/min/mg prot.; ER 11. In this case,

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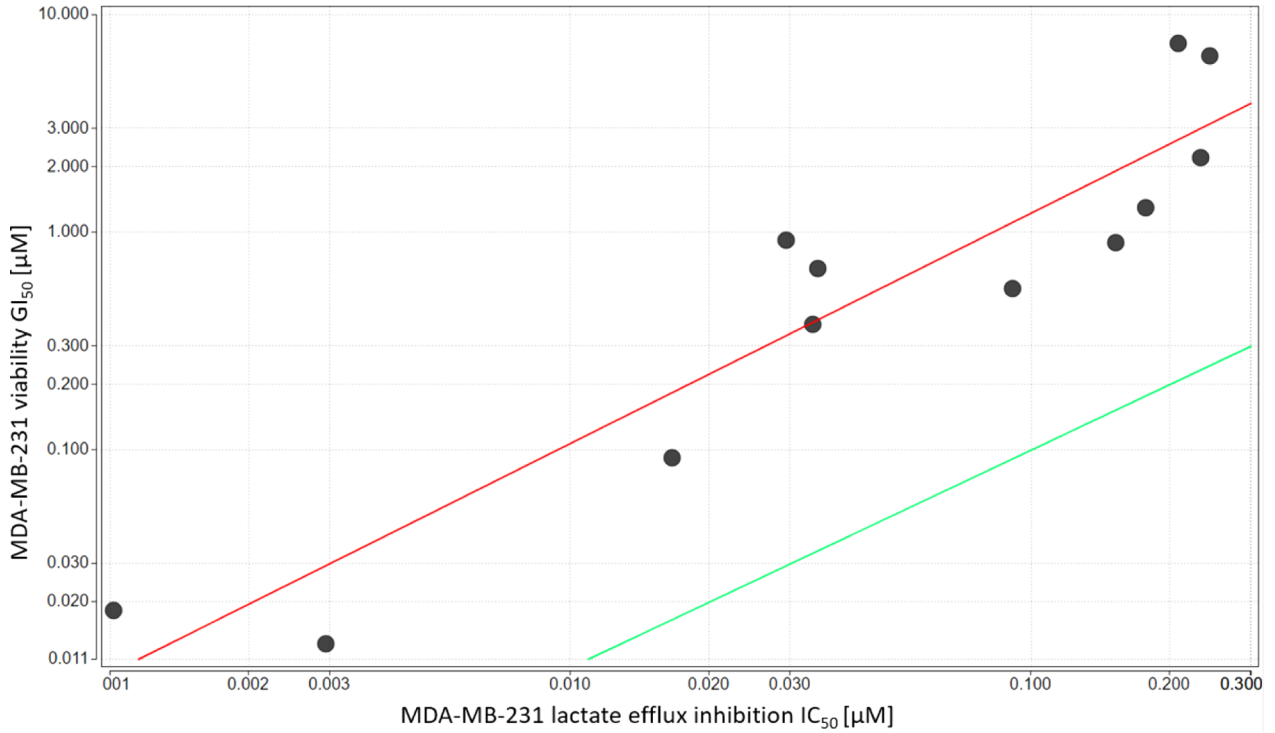

Figure 5. Correlation (red line) of 4 h lactate efflux inhibition (*X*-axis) with a 6 d viability effect (*Y*-axis) of compounds from Table 7 in MDA-MB-231 cells (Supporting Information Table S1). R^2: 0.87. 10-fold off-set to diagonal (green line).

the cellular activity also increased. Extension to the ethyl ether **18l** improved activity even further. From benzoic acid pairs such as **17m**/**17n** (Table 5), it was learned that methoxy substitution of ring C could have a negative impact on potency. The methylamine, on the other hand, suggested a favorable impact (**17k**). Because of this inconsistency, similar substituents were investigated on the picolinic acid ring C. For both the methyl ether **18m** as wells as the methylamine **18o,** potency could be increased to an IC_{50} value of 3 nM. Halogen substitution on ring B improved activity for the ring C benzoates (not discussed above) and was analyzed for the picolinates, too. The **18n** chlorine-substituted analog of **18m** was tested with an IC_{50} of 1 nM in the lactate efflux assay and proved to have a favorable property profile (vide infra). Explanations for the high activity could be a specific van der Waals interaction or the appropriate positioning of transporter-interacting moieties on a rigid scaffold, which avoids negative entropic penalties (preferred conformation of sulfonamide discussion in Supporting Information, Figure S1).

The original screening hit **9a** (Table 1) had demonstrated that lactate accumulation (IC_{50}: 0.7 μM) could lead to a viability effect (GI_{50}: 5.9 μM). Accordingly, optimized derivatives from Table 7 were investigated with respect to their effect on MDA-MB-231 cell viability. Figure 5 depicts that in MDA-MB-231, inhibition of lactate efflux (IC_{50}; *x*-axis) correlates well with cell viability (GI_{50} after 144 h; *y*-axis) with an off-set of around 10 (Supporting Information, Table S1).

Virtually all derivatives from Table 7 had intrinsic clearance values below 10 μL/min/mg prot. in mouse liver microsomes. Only derivatives **18h** and **18n** were in the range of 20 μL/min/mg prot. and the 5-methyl derivative **18f** showed high intrinsic clearance. Most analogues showed a higher clearance in hepatocyte incubation than with microsomes, indicating phase II metabolism, possibly via formation of an acyl glucuronide metabolite. The acid function in ring C, in combination with the sulfonamide bridge, resulted in low to moderate permeability (frequently below $1 \times 10 \times 10^{-6}$ cm/s in Caco-2 cells). This caused transporter-mediated elimination, manifested by underestimation of in vivo clearance ranging from 4- to 16-fold from in vitro systems. This was further supported by elevated fecal levels (20−40% of the dose) after an IV dose indicating biliary elimination. **18h** and **18o** also showed a higher volume of distribution than expected for the ion-class, presumably due to enterohepatic recirculation (Table 7).[34]

To optimize the still limited permeability of derivatives based on the acetylene scaffold, both moieties, the acid and the sulfonamide, were probed with isosteres and bridge alternatives to reduce the H-bond donor count. This work did not provide compounds with improved properties and is described in the Supporting Information.

Biophysical Target-Binding Confirmation. Solute carriers such as MCT4 are challenging treatment targets not only due to subtype plurality and linked selectivity issues, but also because, as membrane-bound targets, no enzymatic assay could be established, and only cellular testing was possible. In addition, confirmation of target engagement was difficult given that biophysical measurements for targets that require specific cellular environments often also require target-specific experimental conditions. Thus, fluorescence cross-correlation spectroscopy (FCCS) was selected to investigate target interaction.[35] Although GFP-tagged expression of the MCT4 transporter and solubilization of an active conformation were two major hurdles, the SAR work around compound **18b** suggested that the quinoline moiety could provide an exit vector for fluorescent label attachment. To test this exit vector hypothesis, some probe molecules **18r**−**18u** with different

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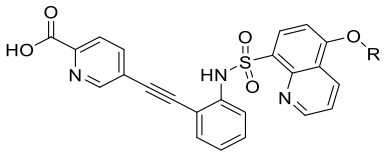
linkers and terminal moieties were prepared and proven to be still active in the cellular assay (Table 8).

Table 8. Probe Molecules and the FCCS Tool 18u



entry	R	IC_{50} [μM][a]
18p	$(C_2H_4O)_2$-Phe	0.04
18q	$(C_2H_4O)_3C_6H_{12}O$-Bzl	0.24
18r	$C_2H_4O_2$-(4-NO_2-Phe)	0.57
18s	$(C_2H_4O)_3C_2H_4NH$-BOC	0.96
18t	$(C_2H_4O)_3C_2H_4$-NH_2	>25
18u	$(C_2H_4O)_3C_2H_4$-NH-Dye	8.8

[a]Efflux inhibition assay in MDA-MB-231 cells and geometric means of three replicates.

The PEG-linked phenyl ether **18p** showed an IC_{50} value of 40 nM. Different linking moieties and terminal functionalities also provided cellular activity in the micromolar range or even lower (**18q**−**18s**). Based on these results, a fluorescent tracer molecule (**18u**; chemical structure depicted in Supporting Information; Figure S2) was prepared, built on amine **18t**.

For the FCCS experiment, membranes of a stable cell line expressing a C-terminal GFP-fusion of MCT4 were prepared and solubilized using Lauryl Maltose Neopentyl Glycol (LMNG). The addition of increasing amounts of **18u** in a titration experiment did confirm a specific or dose-dependent interaction of the tracer with MCT4-GFP. The equilibrium dissociation constant of the interaction was determined at 9.6 nM (data not shown). This interaction was used to determine the IC_{50}s and K_is of the test compounds in competition experiments. The biophysical orthogonal target-binding results support the assumption that MCT4 was inhibited when altered lactate efflux and accumulation were measured in the MDA-MB-231 cellular assay with the disclosed compounds. The measured binding data for compounds from Table 7 correlated very well with the inhibition of lactate efflux in MDA-MB-231 cells as shown in Figure 6. Considering the significant difference in the complexity of the correlated experiments, functional transporter inhibition in a cellular assay context versus binding to the solubilized protein in a biophysical experimental setup, the on−target activity in the cellular assay could be considered a strong possibility.

Specific Binding of Compounds to the MCT4 Lactate Transporter. HEK293 cells expressing human-MCT4-eGFP were tested by imaging to determine if compounds bind to the cell surface or if they can passively penetrate the membrane. The addition of 10 μM labeled **18u** (Supporting Information, Figure S2) to the cells did result in binding to MCT4-GFP, but only in the presence of 0.05% Triton, which allowed permeabilization of the cells (Figure 7). This binding was shown to be specific because it was displaced by the addition of 10 μM of unlabeled parental compound **18t** (Figure 7C). This result suggested that binding of the compounds to MCT4 occurred at the cytosolic domain of MCT4.

Binding of the compound to the mouse target was also tested in live cells expressing a GFP-mouse-MCT4 clone by confocal microscopy (Supporting Information, Figure S3) and FCCS data confirmed a binding affinity of 301 nM (Supporting Information, Figure S4).

Mechanistic Confirmation by Measurement of Intracellular Lactate Accumulation. Whether the observed modulation of extracellular lactate levels was a result of blocking lactate efflux from the cells was verified by determining intracellular lactate accumulation by a HPLC-based analytical method. As expected, increased intracellular lactate accumulation was observed for compounds that reduced extracellular lactate levels in a dose-dependent manner (data not shown). This method was also used to exclude

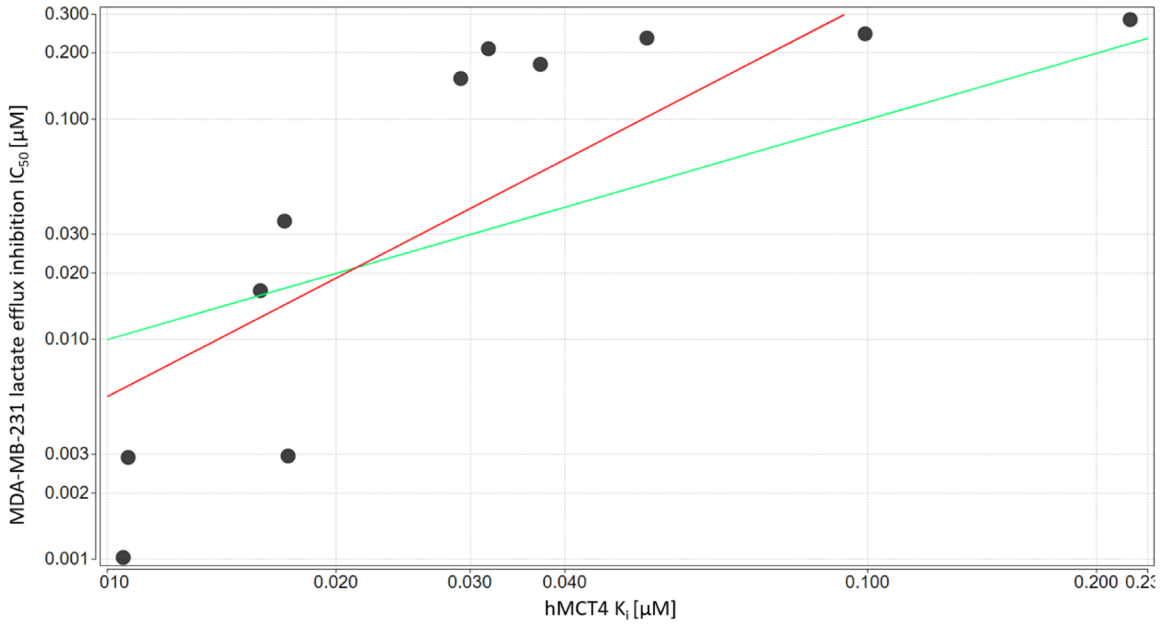


Figure 6. Correlation of cellular lactate efflux inhibition in MDA-MB-231 cells (y-axis) with hMCT4 FCCS binding (x-axis) of compounds from Table 7. Diagonal in green. Regression line in red, R^2: 0.8.

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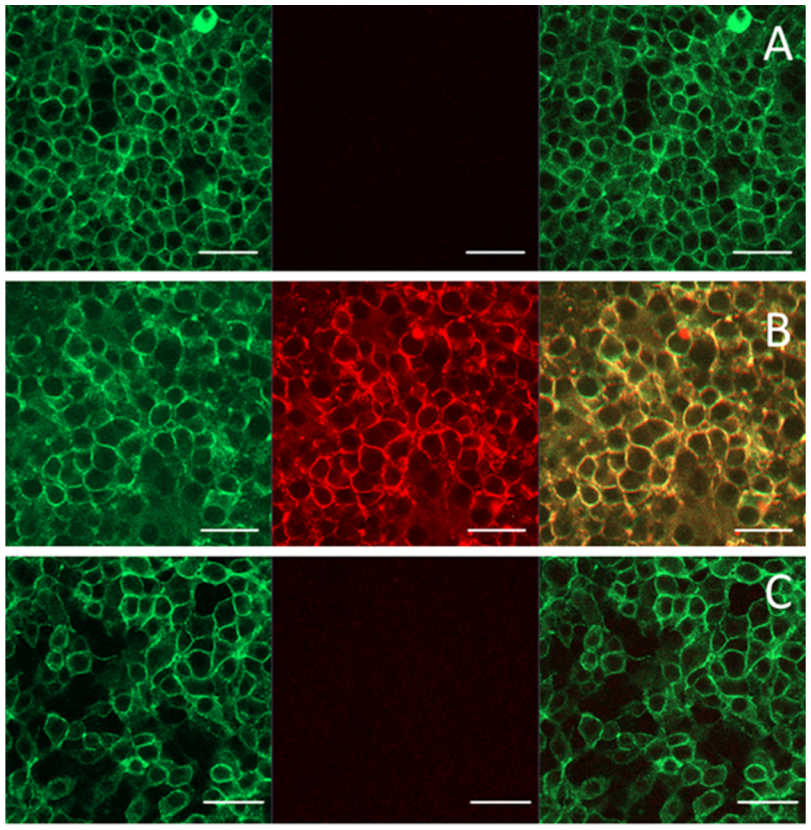

Figure 7. Imaging of HEK293 cells expressing hMCT4-GFP (green) after the addition of 10 μM **18u** (red). (A): no detergent was added, while in (B,C), 0.05% Triton X 100 was added to permeabilize the cells. (C): 10 μM of unlabeled parental **18t** was added. Bars correspond to 50 μm.

compounds showing off-target related extracellular lactate modulation.

In addition, modulation of lactate flux was confirmed by monitoring compound-dependent intracellular concentration changes of ^{14}C-labeled lactic acid (radioactive lactate uptake assay, Evotec) in MDA-MB-231 and in the high MCT1/low MCT4 expressing SNU-398 cells (Table 9). The data correlated well with the results of the extracellular lactate efflux assay, in line with an MCT4-selective profile for the newly synthesized compounds (Table 9).

Table 9. Confirmation of Compound Selectivity by Transporter Assay

	radioactive lactate transporter assay IC_{50} [nM]		lactate efflux assay IC_{50} [nM]	
entry	MDA-MB-231	SNU-398	MDA-MB-231	SNU-398
1	>30,000	7.1	>5000	22
2	>30,000	2.5	>50,000	6.2
18j[a]	18	13,000	100	>50,000
17l[b]	21	2700	150	>50,000
18h[a]	13	8200	40	10,000

[a]Structures shown in Table 7. [b]Structures shown in Table 5.

MCT Isoform Selectivity. The selectivity of described MCT4 inhibitors toward the other MCT isoforms, especially MCT1 and MCT2, was verified by testing extracellular lactate secretion inhibition in selected cell lines with proven predominant expression of given transporters: MDA-MB-231 and NCI-H358 for MCT4, SNU-398 for MCT1, MiaPaCa2 for MCT1 + MCT4, and RT4 for MCT2 + MCT3. In addition, MiaPaCa2 CRISPR Knockout clones for MCT4 and MCT1 were tested. The initially discovered hit compound **9a** inhibited lactate secretion exclusively in cell lines expressing predominantly MCT4 transporter. The trend was maintained during optimization of ethyl-, ethenyl-, and ethynyl linkers (Supporting Information, Table S6).

In Vivo Testing. Compounds **18i** and **18k** were the first seen to be applicable for in vivo studies because of their IC_{50} values for lactate efflux inhibition in MDA-MB-231 of 34 and 29 nM, respectively (Table 7). Derivative **18k** also showed favorable activity in alternative MCT4-high/MCT1-low cell lines (NCI-H358, IC_{50}: 137 nM; NCI-H441, IC_{50}: 239 nM). In addition, the PK profiles of these derivatives were considered to be acceptable. Oral administration of **18i** as homogeneous suspension in 0.5% methocel/0.25% tween20 in 50 mM acetate buffer (pH 5.5) resulted in 45% bioavailability. Dosed at 30 mg/kg, **18i** showed a free exposure exceeding the cellular IC_{50} for approximately 3 h (Supporting Information, Figure S5A). **18k** administered orally as a homogeneous suspension in 0.5% methocel/0.25% tween20 in 50 mM phosphate buffer (pH 7.4) resulted in 35% bioavailability, and administration at 30 mg/kg yielded a free exposure exceeding the MDA-MB-231 IC_{50} for approximately 4 h (Supporting Information, Figure S5B).

Compound tolerability was tested in SCID beige mice upon administration of **18i** and **18k** up to 200 mg/kg once-per-day (QD) for 10 days. Administration of either compound was well tolerated and did not result in body weight loss or any adverse clinical sign (data not shown).

To examine the capability of ethynylsulfonamide compounds to modulate lactate levels and tumor growth in vivo,

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compounds **18i** and **18k** were tested in MCT4-high/MCT1-low/no expressing xenograft models. Treatment of NCI-H358 or NCI-H441 MCT4-high/MCT1-low tumors did not result in the tumoral lactate modulation or antitumor effect (data not shown), suggesting that MCT4 inhibition alone was not sufficient to elicit an effect in MCT1 expressing models. Notably, while a slight to moderate increase of total tumoral lactate could be observed as a consequence of intracellular lactate accumulation in the MCT4-high/MCT1-no MDA-MB-231 model for both compounds **18i** and **18k** (Supporting Information Figure S7A and B), tumor growth inhibition could not be achieved (Supporting Information Figure S7C and D). It was hypothesized that the degree and/or duration of target inhibition was insufficient to obtain a therapeutic effect. Therefore, the search for compounds with improved properties was continued.

Further optimization of acid ring C, linker, middle ring B, bridge, and terminal moiety A with appropriate decoration culminated in compound **18n**. Lead **18n** inhibited lactate efflux in the MDA-MB-231 cell line with an IC_{50} of 1 nM. The on-target activity was confirmed with a K_i of 11 nM, determined via FCCS measurements (Table 7). MCT4 selectivity was validated, as the compound did not inhibit lactate efflux to a similar extent in SNU-398 and MiaPaca2, and only 600-fold less in RT-4 cell lines (Supporting Information; Table S7). No PGE2 affinity could be determined as a main off-target effect for benzoic series (Supporting Information, Table S6). The solubility in PBS (pH: 7.4) was acceptable (58 μg/mL) but dropped significantly at lower pH (SGF; pH: 1; 12 μg/mL). Passive permeability in the Caco-2 assay was low (2.2 10 $\times$ 10^{-6} cm/s) and an efflux ratio of 10 was observed in the same system. In vitro metabolic stability was good in the mouse systems: the compound was stable in mouse microsomes and showed acceptable metabolic stability in mouse hepatocytes (18 μL/min/1 $\times$ 10^6 cells). In line with these results and the modest free fraction in mouse plasma (0.6%), a low clearance was observed in mouse (0.33 L/h/kg), corresponding to approximately 5% of the liver blood flow.

Attempts to administer **18n** via the oral route in mouse using suspensions and unbuffered solutions resulted in modest bioavailability (20%). First pass effects could be excluded as the clearance was low. It was hypothesized that the low solubility at low pH caused the compound to precipitate in the stomach with a dissolution further down in the gastrointestinal tract, inadequate for complete absorption. Therefore, it was transformed into a potassium salt and administered as a solution in 20% kleptose in 50 mM PBS (pH 7.4), which resulted in an increased bioavailability (65%). Dosed at 30 mg/kg, **18n** showed a free exposure exceeding the MDA-MB-231 IC_{50} for 24 h (Figure 8). It is possible that free concentration in tumor was lower as **18n** was a substrate for efflux transporters (Table 10). Assessing free levels in tumors is currently not possible due to heterogeneity (vascularization etc.), but pharmacodynamic effects (see below) were observed in line with observed free exposure. Based on this extensive characterization, we conclude that **18n** is an excellent selective MCT4 inhibitor tool to investigate the biological role of the MCT4 transporter inhibition (Table 10).

Based on the results from **18i** and **18k** in high MCT4 expressing xenograft models, an alternative approach was followed for the optimized compound **18n** to also address the well-described immunosuppressive role of lactate in the tumor microenvironment.[2] The MC38 murine colorectal cancer model was selected as the syngeneic model for its high MCT4/low MCT1 expression. In addition, we found that MC38 tumors accumulate higher levels of lactic acid in comparison to other syngeneic models tested (data not shown).[2] **18n** activity was first characterized in vitro in

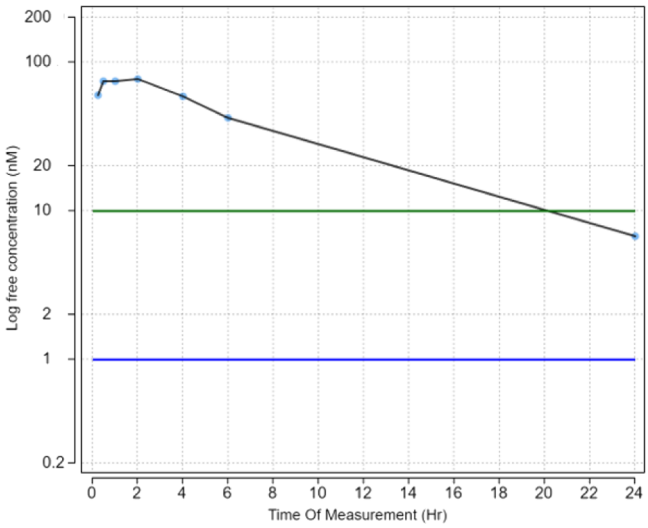

Figure 8. Free plasma concentration in female CD1 mice after a 30 mg/kg oral administration of **18n** formulated in 20% kleptose in 50 mM PBS (pH 7.4). Blue and green lines correspond to the free concentration, related to lactate efflux inhibition IC_{50} in MDA-MB-231 and 10-fold of this value, respectively.

Table 10. Technical Profile of Selective MCT4 Inhibitor 18n

	parameter		value
Phys Chem	MW		538
	log *P*/*D*		3.5/1.5
	*t*PSA		136
	pK_a		4.6
properties	PBS pH 7.4	24 h [μg/mL]	58
	SGF pH 1.2		12
	FaSSIF pH 6.5		268
	FeSSIF pH 5.0		232
	Mic (m/h) [μL/min/mg prot.]		23/250
	Hep (m/h) [μL/min/1 $\times$ 10^6 cells]		18/29
	Fu (m/h) [%]		0.6/0.5
	Caco-2 PP [10 $\times$ 10^{-6} cm/s]; ER		2.2; 10
	HERG [% effect @ 10 μM]		−11
cellular profile	MDA-MB-231	IC_{50}/GI_{50} [nM]	1/11
	NCI-H358		3/−
	SNU-398		>25,000/−
	RT4		638/−
	MiaPaCa		>25,000/4720
PK	CL [L/h/kg]	iv (0.2 mg/kg)	0.33
	AUC [ng/mL $\times$ h]		601
	C_{max} [ng/mL]		489
	$t^{1/2}$ [h]		1
	V_{ss} [L/kg]		0.4
	methocel/tween20 in water	F [%] po (10 mg/kg)	19
	methocel/tween20 in 50 mM Na-citrate pH 3		20
	20% glycofurol in water		27
	20% kleptose in 50 mM phosphate pH 7.4		65

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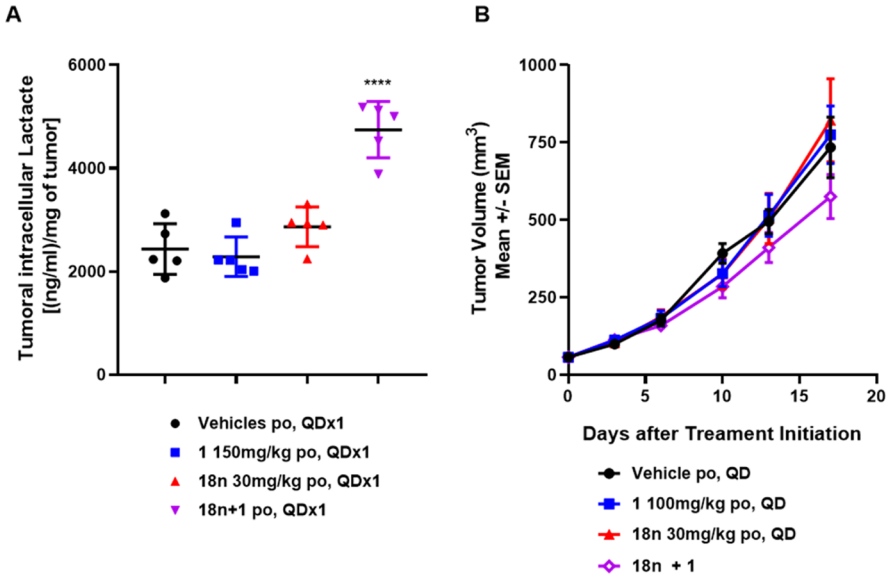

Figure 9. Effect of **18n** treatment on tumoral lactate and tumor growth. (A) Tumoral intracellular lactate levels were measured in MC38 tumor samples collected 2 h after single administration of the indicated compounds or combination. Bars indicate means ± SD. Statistical significance was assessed with One-way ANOVA, followed by Tukey's multiple comparison test. (B) Mice bearing MC38 tumors (n = 10) were treated with vehicle, **18n**, or **1** as a single agent and in combination at the indicated concentrations. Statistical significance was tested with Two-way RM-ANCOVA, followed by post-hoc Tukey multiple pairwise comparisons. po, oral administration; QD, once daily; SD, standard deviation; and SEM, standard error of the mean.

MC38 cells, alone or in combination with MCT1/2 inhibitor **1**, demonstrating that, in this model, modulation of lactate transport and decrease of cellular viability can be achieved exclusively when both MCT1 and MCT4 are inhibited. This effect was confirmed in MC38 MCT1 and MCT4 CRISPR Knockout clones (Supporting Information; Figure S6). In vivo tolerability of **18n** was tested in non-tumor-bearing C57Bl/6 mice; oral administration of **18n** up to 30 mg/kg QD for 10 days was well tolerated without body weight loss or side effects (data not shown). **18n** was then tested in vivo for its ability to modulate tumoral intracellular lactate accumulation. MC38 tumor-bearing mice were treated with a single administration of **18n** as potassium salt, MCT1/2 inhibitor **1**, or a combination of the two at 30 and 150 mg/kg, respectively; animals in the control group were administered with both vehicles. Tumor samples were collected 2 h after single administration and lactate was measured. Only the **18n** + **1** combination group exhibited a significant tumoral intracellular lactate accumulation (Figure 9A), as previously observed in vitro (Supporting Information; Figure S6). Administered compounds were quantified in plasma and tumor from the dosed animals, to confirm exposure (data not shown). To test the antitumor effect of MCT4 inhibition alone or in combination with MCT1/2 inhibition, the efficacy of **18n** as a single agent or in combination with **1** was evaluated using the MC38 model. **18n** showed no significant antitumor activity, neither when administered as monotherapy nor in combination with **1** (Figure 9B).

Similarly, modulation of tumoral lactate with concomitant lack of antitumor response was observed for **18i** and **18k** in the MDA-MB-231 model as well (Supporting Information, Figure S7).

CONCLUSIONS

In summary, the flexible micromolar active amide-screening hit **9a** was optimized to a rigid nanomolar active and in vivo capable sulfonamide **18n**. This highly potent derivative demonstrated lactate efflux inhibition and intracellular lactate accumulation in high MCT4-expressing cell lines in vitro and was found to be inactive in cells, which do not highly express this transporter subtype in comparison to others. Target binding of the compounds to the cytosolic domain of MCT4 was confirmed by FCCS, while off-target activity was not detected. Salt formation and application as solution-formulation with an appropriate vehicle in PK studies demonstrated a free exposure exceeding cellular IC_{50} for 24 h. Thus, **18n** is considered an attractive compound to investigate pharmacological effects of selective MCT4 inhibition in vitro and in vivo. This compound is available upon request.

Initial in vivo studies assessing PD biomarker modulation by **18n** in murine MC38 tumors (MCT4-high/MCT1-low) revealed that an increase in lactate accumulation in tumor cells could only be achieved by concomitant inhibition of MCT4 and MCT1/2. However, even though the combined inhibition of MCT1/2 and MCT4 led to decreased viability in MC38 cell viability in vitro, combination of **18n** with MCT1/2 inhibitor **1** did not translate into a significant tumor growth inhibition in this tumor model. Similar observations were made in the MCT4-high/MCT1-no xenograft model MDA-MB-231, where in vitro findings translated into in vivo tumoral lactate modulation but not into the antitumor effect. Taken together, these data suggest that MCT1 plus MCT4 lactate transport inhibition, while resulting in tumoral lactate modulation, may not be sufficient to achieve antitumor activity in these models.

In line with these results, in vivo tumor growth inhibition was reported for the MCT4 inhibitor AZD0095, not as a single agent but only if combined with VEGFR TKI, αCTLA4, or αPD1 antibodies.[23] More studies are required to establish if this effect is a general limitation of MCT4 inhibitors, with or without MCT1 inhibition, or if a combination approach is required to achieve reduction of tumor growth. In fact, extensive literature has described rationales for combining

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specific MCT inhibitors with investigational or approved treatments with different mechanisms of action, such as checkpoint inhibitors,[9,12] tyrosine kinase inhibitors,[19,20] or radiation.[36] Moreover, combination of MCT inhibitors with molecules able to interfere with cellular metabolism, such as the antidiabetic drug metformin[8] or glutaminase inhibitor CB-839,[22] results in reduction or loss of cellular viability. Consequently, a sequence of experiments was initiated to identify compounds synergistic with MCT4 inhibitors. Furthermore, data for **18n** together with published data indicate that the MCT1/4 expression profile of targeted tumor cells needs to be considered.[23] Overall, **18n** is considered an attractive compound to investigate the pharmacological effects of selective MCT4 inhibition on lactate modulation, but it may require additional investigation into target coverage, tumor model selection, and/or combination partners in order to achieve substantial tumor growth inhibition in vivo.

EXPERIMENTAL SECTION

Chemistry. *General Information.* All reactions were carried out under a nitrogen atmosphere or in sealed vials unless noted otherwise. Dry solvents and reagents were of commercial quality and were used as purchased. Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F254 by fluorescence quenching under UV light or by high-pressure liquid chromatography with subsequent mass detection, except if indicated otherwise. Oxidation reactions carried out with peroxides such as *tert*-butyl hydroperoxide, magnesium monoperoxyphthalate hydrates, or others were analyzed before workup with Merck test MQuant. In the case of detected traces of peroxide, the reaction mixture was treated with sodium thiosulfate accordingly. The purity of the compounds reported in the manuscript was analyzed through the HPLC-MS methodology. In addition, TLC plates were stained using phosphomolybdic acid or potassium permanganate stain. Chromatographic purification of products (flash chromatography) was performed on Isco Combiflash systems using Redisep columns and ethyl acetate (EtOAc)/heptane gradients. Concentration under reduced pressure was performed by rotary evaporation at 40 °C at an appropriate pressure unless otherwise stated. ^{1}H NMR (in DMSO-d_6) and mass spectra are in agreement with the structures and were recorded on a Bruker NMR 400 MHz spectrometer if not stated differently (TMS as an internal standard) and Vacuum Generators VG 70−70 or 70−250 at 70 eV, respectively. High-resolution mass spectroscopy (HRMS) analyses (obtained with a Bruker maXis analyzer) for the final products were within 0.4% of calculated values if not stated otherwise. All compounds reported in the manuscript have a purity ≥95% unless noted otherwise.

General Conditions. LCMS method 1: HPLC; MERCK-HITACHI LaChrom; chromolith performance RP-18e 100−4.6; 3.3 mL/min; solvent A: H_2O + 0.05% HCOOH; solvent B: acetonitrile + 0.04% HCOOH; 220 nm; 0 to 2.0 min: 0% B to 100% B; 2.0 to 2.5 min: 100% B; LCMS method 2: Agilent 1200 Series; Chromolith RP-18e 50−4.6 mm; 3.3 mL/min; solvent A: H_2O + 0.05% HCOOH; solvent B: acetonitrile + 0.04% HCOOH; 220 nm; 0 to 2.0 min: 0% B to 100% B; 2.0 to 2.5 min: 100% B; LCMS Method 3: Column: Chromolith RP-18e 50−4.6 mm; A: H_2O + 0.05% HCOOH |B: MeCN + 0.04% HCOOH/4% → 100% B: 0 → 2,8 min|100% B: 2.8 → 3,3 min; LCMS methods 4: column: Waters Acquity UPLC HSS C18 (2.1 × 50 mm, 1.8 μm); Solvent A: H_2O + 0.1% formic acid; Solvent B: ACN + 0.1% formic acid; Flow: 0.5 mL/min; gradient: 0 min: 95% B, 4 min: 5% B; 5 min: 5% B; 5.2 min: 95% B; 6 min: 95% B; LCMS method 5: column: Kinetex XB C18 (4.6 × 50 mm, 2.6 μm); solvent A: H_2O + 0.1% formic acid; solvent B: ACN + 0.1% formic acid; flow: 0.5 mL/min; gradient: 0 min: 20% B, 6.7 min: 80% B; 7.5 min: 80% B; 7.8 min: 95% B; 9.5 min: 95% B; LCMS method 6: column: Waters Symmetry C18 (3.9 × 150 mm × 5 μm); solvent A: H_2O + 0.1% formic acid; solvent B: ACN + 0.1% formic acid; flow: 1.2 mL/min; gradient: 0 min: 20% B; 20 min: 80% B; 22 min; LCMS 7: Agilent Series; Kinetex EVO C18 5.0 μm; 2.0 mL/min; solvent A: H_2O + 0.05% HCOOH; solvent B: acetonitrile + 0.04% HCOOH; 220 nm; 0 to 6.5 min:1% B to 99% B; 6.5 to 7.0 min: 100% B. LCMS 8: column: CORTECS C18 100A, 2.1 × 50 mm, 2.7 μm; mobile phase A: water/0.1% FA, mobile phase B: acetonitrile/0.1% FA; flow rate: 1.0 mL/min; gradient:10% B to 100% B in 2.0 min, hold 0.6 min; 254 nm; and LCMS 9: column: Ascentis Express C18, 3.0 × 50 mm, 2.7 μm; mobile phase A: H_2O/0.05% TFA, mobile phase B: ACN/0.05% TFA; flow rate: 1.5 mL/min; gradient: 5% B to 100% B in 1.2 min, hold 0.5 min; 254 nm.

*2-(5-{2-[(Naphthalene-2-carbonyl)-amino]-phenyl}-pentyl)-benzoic Acid (**9a**).* Methyl 2-iodobenzoate (1.7 mL; 11.22 mmol) was added into a microwave vial and dissolved in diethylamine (20 mL). Under nitrogen, 3-butin-1-ol **4** (2.6 mL; 33.66 mmol), copper(I) iodide (1.3 g; 6.73 mmol), and bis(triphenylphosphine)palladium(II) chloride (15,2% Pd, 2.4 g; 3.37 mmol) were added and the reaction was stirred for 15 h at RT. The reaction mixture was filtered, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography giving the product **5** as a brown oil (2.2 g; 90% yield).

Methyl 2-(4-hydroxybut-1-yn-1-yl)benzoate **5** (1.8 g; 7 mmol) was dissolved in THF (20 mL). Noblyst P1086 (Pd/C-5%; E101 R; ∼50% H_2O; 14 g; 45 mmol) was added, and the mixture was stirred at RT under hydrogen (500 mL, 22 mmol). The catalyst was filtered and the solution evaporated to dryness under reduced pressure giving methyl 2-(4-hydroxybutyl)benzoate **6a** as a colorless oil (1.6 g; 73% yield).

2-(4-Hydroxy-butyl)-benzoic acid methyl ester (1.6 g; 6.12 mmol) was dissolved in THF (50 mL). The solution was cooled to 0 °C and triphenylphosphine (2.4 mL; 10.71 mmol) and NBS (1.6 g; 9.18 mmol) were added. The reaction was stirred for 15 h at RT. The solvent was removed under reduced pressure and the residue was purified by flash chromatography giving the product **6b** as a colorless oil (1.9 g; 62% yield).

2-(4-Bromo-butyl)-benzoic acid methyl ester (1.9 g; 3.80 mmol) was dissolved in acetonitrile (20 mL). To the solution, triphenylphosphine (0.9 mL; 4.18 mmol) was added, and the reaction was stirred for 4 days at 65 °C. The solvent was removed under reduced pressure and the residue was washed with toluene and dried giving the crude product **6c** as a colorless solid (3 g; content 82%).

4-(2-Methoxycarbonyl-phenyl)-butyl]-triphenyl-phosphonium bromide **6c** (2, 50 g; 3.86 mmol) was dissolved in 1,4-dioxane (50 mL). 2-Nitrobenzaldehyde **7** (5.6 g; 3.86 mmol), potassium carbonate (0.7 g; 5.56 mmol), and water (0.17 mL) were added to the solution and the reaction was stirred under nitrogen overnight at reflux. The reaction mixture was diluted with water and the layers were separated. The water layer was extracted with ethyl acetate two times, the combined organic layers were washed with water and brine, dried over sodium sulfate, filtered, and evaporated to dryness. The residue was purified by flash-chromatography giving the product **8a** as a yellow oil as a cis/trans mixture (1.2 g; 93% content).

Methyl 2-[(4*E*)-5-(2-nitrophenyl)pent-4-en-1-yl]benzoate **8a** (1.1 g; 3.2 mmol) and Noblyst P1086 (1.1 g; Pd/C-5%; E101 R; ∼50% H_2O) were suspended in THF (10 mL) and hydrogen (350 mL) was added at RT overnight. After filtration of the catalyst, the remainder was evaporated to dryness under reduced pressure giving the product **8b** as a yellow oil (921 mg; 92% content).

2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester **8b** (50 mg; 0.14 mmol) was dissolved in DCM (3 mL), triethylamine (54 μL; 0.42 mmol) was added, and the mixture was stirred at RT for 20 min. Then, 2-naphthoyl chloride (28 mg; 0.15 mmol) was added in one portion. The reaction mixture was stirred overnight at RT and then diluted with ethyl acetate and water. The layers were separated and the water layer was extracted two times with ethyl acetate. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and evaporated to dryness giving the product as a white sticky oil (65 mg; 89% yield).

2-(5-{2-[(Naphthalene-2-carbonyl)-amino]-phenyl}-pentyl)-benzoic acid methyl ester (65 mg; 0.12 mmol) was dissolved in methanol (3 mL), and sodium hydroxide solution (c(NaOH) = 1 mol/l (1 N)

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0.50 mL) was added. The reaction was stirred at RT for 2 days, and then HCl was added. After dilution with water and ethyl acetate, the organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by chromatography giving the product as a white solid (17 mg 31%). LCMS method 1: 1.93 min; 438.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.72 (s, 1H), 10.03 (s, 1H), 8.57 (s, 1H), 8.05−7.98 (m, 4H), 7.71 (dd, J = 7.7, 1.5 Hz, 1H), 7.67−7.58 (m, 2H), 7.37−7.33 (m, 1H), 7.32−7.18 (m, 5H), 7.15−7.10 (m, 1H), 2.87−2.80 (m, 2H), 2.68−2.61 (m, 2H), 1.63−1.47 (m, 4H), 1.37−1.27 (m, 2H). HRMS calcd for $C_{29}H_{27}NO_3$, 438.2064; found, 438.2057.

*2-(5-{2-[(Naphthalene-1-carbonyl)-amino]-phenyl}-pentyl)-benzoic Acid (**9b**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (63 mg; 0.17 mmol) and naphthalene-1-carbonyl chloride (28 μL; 0.18 mmol) gave 2-(5-{2-[(Naphthalene-1-carbonyl)-amino]-phenyl}-pentyl)-benzoic acid methyl ester (65 mg; 83% yield). Saponification of this intermediate (72 mg; 0.13 mmol) with sodium hydroxide yielded the product as a white solid (20 mg; 36% yield). LCMS method 2; 1.92 min; 438.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.98−12.43 (m, 1H), 10.02 (s, 1H), 8.56 (s, 1H), 8.05−7.99 (m, 4H), 7.71 (dd, J = 7.7, 1.5 Hz, 1H), 7.67−7.58 (m, 2H), 7.37−7.33 (m, 1H), 7.32−7.17 (m, 5H), 7.14−7.10 (m, 1H), 2.87−2.81 (m, 2H), 2.68−2.61 (m, 2H), 1.63−1.47 (m, 4H), 1.37−1.27 (m, 2H). HRMS calcd for $C_{29}H_{27}NO_3$, 438.2064; found, 438.2059.

*2-(5-{2-[(4-Methyl-naphthalene-1-carbonyl)-amino]-phenyl}-pentyl)-benzoic Acid (**9c**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 4-methyl-1-naphthoic acid (33 mg; 0.18 mmol) gave 2-(5-{2-[(4-methyl-naphthalene-1-carbonyl)-amino]-phenyl}-pentyl)-benzoic acid methyl ester as a clear sticky oil (50 mg; 48%). Saponification of this intermediate with sodium hydroxide yielded the product as white solid (9.40 mg; 24%). LCMS method 3: 2.63 min; 452.00 (M + H). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.78 (s, 1H), 9.96 (s, 1H), 8.31−8.25 (m, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.75 (dd, J = 7.8, 1.5 Hz, 1H), 7.65−7.58 (m, 2H), 7.55 (m, 1H), 7.45 (d, J = 7.6 Hz, 1H), 7.43−7.36 (m, 2H), 7.32−7.18 (m, 5H), 2.92−2.86 (m, 2H), 2.71 (s, 3H), 2.69−2.63 (m, 2H), 1.59 (m, 4H), 1.38 (m, 2H), 1.24 (s, 1H). HRMS calcd for $C_{30}H_{27}NO_3$, 452.2220; found, 452.2218.

*2-[5-(2-Benzoylamino-phenyl)-pentyl]-benzoic Acid (**9d**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and benzoic acid (22 mg; 0.18 mmol) gave methyl 2-[5-(2-benzamidophenyl)pentyl]benzoate as a brown oil (61 mg; 84%). Saponification of this intermediate with sodium hydroxide yielded the product as a white solid (25 mg; 36%). LCMS method 2: 1.772 min; 388.1 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 12.76 (s, 1H), 9.87 (s, 1H), 7.95 (dd, J = 7.9, 1.5 Hz, 2H), 7.73 (dd, J = 7.9, 1.4 Hz, 1H), 7.60−7.55 (m, 1H), 7.50 (t, J = 7.7 Hz, 2H), 7.37 (m, 1H), 7.31−7.16 (m, 6H), 2.87−2.82 (m, 2H), 2.62−2.57 (m, 2H), 1.58−1.47 (m, 4H), 1.30 (p, J = 7.6 Hz, 2H). HRMS calcd for $C_{25}H_{25}NO_3$, 388.1907; found, 388.1899.

*2-{5-[2-(2-Methyl-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9e**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 2-methyl-benzoic acid (24 mg; 0.18 mmol) gave methyl 2-{5-[2-(2-methylbenzamido)phenyl]pentyl}benzoate as a light brown oil (59 mg; 79%). Saponification of this intermediate with sodium yielded the product as a colorless solid (23 mg; 32%). LCMS method 2: 1.821 min; 402.1 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 12.79 (s, 1H), 9.75 (s, 3H), 7.76 (dd, J = 7.8, 1.5 Hz, 3H), 7.45−7.39 (m, 6H), 7.39−7.33 (m, 6H), 7.29 (s, 2H), 7.29−7.24 (m, 13H), 7.24−7.16 (m, 6H), 2.92−2.88 (m, 6H), 2.64−2.59 (m, 6H), 2.40 (s, 9H), 1.56 (h, J = 7.7 Hz, 12H), 1.36 (p, J = 7.5 Hz, 6H). HRMS calcd for $C_{26}H_{27}NO_3$, 402.2064; found, 402.2058.

*2-{5-[2-(4-Methyl-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9f**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 4-methyl-benzoic acid (24 mg; 0.18 mmol) gave methyl 2-{5-[2-(4-methylbenzamido)phenyl]pentyl}benzoate as a light brown oil (60 mg; 79%). Saponification of this intermediate with sodium hydroxide yielded the product as a colorless solid (20 mg; 27%). LCMS method 2: 1.826 min; 402.1 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 12.76 (s, 1H), 9.77 (s, 1H), 7.87−7.83 (m, 2H), 7.74 (dd, J = 7.7, 1.4 Hz, 1H), 7.37 (td, J = 7.5, 1.5 Hz, 1H), 7.31−7.15 (m, 8H), 2.86−2.81 (m, 2H), 2.61−2.56 (m, 2H), 2.38 (s, 3H), 1.57−1.46 (m, 4H), 1.29 (p, J = 7.5 Hz, 2H). HRMS calcd for $C_{26}H_{27}NO_3$, 402.2064; found, 402.2059.

*2-{5-[2-(4-Ethyl-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9g**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 4-ethyl-benzoic acid (27 mg; 0.18 mmol) gave methyl 2-{5-[2-(4-ethylbenzamido)phenyl]pentyl}benzoate as a brown oil (57 mg; 74%). Saponification of this intermediate with sodium hydroxide yielded the product as a colorless solid (35 mg; 47%). LCMS Method 2: 1.890 min; 416.2 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 9.79 (s, 1H), 7.89−7.85 (m, 2H), 7.74 (dd, J = 7.8, 1.4 Hz, 1H), 7.37 (m, 1H), 7.34−7.16 (m, 9H), 2.86−2.82 (m, 2H), 2.68 (q, J = 7.6 Hz, 2H), 2.62−2.57 (m, 2H), 1.57−1.47 (m, 4H), 1.29 (p, J = 7.5 Hz, 2H), 1.21 (t, J = 7.6 Hz, 3H). HRMS calcd for $C_{27}H_{29}NO_3$, 416.2220; found, 416.2215.

*2-{5-[2-(4-Fluoro-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9h**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 4-fluoro-benzoic acid (25 mg; 0.18 mmol) gave methyl 2-{5-[2-(4-fluorobenzamido)phenyl]pentyl}benzoate as a brown oil (69 mg; 92%). Saponification of this intermediate with sodium hydroxide yielded the product as a white solid (51 mg; 70%). LCMS method 2: 1.794; 406.1 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 12.76 (s, 1H), 9.90 (s, 1H), 8.05−7.99 (m, 2H), 7.74 (dd, J = 7.7, 1.4 Hz, 1H), 7.38 (td, J = 7.5, 1.5 Hz, 1H), 7.35−7.30 (m, 2H), 7.30−7.16 (m, 6H), 2.86−2.81 (m, 2H), 2.61−2.56 (m, 2H), 1.57−1.46 (m, 4H), 1.29 (p, J = 7.5 Hz, 2H). HRMS calcd for $C_{25}H_{24}FNO_3$, 406.1813; found, 406.1811.

*2-{5-[2-(2-Fluoro-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9i**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 2-fluoro-benzoic acid (25 mg; 0.18 mmol) gave methyl 2-{5-[2-(2-fluorobenzamido)phenyl]pentyl}benzoate as a brown oil (58 mg; 77%). Saponification of this intermediate with sodium hydroxide yielded the product as a white solid (49 mg; 68%). LCMS method 2: 1.872 min; 388.1 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 12.78 (s, 1H), 9.80 (s, 1H), 7.75 (dd, J = 7.7, 1.4 Hz, 1H), 7.67 (m, 1H), 7.57 (tdd, J = 7.4, 5.1, 1.8 Hz, 1H), 7.44−7.38 (m, 2H), 7.36−7.29 (m, 2H), 7.26 (m, 3H), 7.21 (m, 2H), 2.92−2.87 (m, 2H), 2.65−2.60 (m, 2H), 1.60−1.52 (m, 4H), 1.35 (p, J = 7.5 Hz, 2H). HRMS calcd for $C_{25}H_{24}FNO_3$, 406.1813; found, 406.1809.

*2-{5-[2-(2-Chloro-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9j**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 2-chloro-benzoic acid (28 mg; 0.18 mmol) gave methyl 2-{5-[2-(2-chlorobenzamido)phenyl]pentyl}benzoate as a brown oil (70 mg; 90%). Saponification of this intermediate with sodium hydroxide yielded the product as a white solid (68 mg; 89%). LCMS method 2: 1.818 min; 404.1 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 12.79 (s, 1H), 9.95 (s, 1H), 7.76 (dd, J = 8.0, 1.5 Hz, 1H), 7.54 (m, 2H), 7.49 (m, 1H), 7.46−7.40 (m, 2H), 7.38 (dd, J = 7.7, 1.5 Hz, 1H), 7.29−7.24 (m, 3H), 7.21 (m, 2H), 2.93−2.88 (m, 2H), 2.66−2.61 (m, 2H), 1.57 (h, J = 7.5 Hz, 4H), 1.36 (p, J = 7.5 Hz, 2H). HRMS calcd for $C_{25}H_{24}ClNO_3$, 422.1517; found, 422.1508.

*2-{5-[2-(3-Chloro-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9k**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 3-chloro-benzoic acid (28 mg; 0.18 mmol) gave methyl 2-{5-[2-(3-chlorobenzamido)phenyl]pentyl}benzoate as a yellow oil (69 mg; 90%). Saponification of this intermediate with sodium hydroxide yielded the product as a white solid (67 mg; 89%). LCMS method 2: 1.866 min; 404.0 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 12.76 (s, 1H), 10.01 (s, 2H), 7.98 (t, J = 1.9 Hz, 2H), 7.90 (d, J

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= 7.7 Hz, 2H), 7.74 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.65 (m, 2H), 7.54 (t, *J* = 7.9 Hz, 2H), 7.37 (m, 2H), 7.30−7.19 (m, 10H), 7.18 (dd, *J* = 7.6, 1.3 Hz, 2H), 2.87−2.82 (m, 4H), 2.61−2.56 (m, 4H), 1.58−1.47 (m, 8H), 1.30 (p, *J* = 7.5 Hz, 4H). HRMS calcd for $C_{25}H_{24}ClNO_3$, 422.1517; found, 422.1515.

*2-{5-[2-(3,4-Dichloro-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9l**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 3,4-dichloro-benzoic acid (34 mg; 0.18 mmol) gave methyl 2-{5-[2-(3,4-dichlorobenzamido)phenyl]pentyl}benzoate as an orange oil (53 mg; 85%). Saponification of this intermediate with sodium hydroxide yielded the product as a white solid (47 mg; 57%). LCMS method 2: 1.961 min; 438.0 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 10.06 (s, 1H), 8.17 (d, *J* = 2.1 Hz, 1H), 7.90 (m, 1H), 7.78 (t, *J* = 8.5 Hz, 1H), 7.73 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.37 (m, 1H), 7.31−7.26 (m, 2H), 7.25 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.24−7.20 (m, 2H), 7.16 (dd, *J* = 7.7, 1.3 Hz, 1H), 2.86−2.81 (m, 2H), 2.60−2.55 (m, 2H), 1.57−1.46 (m, 4H), 1.30 (p, *J* = 7.5 Hz, 2H). HRMS calcd for $C_{25}H_{23}Cl_2NO_3$, 456.1128; found, 456.1116.

*2-{5-[2-(2,6-Dichloro-benzoylamino)-phenyl]-pentyl}-benzoic Acid (**9m**).* The synthesis was carried out as described for **9a**. 2-[5-(2-Amino-phenyl)-pentyl]-benzoic acid methyl ester (57 mg; 0.18 mmol) and 2,6-dichloro-benzoic acid (33 mg; 0.18 mmol) gave methyl 2-{5-[2-(2,6-dichlorobenzamido)phenyl]pentyl}benzoate as an orange oil (52 mg; 85%). Saponification of this intermediate with sodium hydroxide yielded the product as a white solid (45 mg; 57%). LCMS method 2: 1.854 min; 438.1 (M + H). ^{1}H NMR (700 MHz, DMSO-d_6): δ 10.16 (s, 1H), 7.76 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.49 (dd, *J* = 8.7, 7.5 Hz, 1H), 7.43 (td, *J* = 7.4, 1.5 Hz, 1H), 7.40 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.29−7.25 (m, 3H), 7.23 (td, *J* = 7.5, 1.8 Hz, 1H), 7.20 (td, *J* = 7.3, 1.5 Hz, 1H), 2.92−2.88 (m, 2H), 2.67−2.62 (m, 2H), 1.56 (tq, *J* = 14.8, 7.6, 6.8 Hz, 4H), 1.36 (p, *J* = 7.7 Hz, 2H). HRMS calcd for $C_{25}H_{23}Cl_2NO_3$, 456.1128; found, 456.1124.

*3-(2-{2-[(Naphthalene-1-carbonyl)-amino]-phenyl}-ethyl)-benzoic Acid (**16a**).* Thionyl chloride (0.47 mL; 6.48 mmol) was dropped into the solution of 3-vinyl-benzoic acid **10** (0.80 g; 5.40 mmol) in methanol (10 mL) over 10 min at 0 °C. After 3 h, the second portion of thionyl chloride (0.47 mL; 6.48 mmol) was added at RT, and the reaction mixture was stirred overnight. Methanol was evaporated, and the residue was poured in water. The pH was adjusted to 7 with 1 M NaOH and the resulting mixture was extracted with diethyl ether (3 × 20 mL). Organic extracts were combined and washed with water and brine, dried over $MgSO_4$, and evaporated to give 3-vinyl-benzoic acid methyl ester **11** (720 mg; 82% yield) as a light brown oil.

2-Bromo-phenylamine **12** (0.15 g; 0.87 mmol), 3-vinyl-benzoic acid methyl ester **11** (0.17 g; 1.05 mmol), palladium(II) acetate (4 mg; 0.02 mmol), tri-*o*-tolyl-phosphane (21 mg; 0.07 mmol), and triethylamine (1 mL) were placed in a reacting vial. The resulting mixture was argonated for 15 min and after capping the vial, the reaction mixture was stirred overnight at 125 °C. After dissolving in DCM and filtration, all solvents were removed under reduced pressure. The brown-orange semisolid product was purified by chromatography giving the product **13** as a yellow-brown oil, which is crystallized after a few minutes. (0.165 g, 75% yield). LC−MS 4: 3.56 min; 254.00 (M + H). ^{1}H NMR (400 MHz, DMSO): δ 8.18 (d, *J* = 1.4 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.81 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.50 (dd, *J* = 16.2, 7.8 Hz, 2H), 7.47 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.08 (d, *J* = 16.1 Hz, 1H), 6.98 (dd, *J* = 6.8, 1.4 Hz, 1H), 6.66 (dd, *J* = 8.1, 1.0 Hz, 1H), 6.55 (d, *J* = 7.4 Hz, 1H), 5.42 (s, 2H), 3.88 (s, 3H).

3-[(*E*)-2-(2-Amino-phenyl)-vinyl]-benzoic acid methyl ester **13** (60 mg; 0.24 mmol) was dissolved in anhydrous DCM (3 mL), followed by triethylamine (0.09 mL; 0.71 mmol). The reaction mixture was stirred at RT for 20 min, and then naphthalene-1-carbonyl chloride (39 μL; 0.25 mmol) was added in one portion. After 6 h at RT, the reaction mixture was diluted with ethyl acetate and the organic layer was washed with 2 M NaOH and water, 2 M HCl and water, saturated $NaHCO_3$, and water and brine. After drying over anhydrous Na_2SO_4 and concentrated under reduced pressure, the crude product was treated with a small volume of diethyl ether, the precipitate was filtered and washed using a funnel with a small volume of diethyl ether to afford 3-((*E*)-2-{2-[(naphthalene-1-carbonyl)-amino]-phenyl}-vinyl)-benzoic acid methyl ester (84 mg; 85% yield) as a white solid. LC−MS 4: 4.02 min; product not ionized.

To a solution of 3-((*E*)-2-{2-[(naphthalene-1-carbonyl)-amino]-phenyl}-vinyl)-benzoic acid methyl ester (50 mg; 0.12 mmol) in methanol (10 mL), palladium on carbon (10% dry, 10 mg; 0.01 mmol) was added. The resulting mixture was subjected to hydrogenation using a Parr apparatus for 3 h. Then, the reaction mixture was filtered through a pad of Celite and silica gel, which was then washed several times with ethyl acetate. The filtrate was evaporated to dryness under reduced pressure, redissolved in a small volume of DCM, and once again evaporated to dryness under reduced pressure to afford 3-(2-{2-[(naphthalene-1-carbonyl)-amino]-phenyl}-ethyl)-benzoic acid methyl ester (49 mg; 95% yield) as a white solid. LC−MS 5: 9.10 min; 410.10 (M + H). ^{1}H NMR (400 MHz, DMSO): δ 10.17 (s, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.09 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 7.7 Hz, 1H), 7.84 (d, *J* = 1.4 Hz, 1H), 7.82−7.76 (m, 2H), 7.65−7.48 (m, 5H), 7.41 (d, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.30 (d, *J* = 7.1 Hz, 1H), 7.24 (d, *J* = 7.1 Hz, 1H), 3.80 (s, 3H), 2.99 (s, 4H).

To a solution of 3-(2-{2-[(naphthalene-1-carbonyl)-amino]-phenyl}-ethyl)-benzoic acid methyl ester (40 mg; 0.10 mmol) in methanol (3 mL) and water (3 mL), sodium hydroxide (1 M in water, 0.49 mL; 0.97 mmol) was added. The resulting mixture was heated at 45 °C for 3 h. Methanol was evaporated, and the aqueous solution was acidified with 2 M HCl. The solution was extracted with ethyl acetate. The organic layers were combined, washed with a small amount of water and brine, dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure. The residue was redissolved in a small volume of DCM, which was once again evaporated to dryness under reduced pressure affording the product **16a** (37 mg; 0.09 mmol; 89% yield) as a white solid. LC−MS 5: 7.80 min; 394.10 (M − H). ^{1}H NMR (400 MHz, DMSO): δ 12.85 (s, 1H), 10.17 (s, 1H), 8.26 (d, *J* = 7.8 Hz, 1H), 8.07 (d, *J* = 8.2 Hz, 1H), 8.02 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.87 (d, *J* = 1.4 Hz, 1H), 7.80 (d, *J* = 7.0 Hz, 1H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.62 (m, 1H), 7.59−7.53 (m, 2H), 7.51 (d, *J* = 7.5 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.41−7.34 (m, 2H), 7.30 (d, *J* = 7.0 Hz, 1H), 7.24 (d, *J* = 7.0 Hz, 1H), 3.02−2.98 (m, 4H). HRMS calcd for $C_{26}H_{21}NO_3$, 396.1594; found, 396.1583.

*3-{2-[2-(Naphthalene-1-sulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16b**).* The compound was prepared as described for **16a** with the exception that the respective sulfonylchloride was used instead of the carboxylchoride. 3-{(*E*)-2-[2-(Naphthalene-1-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester (38 mg; 0.09 mmol) yielded the final product in 76% (25 mg) as beige foam. LC−MS 4: 3.722 min; 432.1 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.90 (s, 1H), 10.02 (s, 1H), 8.74 (d, *J* = 8.4 Hz, 1H), 8.20 (d, *J* = 8.2 Hz, 1H), 8.04 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.01 (dd, *J* = 7.3, 1.2 Hz, 1H), 7.75 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.71 (s, 1H), 7.67−7.53 (m, 3H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.20−7.13 (m, 2H), 7.09 (td, *J* = 7.4, 1.5 Hz, 1H), 7.03 (td, *J* = 7.6, 1.8 Hz, 1H), 6.86 (dd, *J* = 7.9, 1.4 Hz, 1H), 2.59 (s, 4H). HRMS calcd for $C_{25}H_{21}NO_4S$, 432.1264; found, 432.1261.

*4-{2-[2-(Naphthalene-1-sulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16c**).* The compound was prepared as described for **16b**. Methyl 4-[2-[2-(naphthalene-1-sulfonamido)phenyl]ethenyl]benzoate (30 mg; 0.07 mmol) yielded the final product in 83% (24 mg) as white foam. LC−MS 3: 1.76 min; 432.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.77 (s, 1H), 9.96 (s, 2H), 8.80−8.72 (m, 3H), 8.22 (dd, *J* = 8.4, 1.3 Hz, 3H), 8.13−8.04 (m, 3H), 8.01 (dd, *J* = 7.4, 1.2 Hz, 3H), 7.82−7.75 (m, 6H), 7.72−7.61 (m, 6H), 7.57 (dd, *J* = 8.2, 7.3 Hz, 3H), 7.17−6.99 (m, 15H), 6.87 (dd, *J* = 7.9, 1.4 Hz, 3H), 2.66−2.53 (m, 12H). HRMS calcd for $C_{25}H_{21}NO_4S$, 432.1264; found, 432.1263.

*4-{2-[2-(Naphthalene-2-sulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16d**).* The compound was prepared as described for **16b**. Methyl 4-[2-[2-(naphthalene-2-sulfonamido)phenyl]ethenyl]-benzoate (31 mg; 0.07 mmol) yielded the final product in 79% (23 mg) as white foam. LC−MS 3: 1.78 min; 432.00 (M + H). ^{1}H NMR

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(400 MHz, DMSO-d_6): δ 12.75 (s, 1H), 9.75 (s, 2H), 8.32 (d, J = 1.8 Hz, 3H), 8.14−8.07 (m, 6H), 8.06−8.00 (m, 3H), 7.81−7.74 (m, 9H), 7.70 (m, 3H), 7.65 (m, 3H), 7.20 (dd, J = 7.6, 1.7 Hz, 3H), 7.12 (dd, J = 8.7, 7.3 Hz, 9H), 7.06 (m, 3H), 6.91 (dd, J = 7.9, 1.4 Hz, 3H), 2.82−2.74 (m, 6H), 2.74−2.65 (m, 6H). HRMS calcd for $C_{25}H_{21}NO_4S$, 432.1264; found, 432.1264.

*2-{2-[2-(Naphthalene-1-sulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16e**).* The compound was prepared as described for **16b**. Methyl 4-[2-[2-(naphthalene-2-sulfonamido)phenyl]ethenyl]-benzoate (30 mg; 0.07 mmol) yielded the final product in 78% (22 mg) as white foam. LC−MS 3: 1.894 min; 432.0 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.92−12.84 (m, 1H), 9.84−9.78 (m, 1H), 8.77−8.71 (m, 1H), 8.22−8.18 (m, 1H), 8.08−8.03 (m, 1H), 8.01 (dd, J = 7.3, 1.2 Hz, 1H), 7.80 (dd, J = 7.8, 1.5 Hz, 1H), 7.66−7.60 (m, 2H), 7.59−7.54 (m, 1H), 7.40 (td, J = 7.5, 1.5 Hz, 1H), 7.28 (td, J = 7.5, 1.3 Hz, 1H), 7.14 (dd, J = 7.6, 1.9 Hz, 1H), 7.09 (td, J = 7.3, 1.5 Hz, 1H), 7.04 (td, J = 7.5, 2.0 Hz, 1H), 6.93−6.89 (m, 1H), 6.90−6.86 (m, 1H), 2.88−2.81 (m, 2H), 2.58−2.52 (m, 2H). HRMS calcd for $C_{25}H_{21}NO_4S$, 432.1264; found, 432.1264.

*2-{5-[2-(Naphthalene-1-sulfonylamino)-phenyl]-pentyl}-benzoic Acid (**10**).* Synthesis was carried out as described for **9a** with the exception that the respective sulfonylchloride was used and not the carboxylchloride. Methyl 2-{5-[2-(naphthalene-1-sulfonamido)-phenyl]pentyl}benzoate yielded the product (25 mg) as a colorless solid. LC−MS 3: 1.937 min; 456.0 (M-OH); 496.0 (M + Na). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.86−12.69 (m, 1H), 9.91−9.79 (m, 1H), 8.76−8.71 (m, 1H), 8.18 (d, J = 8.2 Hz, 1H), 8.09−8.04 (m, 1H), 7.96 (dd, J = 7.4, 1.3 Hz, 1H), 7.77 (dd, J = 7.8, 1.5 Hz, 1H), 7.69−7.61 (m, 2H), 7.53 (t, J = 7.8 Hz, 1H), 7.47 (td, J = 7.5, 1.5 Hz, 1H), 7.31−7.23 (m, 2H), 7.10−6.99 (m, 3H), 6.98−6.93 (m, 1H), 2.82−2.74 (m, 2H), 2.23−2.13 (m, 2H), 1.32−1.22 (m, 2H), 1.00−0.90 (m, 2H), 0.87−0.77 (m, 2H). HRMS calcd for $C_{28}H_{27}NO_4S$, 474.1734; found, 474.1721.

*3-{2-[2-(Naphthalene-2-sulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16f**).* 3-{(*E*)-2-[2-(Naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester (42 mg; 0.08 mmol) was dissolved in ethanol (10 mL) under argon in a three-necked flask, which was equipped with a hydrogen baloon and argon and vacuum pins. After palladium on carbon (10%, 40 mg; 0.04 mmol) was added, the flask was evacuated and back filled with argon three times. Then, the hydrogen valve was opened and the reaction mixture was stirred overnight at RT vigorously. Then, the catalyst was filtered off and the filtrate was evaporated to give 3-{2-[2-(naphthalene-2-sulfonylamino)-phenyl]-ethyl}-benzoic acid methyl ester as a crude product (41 mg; 0.09 mmol). Without further purification, the crude was dissolved in ethanol (4 mL), and 5 N NaOH (0.34 mL; 1.69 mmol; 20.00 equiv) was added under stirring. The reaction mixture was stirred overnight and then diluted with water, and acidified with 2 M HCl. The precipitate was taken up in ethyl acetate and extracted with water. After repeated re-extraction with ethyl acetate, the combined organic phases were washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography giving 3-{2-[2-(naphthalene-2-sulfonylamino)-phenyl]-ethyl}-benzoic acid (29 mg; 0.06 mmol; 75% yield) as a white solid. LCMS 5: 8.30 min; 432.4 (M + H); 430.30 (M − H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.83 (s, 1H), 8.31 (d, J = 1.9 Hz, 1H), 8.14−8.06 (m, 1H), 8.05−7.99 (m, 1H), 7.82−7.78 (m, 2H), 7.76 (dt, J = 7.6, 1.5 Hz, 1H), 7.69 (ddd, J = 8.2, 6.9, 1.4 Hz, 1H), 7.64 (ddd, J = 8.2, 6.8, 1.4 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.30−7.22 (m, 3H), 7.13 (td, J = 7.4, 1.5 Hz, 1H), 7.06 (td, J = 7.6, 1.7 Hz, 1H), 6.90 (dd, J = 7.9, 1.4 Hz, 1H), 2.83−2.73 (m, 2H), 2.74−2.64 (m, 2H). HRMS calcd for $C_{25}H_{21}NO_4S$, 432.1264; found, 432.1264.

*3-[2-(2-Benzenesulfonylamino-phenyl)-ethyl]-benzoic Acid (**16g**).* The compound was prepared as described for **16f** and obtained as a colorless solid.

LCMS 3: 1.664 min; 382.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.88−12.81 (m, 1H), 9.68 (s, 1H), 7.81−7.79 (m, 1H), 7.79−7.75 (m, 1H), 7.72−7.68 (m, 2H), 7.65−7.60 (m, 1H), 7.57−7.51 (m, 2H), 7.43−7.38 (m, 2H), 7.24 (dd, J = 7.5, 1.8 Hz, 1H), 7.14 (td, J = 7.4, 1.6 Hz, 1H), 7.09 (td, J = 7.6, 1.9 Hz, 1H), 6.92 (dd, J = 7.7, 1.5 Hz, 1H), 2.78−2.66 (m, 4H). HRMS calcd for $C_{21}H_{19}NO_4S$, 382.1108; found, 382.1099.

*3-{2-[2-(4-Fluoro-benzenesulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16h**).* The compound was prepared as described for **16f** and obtained as a white solid. LCMS 3: 1.69 min; 400.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.93−12.77 (m, 1H), 9.85−9.73 (m, 1H), 7.82−7.80 (m, 1H), 7.80−7.75 (m, 1H), 7.71−7.67 (m, 2H), 7.64−7.60 (m, 2H), 7.44−7.38 (m, 2H), 7.27 (dd, J = 7.6, 1.8 Hz, 1H), 7.16 (td, J = 7.4, 1.6 Hz, 1H), 7.12 (td, J = 7.6, 1.8 Hz, 1H), 6.89 (dd, J = 7.7, 1.6 Hz, 1H), 2.82−2.69 (m, 4H). HRMS calcd for $C_{21}H_{18}FNO_4S$, 400.1013; found, 400.1008.

*3-{2-[2-(5-Fluoro-2-methyl-benzenesulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16i**).* The compound was prepared as described for **16f** and obtained as an amorphous colorless solid.

LCMS 3: 1.75 min; 414.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.12−12.52 (m, 1H), 10.17−9.54 (m, 1H), 7.83−7.80 (m, 1H), 7.80−7.77 (m, 1H), 7.46−7.35 (m, 5H), 7.27 (dd, J = 7.6, 1.8 Hz, 1H), 7.18 (td, J = 7.5, 1.5 Hz, 1H), 7.11 (td, J = 7.6, 1.7 Hz, 1H), 6.85 (dd, J = 7.9, 1.4 Hz, 1H), 2.81−2.74 (m, 4H), 2.50−2.48 (m, 3H). HRMS calcd for $C_{22}H_{20}FNO_4S$, 414.1170; found, 414.1160.

*3-{2-[2-(4-Methoxy-2,3-dimethyl-benzenesulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16j**).* The compound was prepared as described for **16f**. 3-[2-(2-Amino-phenyl)-ethyl]-benzoic acid methyl ester (60 mg; 0.22 mmol) and 4-methoxy-2,3-dimethylbenzene-1-sulfonyl chloride (58 mg; 0.25 mmol) yielded a product as a white solid in 36% yield (37 mg) over the last two steps. LC−MS 2: 1.79 min; 440.0 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.86 (s, 1H), 9.52 (s, 1H), 7.83−7.81 (m, 1H), 7.81−7.75 (m, 1H), 7.58 (d, J = 8.9 Hz, 1H), 7.42−7.39 (m, 2H), 7.22 (dd, J = 7.4, 2.0 Hz, 1H), 7.14−7.05 (m, 2H), 6.92 (dd, J = 7.6, 1.7 Hz, 1H), 6.88 (d, J = 8.9 Hz, 1H), 3.80 (s, 3H), 2.82−2.71 (m, 4H), 2.47 (s, 3H), 2.03 (s, 3H). HRMS calcd for $C_{24}H_{25}NO_5S$, 440.1526; found, 440.1520.

*3-{2-[2-(2,5-Dichloro-benzenesulfonylamino)-phenyl]-ethyl}-benzoic Acid (**16k**).* The compound was prepared as described for **16f.** LCMS 3: 1.80 min; 452.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.05−12.57 (m, 1H), 10.43−9.90 (m, 1H), 7.86−7.83 (m, 1H), 7.81−7.77 (m, 2H), 7.73−7.71 (m, 1H), 7.47−7.37 (m, 3H), 7.29 (dd, J = 7.6, 1.7 Hz, 1H), 7.19 (td, J = 7.5, 1.4 Hz, 1H), 7.12 (td, J = 7.6, 1.7 Hz, 1H), 6.88 (dd, J = 7.8, 1.4 Hz, 1H), 2.91−2.79 (m, 4H). HRMS calcd for $C_{21}H_{17}Cl_2NO_4S$, 450.0328; found, 450.0315.

*3-{(E)-2-[2-(4-Methoxy-2,3-dimethyl-benzenesulfonylamino)-phenyl]-vinyl}-benzoic Acid (**15a**).* To a solution of 3-[(*E*)-2-(2-amino-phenyl)-vinyl]-benzoic acid methyl ester (60 mg; 0.22 mmol) in acetonitrile (5 mL) were added 4-methoxy-2,3-dimethyl-benzenesulfonyl chloride (61 mg; 0.25 mmol) and sodium hydrogen carbonate (23 mg; 0.27 mmol). The reaction was stirred for 7 days at RT. Methanol (5 mL) and sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (2.3 mL) were added and the mixture was stirred for 6 h at RT. The reaction mixture was evaporated to dryness. The residue was purified by chromatography giving the product in 53% (53 mg) yield as an amorphous white solid. LCMS 2: 1.78 min; 438.00 (M + H). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.10−12.99 (m, 1H), 9.81 (s, 1H), 8.10−8.08 (m, 1H), 7.84 (dt, J = 7.6, 1.4 Hz, 1H), 7.68−7.64 (m, 1H), 7.61−7.58 (m, 1H), 7.52−7.48 (m, 2H), 7.34 (d, J = 16.2 Hz, 1H), 7.26−7.18 (m, 3H), 6.99 (d, J = 16.2 Hz, 1H), 6.75 (d, J = 8.9 Hz, 1H), 3.67 (s, 3H), 2.40 (s, 3H), 1.85 (s, 3H). HRMS calcd for $C_{24}H_{23}NO_5S$, 438.1370; found, 438.1366.

*3-[(E)-2-[2-(naphthalene-1-sulfonamido)phenyl]ethenyl]benzoic Acid (**15b**).* Step 1. Thionyl chloride (0.59 mL; 8.10 mmol) was dropped into the mixture of 3-vinyl-benzoic acid **10** (1.00 g; 6.75 mmol) in anhydrous methanol (10 mL) over 10 min at 0 °C and warmed up to RT over 3 h. Then, a second portion of thionyl chloride (0.59 mL; 8.10 mmol) was added at RT. After 48 h, methanol was evaporated, and the residue was poured in water. The pH was adjusted to 7 with 1 M NaOH and the resulting mixture was extracted with diethyl ether (3 × 20 mL). Organic extracts were combined and washed with water and brine, dried over $MgSO_4$, and evaporated to give 3-vinyl-benzoic acid methyl ester **11** (1.05 g; 6.44 mmol; 95.5%) as a light brown oil. LCMS 2: 3.56 min.

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Step 2. 3-Vinyl-benzoic acid methyl ester (0.95 g; 5.86 mmol), triethylamine (0.30 mL), and 2-bromo-phenylamine **12** (0.50 g; 2.91 mmol) were placed in a reacting vial. The mixture was purged with argon for 15 min and *trans*-{bis(acetato)}bis[o-{(di-{o-tolylphosphino)}benzyl]}dipalladium(II) (Herrmann's palladacycle) (81.75 mg; 0.09 mmol) was added and vial was capped. The reaction mixture was stirred overnight at 120 °C, dissolved in DCM, and filtered. The filtrate was concentrated and residual triethylamine was coevaporated with diethyl ether. The brown-orange gummy residue was purified by chromatography to give 3-[(*E*)-2-(2-amino-phenyl)-vinyl]-benzoic acid methyl ester **13** (0.21 g; 28%) as a light brown semisolid. LCMS 4: 3.58 min.

Step 3. 3-[(*E*)-2-(2-Amino-phenyl)-vinyl]-benzoic acid methyl ester (0.05 g; 0.20 mmol) was dissolved in acetonitrile (5 mL) and $NaHCO_3$ (19.90 mg; 0.24 mmol) was added, followed by naphthalene-1-sulfonyl chloride (49.22 mg; 0.22 mmol). The reaction mixture was stirred at RT for 48 h, then it was diluted with Et_2O and extracted with water and brine. Organic extract was dried over Na_2SO_4, evaporated, and residue was purified by chromatography to give 3-{(*E*)-2-[2-(naphthalene-1-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester (60 mg; 68%) as a beige film. LCMS 4: 4.15 min; 422.1 [M − H].

Step 4. 3-{(*E*)-2-[2-(Naphthalene-1-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester (20.00 mg; 0.04 mmol) was stirred overnight in 5 M NaOH (2 mL) and MeOH (1 mL). The reaction mixture was poured into 5.5 mL of 2 M HCl. The acidic suspension of crude product was extracted twice with ethyl acetate. Combined organic extracts were washed with water and brine, then dried over Na_2SO_4, and filtered. The filtrate was evaporated in vacuo to obtain the product (19 mg; 96%) as a beige solid. LCMS 5: 8.2 min; 428.2 [M − H]. ^{1}H NMR (400 MHz, DMSO): δ: 13.04 (s, 1H), 10.36 (s, 1H), 8.81 (d, J = 8.7 Hz, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.98−7.90 (m, 3H), 7.81 (d, J = 7.7 Hz, 1H), 7.71−7.64 (m, 1H), 7.58 (dd, J = 7.7, 1.4 Hz, 1H), 7.55−7.44 (m, 2H), 7.40 (t, J = 7.7 Hz, 1H), 7.30−7.13 (m, 4H), 7.06 (d, J = 16.3 Hz, 1H), 6.87 (d, J = 16.2 Hz, 1H). HRMS calcd for $C_{25}H_{18}NO_4S^-$, 428.0962; found, 428.0956.

*3-[(E)-2-[2-(Naphthalene-2-sulfonamido)phenyl]ethenyl]benzoic Acid (**15c**).* Step 1. 3-[(*E*)-2-(2-Amino-phenyl)-vinyl]-benzoic acid methyl ester (0.05 g; 0.20 mmol), naphthalene-2-sulfonyl chloride (49.22 mg; 0.22 mmol), and $NaHCO_3$ (19.90 mg; 0.24 mmol) were stirred at RT for 18 h in acetonitrile (5 mL). The reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated and 3-{(*E*)-2-[2-(naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester was isolated by chromatography (62.90 mg 64%) as a beige solid. LCMS 4: 4.12 min.

Step 2. 3-{(*E*)-2-[2-(Naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester (20 mg; 0.04 mmol) was stirred overnight in a mixture of 5 M NaOH (2 mL) and MeOH (1 mL). Then, it was acidified with 5.5 mL of 2 M HCl. The resulting foggy mixture was extracted twice with ethyl acetate. The organic extract was washed with water, brine, and dried over Na_2SO_4. Evaporation gave the product (15.30 mg; 85%) as a beige gassy solid. LCMS 5: 8.2 min; 428.1 [M − H]; ^{1}H NMR (400 MHz, DMSO): δ: 13.07 (s, 1H), 10.12 (s, 1H), 8.23 (d, J = 1.4 Hz, 1H), 8.01−7.90 (m, 3H), 7.86−7.77 (m, 2H), 7.71 (dd, J = 8.7, 1.8 Hz, 1H), 7.68−7.63 (m, 1H), 7.54 (dtd, J = 16.2, 7.0, 1.2 Hz, 2H), 7.43−7.34 (m, 2H), 7.27−7.18 (m, 4H), 6.89 (d, J = 16.3 Hz, 1H). HRMS calcd for $C_{25}H_{18}NO_4S^-$, 428.0962; found, 428.0956.

*3-[(Z)-2-[2-(Naphthalene-2-sulfonamido)phenyl]ethenyl]-benzoic Acid (**15d**).* Step 1. Triphenylphosphine (2.58 g; 9.82 mmol) was added to the solution of methyl 3-bromomethyl-benzoate (2.50 g; 10.91 mmol) in anhydrous acetonitrile (50 mL). The mixture was argonated for 5 min, and the vessel was capped and heated with stirring for 3 h at 85−90 °C. Then, the reaction mixture was cooled to RT, and 50 mL of Et_2O was added. The mixture was stirred at RT for 2 h. During stirring, a white precipitate forms. After that, the reaction mixture was filtered and the white solid was transferred into a 150 mL reactor. Anhydrous acetonitrile (50 mL) was added, followed by 2-nitro-benzaldehyde (1.48 g; 9.82 mmol). The mixture was argonated for 10 min, 1,8-diazabicyclo[5.4.0]undec-7-ene and DBU (1.47 mL; 9.82 mmol) were added, the vessel was capped, and the mixture was heated with stirring for 3 h at 85−90 °C. The reaction mixture was evaporated, the residue was dissolved in DCM, and the product was adsorbed on silica gel. The solvent was evaporated and the residue was placed in a filtering funnel. The product was eluted with Et_2O. The filtrate was evaporated to give crude methyl 3-[2-(2-nitrophenyl)vinyl]benzoate (2.66 g; 86%) as a yellow solid. LCMS 4: 3.74 min, 3.84 min; 40.4%; 57.1% of *E* and *Z* isomers (not specified which is which).

Step 2. Methyl 3-[(*E*)-2-(2-nitrophenyl)vinyl]benzoate (0.56 g; 1.00 mmol) was dissolved in ethanol (25 mL). Iron dust (0.58 g; 10 mmol) and ammonium chloride (1.10 g; 20 mmol) were added. The mixture was stirred and heated to reflux for 3 h. The inorganic precipitate was filtered off, the filtrate was diluted with ethyl acetate, and extracted with water. The organic layer was dried over Na_2SO_4 and evaporated to give crude methyl 3-[(*E*/*Z*)-2-(2-aminophenyl)-vinyl]benzoate (0.531 g; 82.7%) as a light brown foam. LCMS 4: 3.43 min.

Step 3. The mixture of methyl 3-[(*E*)-2-(2-aminophenyl)vinyl]-benzoate and methyl 3-[(*Z*)-2-(2-aminophenyl)vinyl]benzoate (531 mg; 0.82 mmol) was dissolved in acetonitrile (25 mL) and $NaHCO_3$ (206 mg; 2.45 mmol) was added, followed by naphthalene-2-sulfonyl chloride (445 mg; 1.96 mmol). The mixture was stirred overnight, diluted with ethyl acetate, and extracted with water to remove inorganics. The organic extract was washed with brine and dried over Na_2SO_4, then evaporated to give 700 mg of brown oil, which was purified by chromatography. The obtained foam (543 mg) was crystallized from 8 mL of MeOH. The precipitate was filtered off, washed with cold methanol, and dried in vacuo to give 3-{(*E*)-2-[2-(naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester (175 mg; 46%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.10 (s, 1H), 8.23 (d, J = 1.9 Hz, 1H), 7.96 (dd, J = 8.0, 1.3 Hz, 1H), 7.94−7.88 (m, 2H), 7.81 (dt, J = 6.9, 1.6 Hz, 2H), 7.69 (dd, J = 8.7, 1.9 Hz, 1H), 7.67−7.64 (m, 1H), 7.56 (ddd, J = 8.2, 6.9, 1.5 Hz, 1H), 7.51 (ddd, J = 8.1, 6.9, 1.4 Hz, 1H), 7.46−7.37 (m, 2H), 7.30−7.24 (m, 2H), 7.24−7.16 (m, 2H), 6.89 (d, J = 16.2 Hz, 1H), 3.93 (s, 3H).

The filtrate from crystallization was evaporated to dryness to give a brown oil, which was triturated with 4 mL of cold MeOH to precipitate residual *E* isomer, which was filtered off and washed with a small amount of cold MeOH. The filtrate was evaporated to give 3-{(*Z*)-2-[2-(naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester (248 mg; 61%) as a brown gum. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.31 (d, J = 1.8 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 8.7 Hz, H), 7.90−7.85 (m, 1H), 7.80 (dd, J = 8.6, 1.9 Hz, 2H), 7.64−7.52 (m, 4H), 7.51 (d, J = 1.9 Hz, 1H), 7.17−7.11 (m, 2H), 7.06 (t, J = 7.7 Hz, 1H), 6.98−6.94 (m, 2H), 6.89−6.84 (m, 1H), 6.70 (d, J = 12.1 Hz, 1H), 6.52 (d, J = 12.2 Hz, 1H), 3.75 (s, 3H).

Step. 4: 3-{(*Z*)-2-[2-(Naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid methyl ester (50 mg; 0.10 mmol) was dissolved in methanol (2 mL), and 5 N NaOH (0.40 mL; 2.01 mmol) was added. The reaction mixture was stirred for 1 h, then it was diluted with water and acidified to pH 3−4 with 1 M HCl. The precipitate was taken up with ethyl acetate and the water layer was extracted with ethyl acetate. Combined organic extracts were washed with brine and dried over Na_2SO_4. The solvent was evaporated and the resulting oil was purified by chromatography giving 36 mg of a mixture of *E* & *Z* isomers (12:88%). The sample was repurified by chromatography to give 3-{(*Z*)-2-[2-(naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid (16.30 mg; 38%) as an off-white solid. LCMS 6: 14.6 min; 428.4 [M − H]; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.85 (s, 8H), 9.92 (s, 9H), 8.32 (d, J = 1.9 Hz, 10H), 8.23 (d, J = 1.8 Hz, 1H), 8.08−8.00 (m, 20H), 8.00−7.93 (m, 4H), 7.93 ? 7.88 (m, 10H), 7.84−7.77 (m, 13H), 7.71 (dd, J = 8.7, 1.9 Hz, 2H), 7.67−7.48 (m, 44H), 7.39 (d, J = 6.4 Hz, 3H), 7.27−7.16 (m, 6H), 7.13 (ddd, = 8.5, 7.0, 1.7 Hz, 11H), 7.09−7.01 (m, 20H), 6.99−6.84 (m, 32H), 6.67 (d, J = 12.2 Hz, 10H), 6.52 (d, J = 12.3 Hz, 10H). HRMS calcd for $C_{25}H_{18}NO_4S^-$, 428.0962; found, 428.0955.

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*4-[(E)-2-[2-(Naphthalene-2-sulfonamido)phenyl]ethenyl]benzoic Acid (**15e**).* Step 1. Thionyl chloride (0.82 mL; 11.18 mmol) was dropped into 4-vinyl-benzoic acid (1.50 g; 9.31 mmol) in anhydrous methanol (15 mL) over 10 min at 0 °C. After 3 h, the second portion of thionyl chloride (0.61 mL; 8.38 mmol) was added at RT, and the reaction mixture was stirred overnight. Methanol was evaporated, and the residue was poured in water. The pH was adjusted to 7 with 1 M NaOH and the mixture was extracted with diethyl ether (3 × 20 mL). Organic extracts were combined and washed with water and brine, dried over $MgSO_4$, and evaporated to give methyl 4-vinyl-benzoate (1.53 g; 93%) as a brown semi solid. LCMS 4:3.53 min.

Step 2. 2-Bromo-phenylamine (0.43 g; 2.50 mmol), 4-vinyl-benzoic acid methyl ester (0.40 g; 2.27 mmol), palladium(II) acetate (10.74 mg; 0.05 mmol), tri-*o*-tolyl-phosphane (55.31 mg; 0.18 mmol), and triethylamine (4 mL) were argonated for 15 min and the vial was capped. The reaction mixture was stirred overnight at 125 °C, dissolved in DCM, and filtered. The filtrate was concentrated, and the residual triethylamine was coevaporated with diethyl ether. The brown-orange semisolid product was purified by chromatography to give methyl 4-[(*E*)-2-(2-amino-phenyl)-vinyl]-benzoate (385 mg; 67%) as yellow crystals. LCMS 4: 3.57 min; 254 [M + H]; 295 [M + H + ACN].

Step 3. 4-[(*E*)-2-(2-Amino-phenyl)-vinyl]-benzoic acid methyl ester (100 mg; 0.39 mmol), naphthalene-2-sulfonyl chloride (107 mg; 0.47 mmol), and $NaHCO_3$ (49.75 mg; 0.59 mmol) were stirred overnight in acetonitrile (2 mL) at RT. Crude methyl 4-[(*E*)-2-[2-(naphthalene-2-sulfonamido)phenyl]ethenyl]benzoate (product A) was not isolated and already subjected to the saponification step. Crude RM was diluted with methanol, and 5 N NaOH was added. The resulting clear mixture was stirred for 2 h and RM was diluted with water and acidified with 2 M HCl. Formed precipitate of crude 4-{(*E*)-2-[2-(naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid (product B) was taken up to ethyl acetate. The water layer was extracted twice with ethyl acetate. Combined organic extracts were washed with water and brine, then dried over Na_2SO_4, and evaporated. The residue was purified by FCC (DCM → 95:5 DCM/MeOH) to obtain 4-{(*E*)-2-[2-(naphthalene-2-sulfonylamino)-phenyl]-vinyl}-benzoic acid (117.90 mg; 0.26 mmol; 65.4%) as a yellow solid. LC−MS 5: 8.2 min; 428.2 [M − H], 430.3 [M + H] ^{1}H NMR (400 MHz, DMSO-d_6) d: 12.89 (s, 1H), 10.07 (s, 1H), 8.03 (d, *J* = 8.7 Hz, 1H), 7.98 (d, *J* = 8.7 Hz, 1H), 7.87 (d, *J* = 7.4 Hz, 1H), 7.85−7.81 (m, 2H), 7.73 ? 7.64 (m, 2H), 7.61 (ddd, *J* = 8.2, 6.9, 1.4 Hz, 1H), 7.56 (ddd, *J* = 8.2, 6.8, 1.4 Hz, 1H), 7.34−7.28 (m, 2H), 7.28−7.24 (m, 2H), 7.23−7.17 (m, 2H), 6.92 (d, *J* = 16.3 Hz, 1H). HRMS calcd for $C_{25}H_{18}NO_4S^-$, 428.0962; found, 428.09553.

*4-[2-(2-Benzenesulfonamidophenyl)ethynyl]benzoic Acid (**17a**).* Step 1. A screw cap vessel was charged with 4-bromo-benzoic acid methyl ester (15.55 g; 0.07 mol), 2-ethynylphenylamine (7 mL; 0.06 mol), and diisopropylamine (13 mL; 0.090 mol). The resulting mixture was purged with argon for 10 min. Then, copper(I) iodide (569 mg; 3.00 mmol) and tetrakis(triphenylphosphine)palladium(0) (3.5 g; 3 mmol) were added and the reaction mixture was stirred at 70 °C overnight, diluted with ethyl acetate, and washed with water. Combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and evaporated in vacuo. The crude product was crystallized from toluene to give 4-(2-amino-phenylethynyl)-benzoic acid methyl ester (9.42 g; 58%) as a yellow solid. The filtrate after crystallization was concentrated in vacuo, and an additional amount of product was isolated by chromatography to give: 4-(2-amino-phenylethynyl)-benzoic acid methyl ester (4.40 g; 28%) as a yellow solid. LCMS 4: 3.68 min; 252.0 [M + H].

Step 2. 4-(2-Amino-phenylethynyl)-benzoic acid methyl ester (50 mg; 0.20 mmol), benzenesulfonyl chloride (52.72 mg; 0.30 mmol), and sodium bicarbonate (25.07 mg; 0.30 mmol) were dissolved in anhydrous acetonitrile (5 mL). The reaction mixture was stirred at RT for 48 h. The reaction mixture was diluted with ethyl acetate and washed with water twice. The organic layer was washed with brine, dried over sodium sulfate, and evaporated under reduced pressure. Crude 4-(2-benzenesulfonylamino-phenylethynyl)-benzoic acid methyl ester (56 mg; 48.9%) obtained as a white powder was used in the next step without purification. LCMS 4: 3.48 min; 390.00 [M − H].

Step 3. 4-(2-Benzenesulfonylamino-phenylethynyl)-benzoic acid methyl ester (56 mg; 0.10 mmol) was dissolved in THF (3 mL) and water (1 mL). Then, lithium hydroxide monohydrate (25 mg; 0.60 mmol) was added, and the reaction mixture was stirred at RT overnight. Then, THF was evaporated, the remainder was acidified to pH = 5, and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by chromatography to give the product (30 mg; 38%) as a white powder. LCMS 5: 6.3 min; 376.5; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.13 (s, 1H), 10.08 (s, 1H), 8.00 (d, *J* = 8.4 Hz, 2H), 7.72−7.70 (m, 2H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.56−7.52 (m, 1H), 7.48 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.44−7.39 (m, 3H), 7.33 (dd, 1H), 7.25 (td, *J* = 7.6, 1.2 Hz, 1H). HRMS calcd for $C_{21}H_{14}NO_4S^-$, 376.0649; found, 376.0646.

*4-[2-(3-Methoxy-benzenesulfonylamino)-phenylethynyl]-benzoic Acid (**17b**).* 4-(2-Amino-phenylethynyl)-benzoic acid methyl ester (51 mg; 0.20 mmol) was dissolved with sodium bicarbonate (25 mg; 0.30 mmol) in acetonitrile (5 mL), 3-methoxy-benzenesulfonyl chloride (0.04 mL; 0.27 mmol) was added, and the reaction mixture was stirred at RT for 72 h. After evaporating, the crude product 4-[2-(3-methoxy-benzenesulfonylamino)-phenylethynyl]-benzoic acid methyl ester was obtained (94 mg; 0.15 mmol) as a brown solid. The residue was suspended in methanol (2 mL) and treated with sodium hydroxide solution (5 M, 4 mL) to pH 12 and stirred at RT for 2 h. Then, the formed solution was acidified with hydrochloric acid (10% wt) to pH 3 and extracted three times by ethyl acetate. Combined organics extracts were dried over anhydrous Na_2SO_4. After evaporating, the crude was purified by chromatography giving the product in 27% (22 mg) yield as a white solid. LCMS 5: 7.90 min; 406.00 (M − H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.16 (s, 1H), 10.06 (s, 1H), 8.01−7.95 (m, 2H), 7.63−7.57 (m, 2H), 7.49 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.42 (ddd, *J* = 8.2, 7.3, 1.6 Hz, 1H), 7.38−7.32 (m, 2H), 7.29−7.20 (m, 2H), 7.18 (dd, *J* = 2.6, 1.7 Hz, 1H), 7.09 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H), 3.60 (s, 3H). HRMS calcd for $C_{22}H_{17}NO_5S$, 408.0900; found, 408.0889.

*4-[2-(4-Methoxy-benzenesulfonylamino)-phenylethynyl]-benzoic Acid (**17c**).* The compound was prepared as described for **17b**. 4-[2-(4-Methoxy-benzenesulfonylamino)-phenylethynyl]-benzoic acid methyl ester (164 mg; 0.24 mmol) yielded 35% (36 mg) of the product as a rose solid. LCMS 2: 1.72 min; 408.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.30−12.96 (m, 1H), 9.85 (s, 1H), 8.01−7.97 (m, 2H), 7.64−7.62 (m, 2H), 7.62−7.60 (m, 2H), 7.47 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.42−7.37 (m, 1H), 7.32 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.23 (td, *J* = 7.5, 1.4 Hz, 1H), 6.93−6.89 (m, 2H), 3.69 (s, 3H). HRMS calcd for $C_{22}H_{17}NO_5S$, 408.0900; found, 408.0890.

*4-[2-(4-Methoxy-2,3-dimethyl-benzenesulfonylamino)-phenylethynyl]-benzoic Acid (**17d**).* The compound was prepared as described for **17b**. 4-(2-Amino-phenylethynyl)-benzoic acid methyl ester (80 mg; 0.23 mmol) yielded 27% (32 mg) of the product as a white solid. LC−MS 2: 1.86 min; 436.00 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.10 (s, 1H), 9.85 (s, 1H), 8.00−7.95 (m, 2H), 7.65 (d, *J* = 8.9 Hz, 1H), 7.63−7.58 (m, 2H), 7.49−7.44 (m, 1H), 7.39−7.32 (m, 2H), 7.20−7.15 (m, 1H), 6.83 (d, *J* = 9.0 Hz, 1H), 3.76 (s, 3H), 2.45 (s, 3H), 1.93 (s, 3H). HRMS calcd for $C_{24}H_{21}NO_5S$, 436.1213; found, 436.1206.

*4-[2-(2,5-Dichloro-benzenesulfonylamino)-phenylethynyl]-benzoic Acid (**17e**).* 4-(2-Amino-phenylethynyl)-benzoic acid methyl ester (140 mg; 0.56 mmol), sodium bicarbonate (52 mg; 0.61 mmol), and 2,5-dichlorobenzenesulfonyl chloride (151 mg; 0.61 mmol) were mixed in CH_3CN (4.20 mL), and the reaction mixture was stirred at 60 °C for one night. DCM was added followed by HCl 1 M. The aqueous layer was extracted once again with DCM. Combined organic layers were dried over Na_2SO_4. Solvents were removed under vacuum to afford the ester intermediate. It was dissolved in THF (5 mL) and MeOH (5 mL), and NaOH (500 μL; 5 M; 2.50 mmol) was added. The reaction was stirred at 45 °C for one night and the solvents were removed under vacuum. Aqueous workup was performed: DCM (5 mL) and water (3 mL) were added; HCl 5 M (>500 μL) was added

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to reach pH = 1. The aqueous phase was extracted once again with DCM. The solvent was removed under vacuum to afford the desired product in 54% (133 mg) yield as a pink solid. ^{1}H NMR (300 MHz, DMSO): δ 13.20 (br s, 1H), 10.53 (s, 1H), 8.02−7.92 (m, 2H), 7.86 (d, J = 2.0 Hz, 1H), 7.64−7.51 (m, 5H), 7.42 (dd, J = 7.3, 1.5 Hz, 1H), 7.39−7.23 (m, 2H). HRMS calcd for $C_{21}H_{13}Cl_2NO_4S$, 446.0015; found, 445.9993.

*4-{2-[2-(Naphthalene-1-sulfonamido)phenyl]ethynyl}benzoic Acid (**17f**).* 4-(2-Amino-phenylethynyl)-benzoic acid methyl ester (80 mg; 0.31 mmol) was dissolved in anhydrous pyridine (1 mL) and naphthalene-1-sulfonyl chloride (95 mg; 0.42 mmol) was added. Then reaction was stirred at RT for overnight, 15 mL of water was added and acidified with 2 M HCl solution to pH 3. The mixture was extracted with ethyl acetate, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by chromatography to yield 4-[2-(naphthalene-1-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (127 mg; 87%) as a white solid. LCMS 4: 4.44 min; 440 [M − H].

The ester was dissolved in THF (5 mL), then water (2 mL) and lithium hydroxide monohydrate (34 mg; 0.41 mmol) was added, and the mixture was stirred at RT overnight. The THF was evaporated and 15 mL of water was added to the residue. The mixture was acidified with 2 M hydrochloric acid to pH 3 and extracted three times with ethyl acetate. The combined organics extracts were dried over anhydrous Na_2SO_4. Evaporation resulted in the product (119 mg; 88%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ: 13.15 (s, 1H), 10.37 (s, 1H), 8.67 (d, J = 8.6 Hz, 1H), 8.15 (d, J = 8.2 Hz, 1H), 8.09 (dd, J = 7.4, 1.3 Hz, 1H), 7.98 (dd, J = 8.0, 1.5 Hz, 1H), 7.96−7.91 (m, 2H), 7.58−7.44 (m, 3H), 7.43−7.39 (m, 3H), 7.38−7.33 (m, 2H), 7.19 (dq, J = 8.2, 4.4 Hz, 1H). LC−MS 5: 6.7 min; 428.0 [M + H]; HRMS calcd for $C_{25}H_{16}NO_4S^-$, 426.0806; found, 426.0805.

*2-Fluoro-4-[2-(naphthalene-1-sulfonylamino)-phenylethynyl]-benzoic Acid (**17g**).* Step 1. 2-Ethynyl-phenylamine (0.10 mL; 0.84 mmol) and 4-bromo-2-fluoro-benzoic acid methyl ester (0.19 mL; 1.25 mmol) were added to the vessel containing acetonitrile (6 mL) and diisopropyl-amine (0.18 mL; 1.25 mmol). The resulting mixture was argonated for 10 min. Then, copper(I) iodide (7.97 mg; 0.04 mmol) and tetrakis(triphenylphosphine)palladium(0) (48.33 mg; 0.04 mmol) were added. The reaction mixture was stirred at 100 °C for 1 h and then at 70 °C for 3 h. The reaction mixture was diluted with ethyl acetate and filtrated. The filtrate was evaporated and the residue was purified by chromatography giving 4-(2-aminophenylethynyl)-2-fluoro-benzoic acid methyl ester (159 mg; 71%) as a yellow solid. LCMS 4: 3.78 min; 270.30 [M + H].

Step 2. Naphthalene-1-sulfonyl chloride (37 mg; 0.16 mmol) was added to a mixture of 4-(2-aminophenylethynyl)-2-fluoro-benzoic acid methyl ester (40 mg; 0.15 mmol) in pyridine (0.5 mL). The reaction was carried out for 5h at 70 °C, quenched with water, and acidified with 2 M HCl. The crude product was extracted with ethyl acetate. The solvent was evaporated to give 2-fluoro-4-[2-(naphthalene-1-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (74 mg; 99%) as a brown solid. LCMS 4: = 4.37 min; 458.0 [M − H].

Step 3. The ester (74 mg; 0.15 mmol) was dissolved in methanol (5 mL) and NaOH (1.11 mL; 2.21 mmol) was added. The reaction mixture was stirred overnight at RT, diluted with ethyl acetate, washed with acidified water (pH = 5), and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated. The crude product was purified by chromatography to yield the product (35 mg; 53%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.48 (s, 1H), 10.48 (s, 1H), 8.71−8.64 (m, 1H), 8.17−8.10 (m, 1H), 8.04 (dd, J = 7.4, 1.2 Hz, 1H), 7.99−7.93 (m, 1H), 7.84 (t, J = 8.1 Hz, 1H), 7.54−7.33 (m, 6H), 7.22−7.13 (m, 3H). LC−MS 5: 6.7 min; 444.0 [M − H]; HRMS calcd for $C_{25}H_{15}FNO_4S^-$, 444.0711; found, 444.0706.

*4-{2-[2-(naphthalene-2-sulfonamido)phenyl]ethynyl}benzoic Acid (**17h**).* The compound was prepared as descried for **17f**. 4-(2-Amino-phenylethynyl)-benzoic acid methyl ester (51 mg; 0.20 mmol) and naphthalene-2-sulfonyl chloride (50 mg; 0.22 mmol) yielded 4-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (92 mg; 83%), which was saponified to the white solid product (19 mg; 22%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.15 (s, 1H), 10.20 (s, 1H), 8.36 (d, J = 1.8 Hz, 1H), 8.00−7.96 (m, 1H), 7.93 (d, J = 1.9 Hz, 1H), 7.91 (d, J = 1.6 Hz, 2H), 7.90 (d, J = 1.8 Hz, 1H), 7.71 (dd, J = 8.7, 1.9 Hz, 1H), 7.63 (ddd, J = 8.2, 6.8, 1.3 Hz, 1H), 7.55 (ddd, J = 8.1, 6.8, 1.2 Hz, 1H), 7.46−7.43 (m, 3H), 7.42−7.38 (m, 2H), 7.24 (ddd, J = 7.6, 6.4, 2.3 Hz, 1H). LC−MS 5: 8.5 min; 428.0 [M + H]; HRMS calcd for $C_{25}H_{16}NO_4S^-$, 426.0806; found, 426.0804.

*3-{2-[2-(Naphthalene-2-sulfonamido)phenyl]ethynyl}benzoic Acid (**17i**).* 3-(2-Amino-phenylethynyl)-benzoic acid methyl ester (37 mg; 0.15 mmol), naphthalene-2-sulfonyl chloride (50 mg; 0.22 mmol), and $NaHCO_3$ (19 mg; 0.22 mmol) were stirred overnight at RT in acetonitrile (2 mL). Second portion of naphthalene-2-sulfonyl chloride (17 mg; 0.07 mmol) and $NaHCO_3$ (12 mg; 0.15 mmol) were added and stirring was continued for additional 24 h. The reaction mixture was diluted with ethyl acetate, and washed with water and brine. The organic extract was dried over Na_2SO_4 and evaporated. The oily residue was purified by chromatography to give 3-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (28 mg; 43%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.24 (s, 1H), 8.32 (d, J = 1.9 Hz, 1H), 8.01 (td, J = 1.7, 0.6 Hz, 1H), 7.98−7.93 (m, 1H), 7.93−7.83 (m, 3H), 7.68 (dd, J = 8.7, 1.9 Hz, 1H), 7.59 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H), 7.55 (dt, J = 7.7, 1.5 Hz, 1H), 7.52−7.47 (m, 2H), 7.44−7.39 (m, 3H), 7.23 (dt, J = 7.5, 4.4 Hz, 1H), 3.93 (s, 3H). LCMS 5: 7.7 min; 440.1 [M − H]; HRMS calcd for $C_{25}H_{16}NO_4S^-$, 426.0806; found, 426.0802.

The ester (21 mg; 0.05 mmol) was added to a mixture of 5 N NaOH (0.2 mL; 0.95 mmol) and methanol (2 mL). The suspension became a solution within 10 min of stirring at RT. After 1 h, the reaction mixture was diluted with water and neutralized with 1 M HCl. The formed precipitate was taken up to AcOEt. The organic layer was extracted with brine, dried over Na_2SO_4, and evaporated to give 40 mg of oily residue, which was purified by chromatography. Proper fractions were combined and evaporated to give the product (15 mg; 73%) as a beige solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ: 13.24 (s, 1H), 10.25 (s, 1H), 8.33 (d, J = 1.8 Hz, 1H), 8.08 (t, J = 1.7 Hz, 1H), 7.96−7.86 (m, 4H), 7.71 (dd, J = 8.7, 1.9 Hz, 1H), 7.60 (ddd, J = 8.2, 6.8, 1.3 Hz, 1H), 7.55−7.46 (m, 3H), 7.44−7.37 (m, 3H), 7.26−7.18 (m, 1H). LC−MS 5: 8.5 min; 428.0 [M + H]; HRMS calcd for $C_{25}H_{16}NO_4S^-$, 426.0806; found, 426.0802.

*4-{2-[2-(Quinoline-8-sulfonamido)phenyl]ethynyl}benzoic Acid (**17j**).* Quinoline-8-sulfonyl chloride (95 mg; 0.42 mmol) was added to a mixture of 4-(2-amino-phenylethynyl)-benzoic acid methyl ester (70 mg; 0.28 mmol) and $NaHCO_3$ (35.10 mg; 0.42 mmol) in acetonitrile (5 mL). The reaction was carried out at RT. After 72 h, the reaction was charged with an additional amount of quinoline-8-sulfonyl chloride (44 mg; 0.20 mmol) and another one after 6 days with quinoline-8-sulfonyl chloride (19 mg; 0.08 mmol). The reaction was quenched with water after stirring for 8 days at RT and the product was extracted with ethyl acetate from the water phase, which was acidified with 2 M HCl. The combined organic phase was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by chromatography to yield 4-[2-(quinoline-8-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (116 mg; 92%) as a nude solid. LC−MS 4: 4.09 min; 443.3 [M + H]; 441.1 [M − H]. The ester (60 mg; 0.13 mmol) was dissolved in methanol (3 mL), and 5 M NaOH (0.5 mL; 2.66 mmol) was added. The reaction mixture was stirred for 2.5 h at 60 °C, diluted with ethyl acetate, washed with water in the presence of 2 M HCl, and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated. The crude product was purified by chromatography to give the product (30 mg; 53%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.21 (s, 1H), 9.26 (s, 1H), 8.79 (dd, J = 4.3, 1.8 Hz, 1H), 8.47 (dd, J = 8.4, 1.8 Hz, 1H), 8.39 (dd, J = 7.3, 1.5 Hz, 1H), 8.28 (dd, J = 8.3, 1.5 Hz, 1H), 8.02 (d, J = 8.3 Hz, 2H), 7.74 (dd, J = 8.3, 7.3 Hz, 1H), 7.64−7.58 (m, 2H), 7.55 (dd, J = 8.3, 4.3 Hz, 1H), 7.43 (ddd, J = 18.1, 8.0, 1.3 Hz, 2H), 7.30 (ddd, J = 8.5, 7.5, 1.6 Hz, 1H), 7.07 (td, J = 7.6, 1.2 Hz, 1H). LC−MS 5: 6.6 min; 426.7 [M − H]; HRMS

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calcd for $C_{24}H_{17}N2O_4S^+$, 429.0909; found, 429.0901, calcd for $C_{24}H_{15}N_2O_4S^-$, 427.0758; found, 427.0750.

*2-(Methylamino)-4-{2-[2-(7-methylquinoline-8-sulfonamido)-phenyl]ethynyl}benzoic Acid (**17k**).* Step 1. 2-Ethynyl-phenylamine (0.1 mL; 0.58 mmol) and 4-bromo-2-methylamino-benzoic acid methyl ester (120 mmol) were dissolved in anhydrous acetonitrile (4 mL) and the mixture was argonated for 10 min. Then, copper(I) iodide (3 mg; 0.01 mmol) and tetrakis(triphenylphosphine)-palladium(0) (17 mg; 0.01 mmol) were added and the reaction mixture was stirred at 65 °C overnight. After dilution with ethyl acetate and filtration, the residue was purified by chromatography to afford 4-(2-amino-phenylethynyl)-2-methylamino-benzoic acid methyl ester (100 mg; 70%) as a yellow solid. LCMS 4: 4.17 min; m/z = 281.15 [M + H].

Step 2. 4-(2-Amino-phenylethynyl)-2-methylamino-benzoic acid methyl ester (100 mg; 0.35 mmol) was dissolved in pyridine (2 mL), and 7-methyl-quinoline-8-sulfonyl chloride (100 mg; 0.42 mmol) was added. The reaction mixture was stirred at RT overnight. The solvent was coevaporated with toluene, and the crude was purified by chromatography to afford 2-methylamino-4-[2-(7-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (150 mg; 0.31 mmol) as a yellow solid. LCMS 4: 4.68 min; 486.25 [M + H]; 484.25 [M − H].

Step 3. To a stirred solution of 2-methylamino-4-[2-(7-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (150 mg; 0.31 mmol) in water (0.5 mL) and THF (0.5 mL), lithium hydroxide (37 mg; 1.53 mmol) was added. The reaction mixture was stirred at RT for 6 h and NaOH (61 mg; 1.53 mmol) was added. The reaction mixture was stirred at RT overnight. A second portion of NaOH (245 mg; 6.12 mmol) and MeOH (3 mL) was added, and the reaction mixture was stirred at 50 °C for 6 h. The reaction mixture was neutralized with HCl 1 N, and the aqueous layer was extracted with DCM, dried with Na_2SO_4, and evaporated. The crude was purified by chromatography to yield the product (101 mg; 70%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.68 (s, 1H), 8.80 (dd, J = 4.3, 1.8 Hz, 1H), 8.43 (dd, J = 8.4, 1.8 Hz, 1H), 8.15 (d, J = 8.5 Hz, 1H), 7.90 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.57−7.48 (m, 2H), 7.42 (dd, J = 7.7, 1.5 Hz, 1H), 7.30 (ddd, J = 8.6, 7.5, 1.6 Hz, 1H), 7.02 (td, J = 7.6, 1.1 Hz, 1H), 6.87 (d, J = 1.5 Hz, 1H), 6.77 (dd, J = 8.1, 1.5 Hz, 1H), 2.92 (s, 3H), 2.90 (s, 3H); not visible signals of the carboxylic protons. LC−MS 5: 7.2 min; 472.1 [M + H]; HRMS calcd for $C_{26}H_{22}N_3O_4S^+$, 472.1326; found, 472.1324; calcd for $C_{26}H_{20}N_3O_4S^-$, 470.1180; found, 470.1174.

*4-{2-[2-(5-Methoxyquinoline-8-sulfonamido)phenyl]ethynyl}-benzoic Acid (**17l**).* 4-(2-Amino-phenylethynyl)-benzoic acid methyl ester (9 g; 33.09 mmol) was suspended in anhydrous pyridine (40 mL) under argon. 5-Methoxy-quinoline-8-sulfonyl chloride (9.38 g; 36.40 mmol) was added in three portions. The resulting mixture was stirred at RT overnight. The reaction mixture was evaporated in vacuo to dryness with toluene. The residue was suspended in water and extracted to ethyl acetate. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The crude product was purified by chromatography to give: 4-[2-(5-methoxy-quinoline-8-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (6.50 g; 38.2%) as a white solid. LCMS 4: 3.89 min; 4.73.1 [M + H]. The ester (6.40 g; 12.46 mmol) was suspended in THF (210 mL). Water (70 mL) and lithium hydroxide monohydrate (2.09 g; 49.84 mmol) were added. The resulting mixture was stirred at 45 °C overnight and THF was removed in vacuo. The remainder was diluted with 200 mL water and adjusted to pH = 6 (2 M HCl). The formed precipitate product was filtered off, washed with water, and dried on air. The product was suspended in 200 mL of ethanol and refluxed for 30 min, then solid was collected upon filtration, rinsed with cold ethanol, and dried in vacuo overnight to obtain the product (5.91 g; 100%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.23 (s, 1H), 9.05 (s, 1H), 8.76 (dd, J = 4.3, 1.8 Hz, 1H), 8.53 (dd, J = 8.5, 1.8 Hz, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.06−8.02 (m, 2H), 7.66−7.63 (m, 2H), 7.54−7.47 (m, 2H), 7.42−7.38 (m, 1H), 7.34−7.27 (m, 1H), 7.15 (d, J = 8.5 Hz, 1H), 7.05 (td, J = 7.5, 1.1 Hz, 1H), 4.04 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.18, 159.75, 151.93, 143.83, 138.96, 133.91, 132.96, 131.95, 131.78, 131.40, 130.67, 130.11, 126.86, 126.51, 124.72, 122.37, 120.77, 119.84, 113.99, 104.35, 94.68, 87.72, 57.15. LC−MS 5: 6.8 min; 459.4 [M + H]; HRMS calcd for $C_{25}H_{19}N_2O_5S^+$, 459.1015; found, 459.1006; calcd for $C_{25}H_{17}N_2O_5S^-$, 457.0864; found, 457.0860.

*4-{2-[2-(7-Methylquinoline-8-sulfonamido)phenyl]ethynyl}-benzoic Acid (**17m**).* 4-(2-Amino-phenylethynyl)-benzoic acid methyl ester (100 mg; 0.39 mmol) was dissolved in anhydrous pyridine (0.5 mL) and 7-methyl-quinoline-8-sulfonyl chloride (103 mg; 0.42 mmol) was added in one portion. The reaction mixture was stirred overnight. An additional portion of 7-methyl-quinoline-8-sulfonyl chloride (50 mg; 0.21 mmol) was added, and the reaction mixture was stirred overnight. A third portion of 7-ethyl-quinoline-8-sulfonyl chloride (20 mg; 0.08 mmol) was added, and stirring continued for overnight. The reaction mixture was diluted with toluene, evaporated, and the crude product was purified by chromatography to give 4-[2-(7-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (96 mg; 53%) as a white solid. LCMS 4: 4.496 min; 457.20 [M + H]; 455.10 [M − H]. The ester (96 mg; 0.20 mmol) was dissolved in THF (3 mL) and water (1 mL), and lithium hydroxide monohydrate (49 mg; 1.16 mmol) was added. The reaction mixture was stirred overnight at RT and partially evaporated, diluted with water, and neutralized with 2 M HCl. The product was extracted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. Crude was purified by chromatography to give the product (70 mg; 40%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.22 (s, 1H), 9.67 (s, 1H), 8.77 (dd, J = 4.3, 1.8 Hz, 1H), 8.41 (dd, J = 8.4, 1.8 Hz, 1H), 8.14 (d, J = 8.5 Hz, 1H), 8.07−8.02 (m, 2H), 7.69−7.65 (m, 2H), 7.61 (d, J = 8.5 Hz, 1H), 7.53−7.47 (m, 2H), 7.43 (dd, J = 7.7, 1.6 Hz, 1H), 7.33−7.27 (m, 1H), 7.03 (td, J = 7.6, 1.2 Hz, 1H), 2.90 (s, 3H). LC−MS 4: 6.8 min; 443.4 [M + H]; HRMS calcd for $C_{25}H_{19}N_2O_4S^+$, 443.1066; found, 443.1060; calcd for $C_{25}H_{17}N_2O_4S^-$, 441.0914; found, 441.0913.

*2-Methoxy-4-{2-[2-(7-methylquinoline-8-sulfonamido)phenyl]-ethynyl}benzoic Acid (**17n**).* Step 1. 2-Ethynyl-phenylamine (0.15 mL; 1.28 mmol), 4-bromo-2-methoxy-benzoic acid methyl ester (345 mg; 1.41 mmol), and diisopropylamine (0.28 mL; 1.92 mmol) were added to the pressure vessel. Then anhydrous acetonitrile (5 mL) was added, and the reaction mixture was bubbled with argon for 10 min. Then, copper(I) iodide (12 mg; 0.06 mmol) and tetrakis-(triphenylphosphine)palladium(0) (74 mg; 0.06 mmol) were added. The reaction mixture was stirred at 60 °C overnight, diluted with ethyl acetate, and filtrated. The filtrate was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude was purified by chromatography to give 4-(2-amino-phenylethynyl)-2-methoxy-benzoic acid methyl ester (326 mg; 87%) as a yellow solid. LCMS 4: RT = 1.45 min; 282.7 [M + H].

Step 2. A mixture of 4-(2-amino-phenylethynyl)-2-methoxy-benzoic acid methyl ester (100 mg; 0.34 mmol), 7-methyl-quinoline-8-sulfonyl chloride (99 mg; 0.41 mmol), and anhydrous pyridine (0.5 mL) was stirred overnight at RT. The reaction mixture was diluted with toluene and evaporated under reduced pressure. The crude was purified by chromatography provide to: 2-methoxy-4-[2-(7-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (145 mg; 86%) as a white solid. LCMS 4: 1.60 min; 487.7 [M + H].

Step 3. 2-Methoxy-4-[2-(7-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-benzoic acid methyl ester (145 mg; 0.30 mmol) was dissolved in THF (6 mL) and water (2 mL); then, lithium hydroxide monohydrate (43 mg; 1.03 mmol) was added, and the reaction mixture was stirred at rt overnight. THF was removed in vacuo, the residue was diluted with additional portion of water, and neutralized with 1 M HCl. The product was extracted to ethyl acetate, the organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by chromatography to provide the product (75 mg; 46%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.87 (s, 1H), 9.68 (s, 1H), 8.80−8.76 (m, 1H), 8.43−8.39 (m, 1H), 8.16−8.13 (m, 1H), 7.74 (d, 1H), 7.61 (d, 1H), 7.53−7.49 (m, 2H), 7.42 (dd, J = 7.7, 1.5 Hz, 1H), 7.33−7.27 (m,

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2H), 7.19 (dd, J = 7.9, 1.4 Hz, 1H), 7.05−7.01 (m, 1H), 3.89 (s, 3H), 2.90 (s, 3H). LCMS 5: 6.7 min; 473.1 [M + H]$^+$; HRMS calcd for $C_{26}H_{21}N_2O_5S^+$, 473.1171; found, 473.1166; calcd for $C_{26}H_{19}N_2O_5S^-$, 471.1020; found, 471.1016.

*5-{2-[2-(Naphthalene-2-sulfonamido)phenyl]ethynyl}pyridine-2-carboxylic Acid (**18a**).* Step 1. 2-Ethynyl-phenylamine (1 mL; 8.37 mmol) and 5-bromo-pyridine-2-carboxylic acid methyl ester (3 mg; 12.55 mmol) were added to the vessel containing acetonitrile (50 mL). Diisopropylamine (1.8 mL; 12.55 mmol) was added, and the resulting mixture was argonated for 10 min. Then, copper(I) iodide (80 mg; 0.42 mmol) and tetrakis(triphenylphosphine)palladium(0) (483 mg; 0.42 mmol) were added, and the reaction mixture was stirred overnight at 70 °C. After cooling, the yellow precipitate was filtered and washed with cold ethyl acetate and hexane. The solid material was dissolved in DCM (250 mL) and washed with water and brine to remove diisopropylamine hydrobromide. The organic layer was dried over Na_2SO_4 and evaporated to give 5-(2-amino-phenylethynyl)-pyridine-2-carboxylic acid methyl ester (2.06 g; 95%) as a yellow solid. LCMS 1: 3.22 min; 253.2 [M + H].

Step 2. Naphthalene-2-sulfonyl chloride (98 mg; 0.43 mmol) was added to a mixture of 5-(2-amino-phenylethynyl)-pyridine-2-carboxylic acid methyl ester (100 mg; 0.39 mmol) in pyridine (0.70 mL). The reaction was carried out at RT overnight and quenched with water. The product was extracted with ethyl acetate from the water phase after acidification with 2 M HCl. The solvent was evaporated to yield 5-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (181 mg; 99%) as a light yellow solid. LCMS 1: 3.85 min; 443.05 [M + H]; 441.05 [M − H].

Step 3. 5-[2-(Naphthalene-2-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (181 mg; 0.39 mmol) was dissolved in methanol (4 mL). Water (2 mL) and lithium hydroxide hydrate (408 mg; 9.71 mmol) were added. RM was stirred overnight at RT. The mixture was acidified with hydrochloric acid and extracted with ethyl acetate. Combined organic layers were dried over Na_2SO_4 and evaporated. The product was isolated by chromatography as a white solid (94 mg; 56%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.37 (s, 1H), 10.23 (s, 1H), 8.64 (d, J = 2.0 Hz, 1H), 8.33 (d, J = 1.8 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.96−7.82 (m, 4H), 7.67 (dd, J = 8.6, 1.9 Hz, 1H), 7.60 (ddd, J = 8.2, 6.7, 1.2 Hz, 1H), 7.54−7.38 (m, 4H), 7.26 (td, J = 7.1, 2.0 Hz, 1H). LC−MS 2: 6.2 min; 429.1 [M + H]$^+$, HRMS calcd for $C_{24}H_{17}N_2O_4S^+$, 429.0909; found, 429.0901; calcd for $C_{24}H_{15}N_2O_4S^-$, 427.0758; found, 427.0754.

*6-{2-[2-(Naphthalene-2-sulfonamido)phenyl]ethynyl}pyridine-2-carboxylic Acid (**19**).* Step 1. 6-Bromo-pyridine-2-carboxylic acid methyl ester (250 mg; 1.16 mmol), 2-ethynyl-phenylamine (0.2 mL; 1.74 mmol), and diisopropylamine (0.3 mL; 2.31 mmol; 2.00 equiv) were dissolved in acetonitrile (2.5 mL) and purged with argon for 10 min. Then, tetrakis(triphenylphosphine)palladium(0) (67 mg; 0.06 mmol) and copper(I) iodide (11 mg; 0.06 mmol) were added and the reaction mixture was heated to 65−70 °C for 18 h. After dilution with ethyl acetate and filtration, 6-(2-amino-phenylethynyl)-pyridine-2-carboxylic acid methyl ester was obtained after chromatography (346 mg; 100%) as a brow-green glue. LCMS 1: 3.29 min; 253.7 [M + H].

Step 2. 6-(2-Amino-phenylethynyl)-pyridine-2-carboxylic acid methyl ester (70 mg; 0.24 mmol), naphthalene-2-sulfonyl chloride (60 mg; 0.26 mmol), and $NaHCO_3$ (24 mg; 0.29 mmol) were suspended in acetonitrile (2 mL) and the mixture was stirred overnight at RT. The reaction mixture was portioned by water and ethyl acetate. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography to give 6-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (49 mg; 46%), which was used directly for saponification. LCMS 1: 3.84 min; 441.1 [M − H].

Step 3. The ester (49 mg; 0.11 mmol) was dissolved in methanol (3 mL) and 5 N NaOH (1 mL; 4.77 mmol) was added. The resulting slurry was refluxed for 2 h to complete ester hydrolysis. The reaction mixture was diluted with water and carefully acidified with 2 M HCl to adjust pH 5. Formed precipitate was taken up in ethyl acetate and the organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography to yield the product (49 mg; 100%) as a yellow-brown glass. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.37 (s, 1H), 10.28 (s, 1H), 8.39 (d, J = 1.8 Hz, 1H), 8.03−7.92 (m, 4H), 7.91−7.86 (m, 1H), 7.77 (dd, J = 8.7, 1.9 Hz, 1H), 7.71 (dd, J = 5.9, 3.0 Hz, 1H), 7.60 (ddd, J = 8.2, 6.8, 1.3 Hz, 1H), 7.55−7.46 (m, 2H), 7.48−7.35 (m, 2H), 7.25 (td, J = 7.4, 1.6 Hz, 1H). LCMS 2: 7.7 min; 429.1 [M + H], 427.00 [M − H]$^-$; HRMS calcd for $C_{24}H_{17}N_2O_4S^+$, 429.0909; found, 429.0902; calcd for $C_{24}H_{15}N_2O_4S^-$, 427.0758; found, 427.0752.

*2-{2-[2-(Naphthalene-2-sulfonamido)phenyl]ethynyl}pyridine-4-carboxylic Acid (**20**).* Step 1. 2-Ethynyl-phenylamine (0.1 mL; 0.84 mmol) and 2-bromoisonicotinic acid methyl ester (271 mg; 1.25 mmol) were dissolved in acetonitrile (6 mL), diisopropylamine (0.2 mL; 1.25 mmol) was added, and the mixture was argonated for 10 min. Then, copper(I) iodide (8 mg; 0.04 mmol) and tetrakis-(triphenylphosphine)palladium(0) (48 mg; 0.04 mmol) were added and the reaction mixture was stirred overnight at 70 °C. The reaction mixture was diluted with ethyl acetate and filtered. The filtrate was evaporated and the residue was purified by chromatography to give 2-(2-amino-phenylethynyl)isonicotinic acid methyl ester (34 mg; 16%) as a yellow solid. LCMS 4: 3.25 min; 253.0 [M + H].

Step 2. 2-(2-Amino-phenylethynyl)-isonicotinic acid methyl ester (34 mg; 0.13 mmol) was suspended with sodium bicarbonate (17 mg; 0.20 mmol) in acetonitrile (4 mL), and naphthalene-2-sulfonyl chloride (41 mg; 0.18 mmol) was added. The reaction was stirred at rt for 72 h and after evaporating, crude 2-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-isonicotinic acid methyl ester (67 mg; 92%) was obtained as a brown solid. LCMS 4: 3.89 min; 441.0 [M − H].

Step 3. The ester (67 mg; 0.12 mmol) was suspended in 3 mL methanol and treated with 5 M solution NaOH to pH 12. The mixture was stirred at RT for 2 h when everything was dissolved. After acidification with 10% solution of HCl to pH 5 and extraction three times with ethyl acetate, the combined organic extracts were dried under anhydrous Na_2SO_4 and evaporated. After chromatography, the product was obtained (7 mg; 12% calculated for two steps) as a bright brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.98 (s, 1H), 10.39 (s, 1H), 8.67 (dd, J = 5.0, 0.9 Hz, 1H), 8.35 (d, J = 1.8 Hz, 1H), 8.14 (dd, J = 1.6, 0.9 Hz, 1H), 7.93−7.84 (m, 3H), 7.77 (dd, J = 5.0, 1.7 Hz, 1H), 7.73 (dd, J = 8.6, 1.9 Hz, 1H), 7.59 (ddd, J = 8.2, 6.8, 1.3 Hz, 1H), 7.53−7.49 (m, 1H), 7.48 (d, J = 1.4 Hz, 1H), 7.47−7.43 (m, 1H), 7.43 (t, J = 1.5 Hz, 1H), 7.29−7.22 (m, 1H). LCMS 5: 7.5 min; 429.0 [M + H], 427.00 [M − H]; HRMS calcd for $C_{24}H_{17}N_2O_4S^+$, 429.0909; found, 429.0903; calcd for $C_{24}H_{15}N_2O_4S^-$, 427.0758; found, 427.0752.

*5-{2-[2-(Naphthalene-2-sulfonamido)phenyl]ethynyl}pyridine-3-carboxylic Acid (**21**).* Step 1. 2-Ethynyl-phenylamine (0.1 mL; 0.84 mmol) and 5-bromo-nicotinic acid methyl ester (272 mg; 1.25 mmol) were dissolved in acetonitrile (6 mL). Diisopropylamine (0.2 mL; 1.25 mmol) was added, and the mixture was argonated for 10 min. Then, copper(I) iodide (8 mg; 0.04 mmol) and tetrakis-(triphenylphosphine)palladium(0) (48 mg; 0.04 mmol) were added, and the mixture was stirred at 70 °C overnight. After dilution with ethyl acetate and filtration, the residue was purified by chromatography giving 5-(2-amino-phenylethynyl)-nicotinic acid methyl ester (174 mg; 83%) as a yellow solid. LCMS 4: 3.32 min; 253.3 [M + H].

Step 2. Naphthalene-2-sulfonyl chloride (61 mg; 0.27 mmol) was added to a mixture of 5-(2-aminophenylethynyl)-nicotinic acid methyl ester (50 mg; 0.20 mmol; 1.00 equiv) and $NaHCO_3$ (25 mg; 0.30 mmol) in acetonitrile (5 mL). The reaction was carried out at rt for 48 h. Then, additional amount of naphthalene-2-sulfonyl chloride (22 mg; 0.10 mmol) was added, and the mixture was stirred at RT for 24 h. After quenching with water and acidification with 2 M HCl, the product was extracted with ethyl acetate. Combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and residue was purified by chromatography to yield 5-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-nicotinic acid methyl ester (76 mg; 86%) as white solid. LCMS 4: 3.86 min; 441.1 [M − H].

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Step 3. The ester (76 mg; 0.17 mmol) was dissolved in methanol (3 mL), and 5 M NaOH (0.7 mL; 3.40 mmol) was added. The reaction mixture was stirred for 3 h, diluted with water, and acidified with hydrochloric acid. After extraction and chromatography, the product was isolated (60 mg; 82%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.70 (s, 1H), 10.25 (s, 1H), 8.98 (d, J = 2.1 Hz, 1H), 8.61 (d, J = 2.1 Hz, 1H), 8.31 (q, J = 2.0 Hz, 2H), 7.85 (dd, J = 8.7, 3.2 Hz, 3H), 7.62 (dd, J = 8.7, 1.9 Hz, 1H), 7.57 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.51−7.40 (m, 4H), 7.29−7.22 (m, 1H). LCMS 5: 7.6 min; 429.0 [M + H], 427.2 [M − H]; HRMS calcd for $C_{24}H_{17}N_2O_4S^+$, 429.0909; found, 429.0904; calcd for $C_{24}H_{15}N_2O_4S^-$, 427.0758; found, 427.0752.

*4-{2-[2-(Naphthalene-2-sulfonamido)phenyl]ethynyl}pyridine-2-carboxylic Acid (**22**).* Step 1. 2-Ethynyl-phenylamine (0.1 mL; 0.84 mmol) and 4-bromo-pyridine-2-carboxylic acid methyl ester (277 mg; 1.25 mmol) were dissolved in acetonitrile (5 mL). Diisopropylamine (0.2 mL; 1.25 mmol) was added, and the resulting mixture was argonated for 10 min. Then, copper(I) iodide (8 mg; 0.04 mmol) and tetrakis(triphenylphosphine)palladium(0) (48 mg; 0.04 mmol) were added, and the reaction mixture was heated for 3 h at 60 °C. After dilution with ethyl acetate and filtration, the residue was purified by chromatography to give 4-(2-aminophenylethynyl)-pyridine-2-carboxylic acid methyl ester (176 mg; 83%) as a yellow solid. LCMS 4: 3.16 min; 253.0 [M + H].

Step 2. Naphthalene-2-sulfonyl chloride (78 mg; 0.35 mmol) was added to 4-(2-aminophenylethynyl)-pyridine-2-carboxylic acid methyl ester (80 mg; 0.31 mmol) dissolved in pyridine (0.5 mL). After reaction at RT overnight, water was added, acidified with 2 M HCl, and the product was extracted with ethyl acetate. The solvent was evaporated and the residue was purified by chromatography to yield 4-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (118 mg; 84%) as a light yellow solid. LCMS 4: 3.80 min; 443.1 [M + H]; 441.0 [M − H].

Step 3. The ester (37 mg; 0.08 mmol) was dissolved in methanol (5 mL), and 5 M NaOH (0.32 mL; 1.62 mmol) was added. The reaction mixture was stirred for 3 h at 50 °C. After acidification with hydrochloric acid and extraction with ethyl acetate, the combined organic layers were dried over Na_2SO_4 and evaporated. The product was purified by chromatography to obtain 4-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid (7 mg; 18%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (br, 1H), 8.64 (d, J = 5.0 Hz, 1H), 8.31 (d, J = 1.8 Hz, 1H), 8.08 (s, 1H), 7.87 (t, J = 7.8 Hz, 3H), 7.68−7.52 (m, 2H), 7.52−7.36 (m, 5H), 7.25 (td, J = 7.5, 7.0, 2.4 Hz, 1H); not visible signal of the acidic proton. LCMS 5: 5.7 min; 429.0 [M + H], 427.2 [M − H]; RMS calcd for $C_{24}H_{17}N_2O_4S^+$, 429.0909; found, 429.0901; calcd for $C_{24}H_{15}N_2O_4S^-$, 427.0758; found, 427.0751.

*6-{2-[2-(Naphthalene-2-sulfonamido)phenyl]ethynyl}pyridine-3-carboxylic Acid (**23**).* Step 1. A mixture of 2-ethynylphenylamine (0.1 mL; 0.68 mmol), 6-bromo-nicotinic acid methyl ester (221 mg; 1.02 mmol), and diisopropylamine (0.15 mL; 1.02 mmol) in acetonitrile (4 mL) was argonated for 10 min, and then heated to 65 °C overnight. The reaction mixture was diluted with ethyl acetate and filtered. The residue was purified by chromatography to give 6-(2-amino-phenylethynyl)-nicotinic acid methyl ester (157 mg; 88%) as a yellow solid. LCMS 4: RT = 3.20 min; 256.60 [M + H].

Step 2. 6-(2-Amino-phenylethynyl)-nicotinic acid methyl ester (60 mg; 0.23 mmol), naphthalene-2-sulfonyl chloride (58 mg; 0.26 mmol), and sodium bicarbonate (29 mg; 0.35 mmol) were suspended in acetonitrile (3 mL). The reaction mixture was stirred at RT for 48 h. A second portion of naphthalene-2-sulfonyl chloride (58 mg; 0.26 mmol) was added. After 24 h, the reaction mixture was evaporated and the residue was dissolved in water and twice extracted with ethyl acetate. Combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The crude product was purified by chromatography to obtain 6-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-nicotinic acid methyl ester (36 mg; 34%) as a yellow powder. LCMS 4: 3.91 min; 443.2 [M + H].

Step 3. The ester (36 mg; 0.08 mmol) was dissolved in THF (3 mL) and water (1 mL), then lithium hydroxide monohydrate (29 mg; 0.70 mmol) was added. The reaction mixture was stirred at RT overnight. Then, THF was removed, the remainder was acidified to pH 5, and extracted to ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The product was purified by chromatography and obtained as a yellow powder (29 mg; 29% counted for steps 2 and 3). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.25 (s, 1H), 10.25 (s, 1H), 8.99 (d, J = 2.1 Hz, 1H), 8.39 (d, J = 1.8 Hz, 1H), 8.26 (dd, J = 8.1, 2.2 Hz, 1H), 7.98 (d, J = 8.2 Hz, 1H), 7.92 (t, J = 8.0 Hz, 2H), 7.75 (dd, J = 8.7, 1.9 Hz, 1H), 7.64−7.59 (m, 2H), 7.56−7.36 (m, 4H), 7.25 (td, J = 7.4, 1.4 Hz, 1H). LCMS 5: 6.2 min; 428.9 [M + H], HRMS calcd for $C_{24}H_{17}N_2O_4S^+$, 429.0909; found, 429.0902; calcd for $C_{24}H_{15}N_2O_4S^-$, 427.0758; found, 427.0752.

*5-{2-[2-(4-Methoxynaphthalene-1-sulfonamido)phenyl]ethynyl}-pyrimidine-2-carboxylic Acid (**24**).* Step 1. 2-Ethynyl-phenylamine (0.1 mL; 0.84 mmol) was added to a solution of 5-bromo-pyrimidine-2-carboxylic acid methyl ester (272 mg; 1.25 mmol) in acetonitrile (3 mL) and diisopropylamine (0.2 mL; 1.25 mmol). The mixture was argonated for 10 min, and then copper(I) iodide (8 mg; 0.04 mmol) and tetrakis(triphenylphosphine)palladium(0) (48 mg; 0.04 mmol) were added. The reaction mixture was stirred for 4.5 h at 80 °C. Then, it was diluted with ethyl acetate and filtered. The filtrate was evaporated and was purified by chromatography to give 5-(2-aminophenylethynyl)-pyrimidine-2-carboxylic acid methyl ester (210 mg; 97%) as a yellow solid. LCMS 4: 2.79 min; 254.3 [M + H].

Step 2. 4-Methoxy-naphthalene-1-sulfonyl chloride (89 mg; 0.34 mmol) was added to a mixture of 5-(2-aminophenylethynyl)-pyrimidine-2-carboxylic acid methyl ester (60 mg; 0.23 mmol) and $NaHCO_3$ (29 mg; 0.34 mmol) in acetonitrile (5 mL). After 12 h at RT, an additional amount of 4-methoxy-naphthalene-1-sulfonyl chloride (56 mg; 0.23 mmol) and $NaHCO_3$ (19 mg; 0.23 mmol) was added, also after 48 h and after 5 days (12 mg; 0.05 mmol). The reaction mixture was quenched with water after 11 days, acidified with 2 M HCl, and the product was extracted with ethyl acetate from the water phase. The combined organic phase was dried over anhydrous Na_2SO_4. The solvent was evaporated and residue was purified by chromatography to yield 5-[2-(4-methoxynaphthalene-1-sulfonylamino)-phenylethynyl]-pyrimidine-2-carboxylic acid methyl ester (18 mg; 15%) as a cream-colored solid. LCMS 4: 3.57 min; 472.2 [M + H].

Step 3. The ester (18 mg; 0.04 mmol) was dissolved in methanol (2 mL), and 5 M NaOH (0.14 mL; 0.71 mmol) was added. The reaction mixture was stirred for 2.5 h at 50 °C, diluted with water in the presence of 2 M HCl, and extracted with ethyl acetate. Combined organic layers were dried over Na_2SO_4 and evaporated. The crude product was purified by chromatography to give the product (8 mg; 49%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.21 (br, 1H), 8.75 (s, 2H), 8.63−8.55 (m, 1H), 8.12−8.04 (m, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.55−7.41 (m, 2H), 7.41−7.28 (m, 3H), 7.20 (td, J = 7.5, 1.3 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 3.95 (s, 3H). LC−MS 5: 6.0 min; 459.8 [M + H], 457.8 [M − H]; HRMS calcd for $C_{24}H_{16}N_3O_5S^-$, 458.0816; found, 458.0817.

*5-{2-[2-(Naphthalene-2-sulfonamido)phenyl]ethynyl}pyrazine-2-carboxylic Acid (**25**).* Step 1. 5-bromo-pyrazine-2-carboxylic acid methyl ester (150 mg; 0.69 mmol), 2-ethynyl-phenylamine (81 mg; 0.69 mmol), and diisopropylamine (0.2 mL; 1.04 mmol) were dissolved in and acetonitrile (2 mL) and argonated. Copper(I) iodide (7 mg; 0.03 mmol) and tetrakis(triphenylphosphine)palladium(0) (40 mg; 0.03 mmol) were added. The resulting mixture was heated for 6 h at 60 °C, diluted with ethyl acetate and DCM, filtered, and evaporated under reduced pressure. The residue was purified by chromatography affording 5-(2-aminophenylethynyl)-pyrazine-2-carboxylic acid methyl ester (95 mg; 54%) as a yellow solid. LCMS 4: 2.95 min; 254.0 [M + H]; 252.1 [M − H].

Step 2. 5-(2-Amino-phenylethynyl)-pyrazine-2-carboxylic acid methyl ester (50 mg; 0.20 mmol), naphthalene-2-sulfonyl chloride (54 mg; 0.24 mmol), and sodium bicarbonate (83 mg; 0.99 mmol) were suspended in acetonitrile (2 mL) for 4 days at RT. An additional

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amount of sodium bicarbonate (41 mg; 0.49 mmol) and naphthalene-2-sulfonyl chloride (22 mg; 0.10 mmol) was added for additional 24 h. The reaction mixture was evaporated, and the residue was portioned by ethyl acetate and water. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated and purified by chromatography to give 5-[2-(naphthalene-2-sulfonylamino)-phenylethynyl]-pyrazine-2-carboxylic acid methyl ester (22 mg; 25%). LCMS 4: 3.41 min; 444.1 [M + H].

Step 3. The ester (22 mg; 0.05 mmol) was dissolved in methanol (5 mL). Water (2 mL) and lithium hydroxide monohydrate (116 mg; 2.76 mmol) were added and the resulting mixture was heated for 2 h at 55 °C. The mixture was diluted with water and acidified with a solution of HCl. The product was extracted to ethyl acetate and the organic layer was washed with water and brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated and purified by chromatography yielding the product as a yellow solid (18 mg; 0.04 mmol; 20% calculated for steps 2 and 3). LCMS 5: 6.1 min; 429.8 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) d: 13.83 (s, 1H), 10.33 (s, 1H), 9.06 (s, 1H), 8.82 (s, 1H), 8.36 (s, 1H), 7.99−7.84 (m, 3H), 7.70 (dd, J = 8.7, 1.6 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.56−7.45 (m, 3H), 7.42 (d, J = 7.9 Hz, 1H), 7.29 (t, J = 7.5 Hz, 1H). HRMS calcd for $C_{23}H_{14}N_3O_4S^-$, 428.0711; found, 428.0704.

6-{2-[2-(5-Methoxyquinoline-8-sulfonamido)phenyl]ethynyl}-pyridazine-3-carboxylic Acid ***26***. Step 1. 6-Bromo-pyridazine-3-carboxylic acid methyl ester (222 mg; 1.02 mmol), 2-ethynylphenylamine (0.1 mL; 0.68 mmol), and diisopropylamine (0.15 mL; 1.02 mmol) were dissolved in acetonitrile (4.8 mL). The mixture was argonated for 15 min. Tetrakis(triphenylphosphine)palladium(0) (39 mg; 0.03 mmol) and copper(I) iodide (6.5 mg; 0.03 mmol) were added and the reaction mixture was stirred at RT overnight. After dilution with ethyl acetate and filtration, the filtrate was washed with water twice, washed with brine, dried over sodium sulfate, and evaporated. The crude product was purified by chromatography to obtain 6-(2-amino-phenylethynyl)-pyridazine-3-carboxylic acid methyl ester (153 mg; 88%). LCMS 4: 3.12 min; 254.0 [M + H].

Step 2. 6-(2-Amino-phenylethynyl)-pyridazine-3-carboxylic acid methyl ester (40 mg; 0.15 mmol) and 5-methoxy-quinoline-8-sulfonyl chloride (47 mg; 0.18 mmol) in pyridine (0.5 mL) were reacted overnight under argon at RT. Then, the reaction mixture was diluted with ethyl acetate, washed with 1 N HCl, washed with water and brine, dried over sodium sulfate, and evaporated. The crude product was purified by chromatography to give 6-[2-(5-methoxy-quinoline-8-sulfonylamino)-phenylethynyl]-pyridazine-3-carboxylic acid methyl ester (63 mg; 84%) as a yellow solid. LCMS 4: 3.59 min; 475.0 [M + H].

Step 3. The ester (63 mg; 0.13 mmol) was dissolved in THF (5 mL) and water (2 mL), then lithium hydroxide monohydrate (19 mg; 0.45 mmol) was added. The reaction mixture was stirred at RT overnight. THF was removed, the remainder was acidified to pH 5, and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and evaporated. The crude was purified by chromatography giving the product (18 mg; 25%) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.07 (s, 1H), 9.30 (s, 1H), 8.90 (dd, J = 4.3, 1.8 Hz, 1H), 8.52 (dd, J = 8.6, 1.8 Hz, 1H), 8.33 (dd, J = 13.8, 8.6 Hz, 2H), 8.07 (d, J = 8.7 Hz, 1H), 7.55−7.48 (m, 3H), 7.39 (td, J = 7.9, 1.6 Hz, 1H), 7.16−7.09 (m, 2H), 4.04 (s, 3H). LCMS 5: 6.4 min; 461.0 [M + H]; HRMS calcd for $C_{23}H_{17}N_4O_5S^+$, 461.0920; found, 461.0914; calcd for $C_{23}H_{15}N_4O_5S^-$, 459.0769; found, 459.0767.

5-[2-(Quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic Acid ***(18b)***. 5-[2-(Quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (60 mg; 0.13 mmol) was dissolved in methanol (3 mL), and lithium hydroxide hydrate (139 mg; 3.31 mmol) and water (1 mL) were added. The product was obtained by chromatography in 91% (53 mg) as a light yellow solid. LCMS 4: 3.27 min; 430.0 [M + H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.41 (s, 1H), 9.43 (s, 1H), 8.81 (dd, J = 4.3, 1.8 Hz, 1H), 8.73 (d, J = 2.0 Hz, 1H), 8.45 (dd, J = 8.4, 1.8 Hz, 1H), 8.37 (dd, J = 7.3, 1.4 Hz, 1H), 8.27 (dd, J = 8.3, 1.5 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 8.01 (dd, J = 8.1, 2.1 Hz, 1H), 7.72 (t, J = 7.8 Hz, 1H), 7.52 (dd, J = 8.4, 4.2 Hz, 1H), 7.45 (ddd, J = 7.7, 3.6, 1.3 Hz, 2H), 7.35 (td, J = 8.3, 7.9, 1.6 Hz, 1H), 7.12 (td, J = 7.5, 1.2 Hz, 1H). HRMS calcd for $C_{23}H_{15}N_3O_4S$, 430.0856; found, 430.0847.

5-[2-(2-Methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic Acid ***(18c)***. To a solution of 5-[2-(2-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (61 mg; 0.11 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (1.1 mL; 2.21 mmol) and stirred for 16 h at RT. The product was isolated by chromatography in 32% (17 mg) yield as an off-white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.49−12.20 (m, 1H), 9.28 (s, 1H), 8.60−8.57 (m, 1H), 8.36 (dd, J = 7.3, 1.4 Hz, 1H), 8.28 (d, J = 8.5 Hz, 1H), 8.19 (dd, J = 8.2, 1.4 Hz, 1H), 8.07−8.03 (m, 1H), 7.88 (dd, J = 8.1, 2.1 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.47 (dd, J = 7.7, 1.5 Hz, 1H), 7.43−7.39 (m, 2H), 7.35 (td, J = 7.6, 1.6 Hz, 1H), 7.13 (td, J = 7.5, 1.2 Hz, 1H), 2.45 (s, 3H). HRMS calcd for $C_{24}H_{17}N_3O_4S$, 444.1013; found, 444.1006.

5-[2-(3-Methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic Acid ***(18d)***. To a solution of 5-[2-(3-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (126 mg; 0.24 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (2.4 mL; 4.71 mmol) and stirred for 16 h at RT. The product was isolated by chromatography in 35% (37 mg) yield as a white solid. LC−MS 2: 1.64 min; 444.00 [M + H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.99−12.78 (m, 1H), 9.39 (s, 1H), 8.68−8.64 (m, 1H), 8.61 (d, J = 2.2 Hz, 1H), 8.21 (dd, J = 7.4, 1.4 Hz, 1H), 8.18−8.13 (m, 2H), 8.12−8.08 (m, 1H), 7.96 (dd, J = 8.1, 2.1 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.50−7.45 (m, 1H), 7.42 (dd, J = 7.8, 1.6 Hz, 1H), 7.39−7.33 (m, 1H), 7.16−7.10 (m, 1H), 2.27 (s, 3H). HRMS calcd for $C_{24}H_{17}N_3O_4S$, 444.1013; found, 444.1006.

5-[2-(4-Methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic Acid ***(18e)***. To a solution of 5-[2-(4-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (103 mg; 0.21 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (2.1 mL; 4.12 mmol) and stirred for 16 h at RT. The product was isolated by chromatography in 17% (16 mg) yield as a light yellow solid. LC−MS 2: 1.63 min; 444.00 [M + H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.38 (s, 1H), 8.66 (dd, J = 2.2, 0.7 Hz, 1H), 8.65 (d, J = 4.4 Hz, 1H), 8.38−8.33 (m, 2H), 8.10 (dd, J = 8.1, 0.9 Hz, 1H), 7.96 (dd, J = 8.1, 2.1 Hz, 1H), 7.72 (dd, J = 8.4, 7.4 Hz, 1H), 7.48−7.45 (m, 1H), 7.43 (dd, J = 7.7, 1.6 Hz, 1H), 7.37−7.31 (m, 2H), 7.11 (td, J = 7.5, 1.2 Hz, 1H), 2.65−2.61 (m, 3H). HRMS calcd for $C_{24}H_{17}N_3O_4S$, 444.1013; found, 444.1005.

5-{2-[2-(5-Methylquinoline-8-sulfonamido)phenyl]ethynyl}-pyridine-2-carboxylic Acid ***(18f)***. To a solution of 5-[2-(5-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (80 mg; 0.15 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (1.5 mL; 3.08 mmol) and stirred for 6 h at RT. The product was isolated by chromatography in 65% (47 mg) yield as a beige solid. LC−MS 2: 1.64 min; 444.00 [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.10 (dd, J = 4.2, 1.7 Hz, 1H), 8.58 (d, J = 1.5 Hz, 1H), 8.46 (dd, J = 8.5, 1.8 Hz, 1H), 8.33 (d, J = 7.4 Hz, 1H), 7.84 (s, 2H), 7.58 (dd, J = 8.5, 4.3 Hz, 1H), 7.50−7.45 (m, 1H), 7.17 (dd, J = 7.6, 1.7 Hz, 1H), 7.12 (dd, J = 8.5, 1.1 Hz, 1H), 6.87 (m, 1H), 6.43 (m, 1H), 2.89 (s, 1H), 2.73 (s, 1H), 2.65 (s, 3H). HRMS calcd for $C_{24}H_{17}N_3O_4S$, 444.1013; found, 444.1008.

5-[2-(6-Methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic Acid ***(18g)***. To a solution of 5-[2-(6-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (151 mg; 0.25 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (2.5 mL; 5.08 mmol) and stirred for 4 days at RT. The product was isolated by chromatography in 31% yield (35 mg) as a white solid. LCMS 2: 1.68 min.; 444.00 [M + H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.26−12.32 (m, 1H), 9.38 (s, 1H), 8.74−8.68 (m, 2H), 8.32 (dd, J = 8.5, 1.7 Hz, 1H), 8.20 (d, J = 1.9 Hz, 1H), 8.13−8.08 (m, 1H), 8.02−7.97

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(m, 2H), 7.49−7.41 (m, 3H), 7.35 (td, J = 7.9, 1.6 Hz, 1H), 7.12 (td, J = 7.6, 1.2 Hz, 1H), 2.53−2.50 (m, 3H). HRMS calcd for $C_{24}H_{17}N_3O_4S$, 444.1013; found, 444.1003.

*5-[2-(7-Methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic Acid (**18h**).* To a solution of 5-[2-(7-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (73 mg; 0.12 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (1.2 mL; 2.44 mmol) and stirred for 16 h at RT. The product was isolated by chromatography in 29% yield (16 mg) as a rose solid. LC−MS 2: 1.66 min; 444.00 [M + H]. ^{1}H NMR (700 MHz, DMSO-d_6): δ 9.74 (s, 1H), 8.82−8.78 (m, 2H), 8.38 (dd, J = 8.2, 1.3 Hz, 1H), 8.16−8.11 (m, 2H), 8.09 (dd, J = 8.0, 2.0 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.51−7.45 (m, 3H), 7.37−7.32 (m, 1H), 7.10−7.06 (m, 1H), 2.87 (s, 3H). HRMS calcd for $C_{24}H_{17}N_3O_4S$, 444.1013; found, 444.1011.

*3-Methyl-5-[2-(7-methyl-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic Acid (**18i**).* Step 1. A mixture of 5-Bromo-3-methyl-pyridine-2-carboxylic acid methyl ester (8 g; 34.77 mmol) and 2-ethynylphenylamine (4.35 mL; 38.25 mmol) in AcCN (50 mL) was argonated. Diisopropylamine (6.84 mL; 52.16 mmol) was added, followed by tetrakis(triphenylphosphine)palladium(0) (2.01 g; 1.74 mmol) and copper(I)iodide (0.33 g; 1.74 mmol) under argon. The resulting mixture was heated for 12 h at 65 °C and cooled down to RT. The formed precipitate was isolated by filtration, washed with n-hexane, and dissolved in DCM. The organic layer was washed twice with water and brine, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated and loaded on a long pad of silica covered with a small layer of Celite. Impurities were eluted using DCM (darker yellow fraction), and then the product was eluted by a mixture of DCM/AcOEt 10% v/v. The fraction was concentrated and the precipitating solid was collected by filtration, washed with n-hexane, and dried to give 5-(2-amino-phenylethynyl)-3-methyl-pyridine-2-carboxylic acid methyl ester (7.72 g; 82%) as a yellow solid. LCMS 4: 1.44 min; 267.30 [M + H].

Step 2. 5-(2-aminophenylethynyl)-3-methyl-pyridine-2-carboxylic acid methyl ester (70 mg; 0.24 mmol) was dissolved in anhydrous pyridine (0.5 mL), and 5-methoxyquinoline-8-sulfonyl chloride (69.30 mg;0.27 mmol) was added in one portion. The reaction mixture was stirred overnight till full consumption of starting material was confirmed by TLC. The reaction mixture was diluted with toluene and evaporated in vacuo. The crude product was purified by FCC (SiHP column, EtOAc gradient in hexane) to give: 5-[2-(5-methoxyquinoline-8-sulfonylamino)-phenylethynyl]-3-methyl-pyridine-2-carboxylic acid methyl ester (103 mg; 85%) as a white solid. LCMS 4: 3.97 min; 488.1 [M + H].

Step 3. 5-[2-(5-Methoxyquinoline-8-sulfonylamino)-phenylethynyl]-3-methyl-pyridine-2-carboxylic acid methyl ester (103 mg; 0.21 mmol eq) was dissolved in THF (5 mL) and water (2 mL). Lithium hydroxide monohydrate (30.77 mg; 0.73 mmol) was added, and the resulting mixture was stirred overnight at RT. Consumption of the starting material was confirmed on TLC. The reaction mixture was partially evaporated and neutralized with 2 M HCl. The precipitate was extracted to ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. Crude was purified by FCC (SiHP column, 0−10% methanol gradient in DCM) to give the product (78 mg; 67%) as a white solid. LCMS 10: 6.5 min; 474.5 [M + H]. ^{1}H NMR (300 MHz, DMSO-d_6): δ: 13.34 (s, 1H), 9.15 (s, 1H), 8.76 (dd, J = 4.3, 1.8 Hz, 1H), 8.52−8.48 (m, 2H), 8.31 (d, J = 8.4 Hz, 1H), 7.79−7.77 (m, 1H), 7.50−7.45 (m, 2H), 7.42−7.38 (m, 1H), 7.36−7.30 (m, 1H), 7.14−7.06 (m, 2H), 4.03 (s, 3H), CH_3—partially covered by DMSO-d_6 signal). HRMS for $C_{25}H_{20}N_3O_5S^+$, 474.1118; found, 474.1116; calcd for $C_{25}H_{18}N_3O_5S^-$, 472.0973; found, 472.0976.

*5-[2-(5-Methoxy-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic Acid (**18j**).* 5-[2-(5-Methoxy-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (42 mg; 0.09 mmol) was dissolved in methanol (2 mL). Water and lithium hydroxide hydrate (92 mg; 2.20 mmol) were added. The reaction mixture was stirred overnight at RT. The product was isolated by chromatography (37 mg; 90%) as a light yellow solid. LCMS 4: 6.10 min; 457.70 [M − H]; 459.80 [M + H].

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.45 (s, 1H), 9.19 (s, 1H), 8.79−8.71 (m, 2H), 8.50 (dd, J = 8.6, 1.8 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 8.04 (dd, J = 8.1, 2.1 Hz, 1H), 7.51−7.43 (m, 2H), 7.42 (dd, J = 7.7, 1.6 Hz, 1H), 7.34 (td, J = 8.4, 8.0, 1.6 Hz, 1H), 7.16−7.04 (m, 2H), 4.03 (s, 3H). HRMS calcd for $C_{24}H_{17}N_3O_5S$, 460.0962; found, 460.0959.

*5-[2-(5-Methoxy-quinoline-8-sulfonylamino)-phenylethynyl]-3-methyl-pyridine-2-carboxylic Acid (**18k**).* Methyl-5-[2-(2-Aminophenyl)ethynyl]-4-methoxypyridine-2-carboxylate (3.50 g; 13.01 mmol) was dissolved in pyridine (20 mL), and 7-methylquinoline-8-sulfonyl chloride (3.77 g; 115.61 mmol) were added. The reaction mixture was stirred at room temperature overnight. The solvent was coevaporated with toluene, and crude was purified by FCC (silica, hexane 100% to DCM 100% to DCM/EtOAc 85:15 gradient) to afford methyl 4-methoxy-5-{2-[2-(7-methylquinoline-8-sulfonamido)phenyl]ethynyl}pyridine-2-carboxylate (5.60 g; 88.0%) as a pale yellow solid. LCMS 4: 3.92 min; 472.2 [M + H], 470.2 [M − H]. To a stirred solution of 3-methyl-5-[2-(7-methylquinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (5.60 g; 10.33 mmol) in water (15 mL) and THF (15 mL) was added lithium hydroxide (1.24 g; 51.66 mmol). The reaction mixture was stirred for 4 h, concentrated in vacuo, and neutralized with 1 N HCl. The aqueous layer was extracted with DCM, dried over Na_2SO_4, and evaporated. The solid was washed with pentane and EtOH to afford the product (4.70 g; 99%) as a white solid. LCMS 10: 6.8 min; 458.0 [M + H]; 456 [M − H]. ^{1}H NMR (300 MHz, DMSO-d_6): δ: 9.73 (s, 1H), 8.80 (dd, J = 4.3, 1.8 Hz, 1H), 8.57 (d, J = 1.9 Hz, 1H), 8.39 (dd, J = 8.3, 1.8 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H), 7.86 (d, J = 2.0 Hz, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.53−7.40 (m, 3H), 7.34 (ddd, J = 8.6, 7.5, 1.6 Hz, 1H), 7.08 (td, J = 7.6, 1.2 Hz, 1H), 2.87 (s, 3H), 2.53 (s, 3H). HRMS calcd for $C_{25}H_{20}N_3O_4S^+$, 458.1169; found, 458.1165; calcd for $C_{25}H_{18}N_3O_4S^-$, 456.1024; found, 456.1030.

*5-{2-[2-(5-Ethoxyquinoline-8-sulfonamido)phenyl]ethynyl}-pyridine-2-carboxylic Acid (**18l**).* To a solution of 5-[2-(5-ethoxy-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (163 mg; 0.20 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (2 mL; 4 mmol) and stirred for 16 h at RT. The product was isolated by chromatography (33%; 88 mg) as a yellow solid. LC−MS 2: 1.71−1.72 min; 474.00 [M + H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.31−12.26 (m, 1H), 9.17 (s, 1H), 8.76 (dd, J = 4.3, 1.8 Hz, 1H), 8.73 (dd, J = 2.1, 0.9 Hz, 1H), 8.49 (dd, J = 8.6, 1.8 Hz, 1H), 8.29 (d, J = 8.4 Hz, 1H), 8.11 (dd, J = 8.1, 0.9 Hz, 1H), 8.02 (dd, J = 8.1, 2.1 Hz, 1H), 7.50−7.44 (m, 2H), 7.41 (dd, J = 7.7, 1.5 Hz, 1H), 7.36−7.30 (m, 1H), 7.11−7.05 (m, 2H), 4.27 (q, J = 7.0 Hz, 2H), 1.44 (t, J = 7.0 Hz, 3H). HRMS calcd for $C_{25}H_{19}N_3O_5S$, 474.1118; found, 474.1102.

*5-{2-[2-(5-Ethoxyquinoline-8-sulfonamido)phenyl]ethynyl}-4-methoxypyridine-2-carboxylic Acid (**18m**).* To a solution of 5-[2-(5-ethoxy-quinoline-8-sulfonylamino)-phenylethynyl]-4-methoxy-pyridine-2-carboxylic acid methyl ester (160 mg; 0.19 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (1.9 mL; 3.85 mmol) and stirred for 4 h at RT. The product was isolated by chromatography (19%; 19 mg) as a light yellow solid. LC−MS 7: 3.76 min; 504.00 [M + H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.06 (s, 1H), 8.65 (dd, J = 4.3, 1.8 Hz, 1H), 8.60 (s, 1H), 8.49 (dd, J = 8.5, 1.8 Hz, 1H), 8.40 (d, J = 8.4 Hz, 1H), 7.77 (s, 1H), 7.54 (d, J = 8.3 Hz, 1H), 7.48 (dd, J = 8.6, 4.3 Hz, 1H), 7.35 (dd, J = 7.7, 1.5 Hz, 1H), 7.28 (td, J = 8.5, 8.0, 1.6 Hz, 1H), 7.14 (d, J = 8.5 Hz, 1H), 6.98 (t, J = 7.5 Hz, 1H), 4.29 (q, J = 6.9 Hz, 2H), 4.13 (s, 3H), 1.45 (t, J = 6.9 Hz, 3H). HRMS calcd for $C_{26}H_{21}N_3O_6S$, 504.1224; found, 504.1215.

*5-{2-[5-Chloro-2-(5-ethoxyquinoline-8-sulfonamido)phenyl]-ethynyl}-4-methoxypyridine-2-carboxylic Acid (**18n**).* Step 1: Methyl-5-bromo-4-meothoxypicolinate (1.5 g; 6.1 mmol) was dissolved in ACN (15 mL) under a nitrogen atmosphere, and trimethylsilylacetylene (1.7 mL, 12.19 mmol) was added, followed by

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diisopropylamine (1.3 mL, 9.14 mmol), copper(I)iodide (116 mg, 0.6 mmol), and tetrakis(triphenylphosphine)-palladium(0) (704 mg, 0.61 mmol). The reaction was stirred for 16 h at 80 °C. After dilution with ethyl acetate and three extractions with water, the organic phase was dried over Na_2SO_4 and evaporated to dryness after filtration. The residue was purified by chromatography, giving methyl 4-methoxy-5-[2-(trimethylsilyl)ethynyl]pyridine-2-carboxylate as a yellow solid (1.5 g, 90%).

Step 2: Silylacetylene (1.5 g, 5.46 mmol) from step 1 was dissolved in methanol (40 mL), and K_2CO_3 (75 mg, 0.55 mmol) was added. The mixture was stirred for 16 h at RT and diluted with ethyl acetate and water. After phase separation, the aqueous phase was extracted three times with ethyl acetate, and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness giving methyl 5-ethynyl-4-methoxypyridine-2-carboxylate as a crude brown solid (851 mg, 72%).

Step 3: 4-Cl-2-I-Aniline (614 mg, 2.33 mmol) and acetylene (753 mg, 3.49 mmol) from step-2 were dissolved in ACN (10 mL) under nitrogen, and diisopropylamine (0.5 m, 3.49 mmol), copper(I)iodide (44 mg, 0.23 mmol), and tetrakis(triphenylphosphine)-palladium(0) (269 mg, 0.23 mmol) were added. The reaction was stirred for 5 days at 80 °C, diluted with ethyl acetate, and washed three times with water, dried over Na_2SO_4, and evaporated to dryness after filtration. The crude was purified by chromatography, giving methyl 5-[2-(2-amino-5-chlorophenyl)ethynyl]-4-methoxypyridine-2-carboxylate as a yellow solid (390 mg, 27%).

Step 4: To a solution of the aniline product from step 3 (50 mg, 0.08 mmol) in pyridine (3 mL) was added 5-ethoxyquinoline-8-sulfonylchloride (65 mg, 0.24 mmol), and the reaction was stirred for 16 h at RT. After evaporation, the product was isolated by chromatography, giving 5-[5-chloro-2-(5-ethoxy-quinoline-8-sulfonylamino)-phenylethynyl]-4-methoxy-pyridine-2-carboxylic acid methyl ester as a brown oil (71 mg, 99%).

Step 5: To a solution of the ester from step 4 (71 mg; 0.08 mmol) in methanol (5 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (0.8 mL; 1.67 mmol) and stirred for 4 h at RT. The product was isolated by chromatography (33%; 15 mg) as a yellow solid. LC−MS 7: 4.27−4.34 min; 538.00−541.00 [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.13 (s, 1H), 8.66 (dd, J = 4.3, 1.8 Hz, 1H), 8.61 (s, 1H), 8.52 (dd, J = 8.5, 1.8 Hz, 1H), 8.37 (d, J = 8.4 Hz, 1H), 7.79 (s, 1H), 7.53 (d, J = 8.9 Hz, 1H), 7.49 (dd, J = 8.5, 4.3 Hz, 1H), 7.44 (d, J = 2.5 Hz, 1H), 7.37 (dd, J = 9.0, 2.5 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 4.30 (q, J = 7.0 Hz, 2H), 4.13 (s, 3H), 1.46 (t, J = 7.0 Hz, 3H). HRMS calcd for $C_{26}H_{20}ClN_3O_6S$, 538.0834; found, 538.0825.

*5-{2-[2-(5-Ethoxyquinoline-8-sulfonamido)phenyl]ethynyl}-3-(methylamino)pyridine-2-carboxylic Acid (**18o**).* To a solution of 5-[2-(5-ethoxy-quinoline-8-sulfonylamino)-phenylethynyl]-3-methylamino-pyridine-2-carboxylic acid methyl ester (209 mg; 0.34 mmol) in methanol (10 mL) was added sodium hydroxide solution c(NaOH) = 2 mol/l (2 N) (3.4 mL; 6.87 mmol) and stirred for 16 h at RT. The product was isolated by chromatography (29%; 50 mg) as a yellow solid. LC−MS 2: 1.71−1.74 min; 503.00 [M + H].

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.13 (s, 1H), 8.78 (dd, J = 4.3, 1.8 Hz, 1H), 8.51 (dd, J = 8.6, 2.1 Hz, 1H), 8.30 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 1.7 Hz, 1H), 7.52−7.44 (m, 2H), 7.41 (d, J = 7.3 Hz, 1H), 7.33 (m, 1H), 7.22 (d, J = 1.7 Hz, 1H), 7.09 (dd, J = 8.3, 6.0 Hz, 2H), 4.28 (q, J = 7.0 Hz, 2H), 2.90 (s, 3H), 1.45 (t, J = 7.0 Hz, 3H). HRMS calcd for $C_{26}H_{22}N_4O_5S$, 503.1384; found, 503.1371.

*5-[2-(2-{4-[2-(2-Phenoxyethoxy)ethoxy]quinoline-8-sulfonamido}phenyl)ethynyl]pyridine-2-carboxylic Acid (**18p**).* To a solution of 5-[2-(4-chloro-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (60 mg; 0.09 mmol) in DMF (4 mL) were added 2-(2-phenoxyethoxy)ethan-1-ol (156 mg; 0.85 mmol) and potassium *tert*-butylate (58 mg; 0.51 mmol). The reaction was stirred for 16 h at RT, evaporated to dryness, and the residue was purified by chromatography giving the product in 19% (10 mg) yield as a yellow solid. LCMS 2: 1.85 min; 610.00 [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.94−12.87 (m, 1H), 9.66−9.12 (m, 1H), 8.68 (dd, J = 2.0, 0.7 Hz, 1H), 8.61 (d, J = 5.3 Hz, 1H), 8.34 (dd, J = 8.4, 1.5 Hz, 1H), 8.31 (dd, J = 7.4, 1.5 Hz, 1H), 8.10 (dd, J = 8.1, 0.6 Hz, 1H), 7.98 (dd, J = 8.1, 2.1 Hz, 1H), 7.62 (dd, J = 8.3, 7.4 Hz, 1H), 7.45−7.42 (m, 2H), 7.36−7.32 (m, 1H), 7.26−7.21 (m, 2H), 7.11 (td, J = 7.6, 1.1 Hz, 1H), 7.00 (d, J = 5.4 Hz, 1H), 6.92−6.87 (m, 3H), 4.36−4.32 (m, 2H), 4.13−4.10 (m, 2H), 3.96−3.93 (m, 2H), 3.88−3.85 (m, 2H). HRMS calcd for $C_{33}H_{27}N_3O_7S$, 610.1642; found, 610.1633.

*5-[2-(2-{4-[(1-Phenyl-2,9,12,15-tetraoxaheptadecan-17-yl)oxy]-quinoline-8-sulfonamido}phenyl)ethynyl]pyridine-2-carboxylic Acid (**18q**).* To a solution of 5-[2-(4-chloro-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (40 mg; 0.05 mmol) in DMF (3 mL) were added 2-{2-[2-(6-benzyloxy-hexyloxy)-ethoxy]-ethoxy}-ethanol (187 mg; 0.54 mmol) and potassium *tert*-butylate (36 mg; 0.32 mmol). The reaction was stirred for 16 h at RT. The reactions were evaporated to dryness and the residue was purified by chromatography giving the product in 63% (27 mg) yield as a light yellow solid. LC−MS 2: 2.05 min; 768.00 [M + H]. ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.69−8.68 (m, 1H), 8.60 (d, J = 5.4 Hz, 1H), 8.36 (dd, J = 8.3, 1.3 Hz, 1H), 8.32 (dd, J = 7.3, 1.3 Hz, 1H), 8.11−8.09 (m, 1H), 7.98 (dd, J = 8.0, 2.1 Hz, 1H), 7.67−7.64 (m, 1H), 7.44−7.42 (m, 2H), 7.35−7.32 (m, 1H), 7.32−7.29 (m, 2H), 7.29−7.26 (m, 2H), 7.26−7.23 (m, 1H), 7.11 (td, J = 7.6, 0.9 Hz, 1H), 6.98 (d, J = 5.4 Hz, 1H), 4.40 (s, 2H), 4.31−4.29 (m, 2H), 3.87−3.85 (m, 2H), 3.63−3.61 (m, 2H), 3.54−3.52 (m, 2H), 3.49−3.47 (m, 2H), 3.40−3.39 (m, 2H), 3.35 (t, J = 6.5 Hz, 2H), 3.29 (t, J = 6.6 Hz, 2H), 1.55−1.44 (m, 2H), 1.42−1.37 (m, 2H), 1.33−1.19 (m, 4H). HRMS calcd for $C_{42}H_{45}N_3O_9S$, 768.2949; found, 768.2931.

*5-[2-(2-{4-[(1-Phenyl-2,9,12,15-tetraoxaheptadecan-17-yl)oxy]-quinoline-8-sulfonamido}phenyl)ethynyl]pyridine-2-carboxylic Acid (**18r**).* To a solution of 5-[2-(4-chloro-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (60 mg; 0.08 mmol) in DMF (4 mL) were added 2-[2-(4-nitro-phenoxy)-ethoxy]-ethanol (198 mg; 0.81 mmol) and potassium *tert*-butylate (55 mg; 0.49 mmol). The reaction was stirred for 16 h at RT. The reactions were evaporated to dryness and the residue was purified by chromatography giving the product in 16% (9 mg) yield as a yellow solid. LC−MS 2: 1.82 min; 655.00 [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.75−12.90 (m, 1H), 9.47−9.33 (m, 1H), 8.67 (dd, J = 2.0, 0.7 Hz, 1H), 8.61 (d, J = 5.3 Hz, 1H), 8.33−8.29 (m, 2H), 8.18−8.14 (m, 2H), 8.10 (dd, J = 8.1, 0.7 Hz, 1H), 7.97 (dd, J = 8.1, 2.1 Hz, 1H), 7.61 (dd, J = 8.3, 7.4 Hz, 1H), 7.45−7.42 (m, 2H), 7.36−7.32 (m, 1H), 7.14−7.09 (m, 3H), 7.00 (d, J = 5.4 Hz, 1H), 4.36−4.32 (m, 2H), 4.30−4.27 (m, 2H), 3.96−3.93 (m, 2H), 3.92−3.89 (m, 2H). HRMS calcd for $C_{33}H_{26}N_4O_9S$, 655.1493; found, 655.1485.

*5-(2-{2-[4-(2-{2-[2-(2-{[(tert-Butoxy)carbonyl]amino}ethoxy)-ethoxy]ethoxy}ethoxy)quinoline-8-sulfonamido]phenyl}ethynyl)-pyridine-2-carboxylic Acid (**18s**).* To a solution of 5-[2-(4-chloro-quinoline-8-sulfonylamino)-phenylethynyl]-pyridine-2-carboxylic acid methyl ester (40 mg; 0.05 mmol) in DMF (3 mL) were added 1-Boc-amino-3,6,9-trioxaundecanyl-11-ol (167 mg; 0.54 mmol) and potassium *tert*-butylate (36 mg; 0.32 mmol). The reaction was stirred for 16 h at RT. The reactions were evaporated to dryness, and the residue was purified by chromatography giving the product in 37% (16 mg) yield as an orange solid. LC−MS 2: 1.72 min; 721.00 [M + H]. ^{1}H NMR (700 MHz, DMSO-d_6): δ 13.77−13.12 (m, 1H), 9.44−9.37 (m, 1H), 8.70−8.69 (m, 1H), 8.61 (d, J = 5.3 Hz, 1H), 8.37 (dd, J = 8.3, 1.5 Hz, 1H), 8.33 (dd, J = 7.3, 1.4 Hz, 1H), 8.11−8.09 (m, 1H), 7.99 (dd, J = 8.0, 2.1 Hz, 1H), 7.68−7.65 (m, 1H), 7.45−7.42 (m, 2H), 7.36−7.33 (m, 1H), 7.13−7.10 (m, 1H), 7.00 (d, J = 5.4 Hz, 1H), 6.75−6.70 (m, 1H), 4.33−4.30 (m, 2H), 3.88−3.86 (m, 2H), 3.65−3.62 (m, 2H), 3.56−3.53 (m, 2H), 3.51−3.48 (m, 2H), 3.46−3.44 (m, 2H), 3.34 (t, J = 6.1 Hz, 2H), 3.05−3.01 (m, 2H), 1.34 (s, 9H). HRMS calcd for $C_{36}H_{40}N_4O_{10}S$, 721.2538; found, 721.2519.

*5-(2-{2-[4-(2-{2-[2-(2-Aminoethoxy)ethoxy]ethoxy}ethoxy)-quinoline-8-sulfonamido]phenyl}ethynyl)pyridine-2-carboxylic Acid (**18t**).* To a solution of 5-{2-[4-(2-{2-[2-(2-*tert*-butoxycarbonylamino-ethoxy)-ethoxy]-ethoxy}-ethoxy)-quinoline-8-sulfonylamino]-phenylethynyl}-pyridine-2-carboxylic acid (371 mg; 0.20 mmol) in 1,4-dioxane (40 mL) was added 4 N HCl in dioxane (5 mL) and

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stirred for 16 h at RT. The reaction was evaporated to dryness, and the residue was purified by chromatography giving the product in 39% (49 mg) yield as a white solid. LC−MS 2: 1.31 min; 621.00 [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.89−12.88 (m, 1H), 9.56−9.23 (m, 1H), 8.69−8.66 (m, 1H), 8.61 (d, J = 5.3 Hz, 1H), 8.36 (dd, J = 8.4, 1.4 Hz, 1H), 8.33 (dd, J = 7.3, 1.4 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 7.99 (dd, J = 8.1, 2.1 Hz, 1H), 7.80−7.69 (m, 2H), 7.69−7.65 (m, 1H), 7.47−7.42 (m, 2H), 7.37−7.32 (m, 1H), 7.15−7.10 (m, 1H), 7.00 (d, J = 5.4 Hz, 1H), 4.34−4.30 (m, 2H), 3.89−3.85 (m, 2H), 3.67−3.63 (m, 4H), 3.57−3.52 (m, 6H), 2.97−2.91 (m, 2H). HRMS calcd for $C_{31}H_{32}N_4O_8S$, 621.2014; found, 621.1999.

*3-{5-[(2-{2-[2-(2-{[8-({2-[2-(6-Carboxypyridin-3-yl)ethynyl]-phenyl}sulfamoyl)quinolin-4-yl]oxy}ethoxy)ethoxy]ethoxy}ethyl)-carbamoyl]pentyl}-2-[(1E,3E)-5-[(2Z)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)-2,3-dihydro-1H-indol-2-ylidene]penta-1,3-dien-1-yl]-3-methyl-5-sulfo-1-(3-sulfonatopropyl)-3H-indol-1-ium (**18u**).* To a solution of 5-{2-[4-(2-{2-[2-(2-amino-ethoxy)-ethoxy]-ethoxy}-ethoxy)-quinoline-8-sulfonylamino]-phenylethynyl}-pyridine-2-carboxylic acid (10 mg; 16 mmol) in THF (2 mL) were added Alexa Fluor 647 (20 mg; 16 mmol) and triethylamine (2.5 μL; 16 mmol). The reaction was stirred for 16 h at RT. The reaction was evaporated to dryness, and the residue was purified by chromatography giving the crude product in 29% (10 mg) yield.

BIOLOGY

Cell Culture Conditions. HEK293 cells were obtained from CLS and cultured in MEM with L-glutamine (31095-029, Gibco) supplemented with 10% fetal bovine serum (FBS) heat inactivated (h.i.) (S0115, Biochrom), 1% non-essential amino acids (NEAA) (11140035, Life Technologies), and 1 mM sodium pyruvate (11360088, Life Technologies). MDA-MB-231 cells were obtained from ATCC and were cultured in DMEM with 1 g/L glucose (31885-023, Gibco) and 1 mM sodium pyruvate ((11360088, Life Technologies) supplemented with 10% FBS h.i, (10270-106, Gibco). MiaPaca2 cells were obtained from ATCC and were cultured in DMEM with 4.5 g/L glucose (41965-039, Gibco) supplemented with 10% FBS h.i. (10270-106, Gibco), 2.5% horse serum h.i. (Gibco, Cat. #16050-122), and 1 mM sodium pyruvate (11360088, Life Technologies). SNU-398 (obtained from ATCC) and RT-4 (obtained from ATCC) cells were cultured in RPMI1640 with 2 g/L glucose (21875-034, Gibco) supplemented with 10% FBS h.i. (10270−106, Gibco) and 1 mM sodium pyruvate (11360088, Life Technologies). Cells were grown at 5% CO_2 and 37 °C in a humidified incubator. NCI-H358 and NCI-H441 cells were obtained from ATCC and cultured in RPMI-1640 (21875-034, Gibco) supplemented with 10% FBS (Corning, 35015CV). MC38 cells were obtained from Scripps Research Institute. For in vitro assays, MC38 cells were cultured in DMEM (41966, Gibco) supplemented with 10% FBS (S0615, Biochrom), 1% NEAA (M7145, Sigma), Pen/Strep (P4333, Sigma), and 0.25 μg/mL Plasmocin (ant-mpp, Invivogene) at 10% CO_2 and 37 °C in a humidified incubator, and harvested with 0.05% Trypsin (25300054, Gibco). For in vivo studies, MC38 cells were cultured in DMEM (Gibco 11865-092), supplemented with 10% FBS (Corning, 35015CV) at 5% CO_2 and 37 °C, passaged 2−21 times before in vivo implantation, and harvested with TrypLE Express (Gibco 12605-010).

Generation of MCT1 and MCT4 CRISPR KO. MC38 cells: 1 × 10^6 MC38 cells were nucleofected using the Nucleofector 2b device (Lonza) and nucleofection reagents Kit V (Lonza, VACA-1003) in combination with Cas9 Alt-R S.p. Cas9 Nuclease V3 (IDT), Alt-R CRISPR-Cas9 tracrRNA (IDT), and crRNA MCT1 (CACCAGCGATCATTACTGGA) or MCT4 (TGATGATAAAACAGCCAAAG). Three days after nucleofection, cells were trypsinized and plated as single cells in 96 well plates. Colonies were picked and screened with next generation sequencing (NGS) to detect possible knock-out (KO) clones. The target region in MCT1 (Fw: CAGGCTTGTGAACCATGGTG, Rev: AGTAGCCACTCACCTCCAAT) or MCT4 (Fw: CTATGGGAGGGGCTGTGG, Rev: GCCATAGAGCATAGCCAGCA) was amplified, cleaned, and sequenced (Illumina, FC-420-1004). MCT1 and MCT4 KO clones were identified, expanded, and frozen. MiaPaCa2 cells: CRISPR KO clones were generated and provided by Horizon Discovery, UK.

Lactate Efflux and Intracellular Accumulation. For lactate efflux measurement, 10,000 cells/well MDA-MB-231, 10,000 cells/well SNU-398, 5000 cells/well MiaPaca2, and 15,000 cells/well RT-4 cells were seeded onto a 384-well plate in complete media and allowed for overnight recovery at 37 °C in the atmosphere of 5% CO_2. On the following day, cells were washed with LCIS buffer (Life Tech, Cat. #A14291DJ) with final wash leaving 24 μL of LCIS buffer above the cells. Test compounds dissolved in DMSO were added to the assay plate using a D300 digital dispenser, and the plate was preincubated for 30 min at RT, protecting from light. D-(+)-Glucose in LCIS was added to an assay plate using a MultiFlo FX dispenser, to obtain a final concentration of 25 mM glucose in every well. Assay plates were incubated for 4 h at 37 °C. Next, 20 μL of medium from above the cells was collected and transferred into a new clear 384-well flat-bottom plate. A reconstituted lactate reagent (P000024, M Dialysis AB) was added to readout well, keeping a sample/reagent volume ratio of 1:1. Plates were briefly centrifuged at 4 °C, and the absorbance at 530 nm at 30 °C was measured using a Spark 20 M Tecan multimode plate reader. For intracellular lactate accumulation, 0.5 × 10^6 cells/well MDA-MB-231, 1 × 10^6 cells/well SNU-398, 0.8 × 10^6 cells/well MiaPaca2, and 0.75 × 10^6 cells/well RT-4 cells were seeded onto 6-well plates in complete growth media and allowed for overnight recovery at 37 °C in the atmosphere of 5% CO_2. On the following day, cells were washed twice with LCIS and 1.35 mL LCIS was added onto the cells. Then, test compounds diluted in DMSO were added into assay plates and preincubated for 30 min at RT, protecting from light. D-(+)-Glucose solution in LCIS was added into assay plates, to obtain a final concentration of 25 mM glucose in every well. Plates were incubated for 4 h at 37 °C. For metabolite collection, cells were washed twice with PBS, 180 μL of methanol was added to the culture dish for 30 s, and then gradually diluted with 125 μL of water for another 30 s. The extracted solution was transferred to a 96-well V-shaped bottom plate and centrifuged at 2300g at 4 °C for 5 min. Determination of lactate was carried out using the UHPLC-ESI-Q-Orbitrap-MS technique.

25,000 MC38 cells/well were seeded onto a 96-well plate (353075, Falcon) in 200 μL complete growth media and allowed for overnight recovery at 37 °C in the atmosphere of 10% CO_2. On the following day, cells were washed once with phosphate buffered saline (PBS) (14190-094, Gibco) and 200 μL of fresh media were added. Test compounds dissolved in DMSO were added into the assay plate using a Tecan D300e Digital Dispenser, and the plate was incubated at 37 °C in the atmosphere of 10% CO_2 for 4 or 24 h for lactate intracellular accumulation or lactate efflux measurement, respectively. For intracellular lactate measurement, the supernatant was discarded, cells were washed once with cold PBS, and

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resuspended in 200 μL of cold Lactate Assay Buffer (ab65331, AbCam), homogenized, and transferred to a fresh 96-well round bottom plate (735-0016, VWR). The plate was briefly centrifuged, and the supernatants were transferred into a fresh 96-well plate (353075, Falcon). For lactate efflux measurement, supernatants were transferred to a fresh 96-well round bottom plate (735-0016, VWR), briefly centrifuged, and supernatants were transferred into a fresh 96-well plate (353075, Falcon). Lactate efflux and intracellular accumulation measurements were performed following manufacturer's instructions (ab65331, AbCam) and the absorbance was measured at 450 nm using the Varioscan Lux (Thermo Scientific) plate reader.

Viability Assay. 180 cells/well MDA-MB-231, 1000 cells/well SNU-398, 80 cells/well MiaPaca2, and 2000 cells/well RT-4 cells were seeded onto a 384-well plate in complete growth media and allowed for overnight recovery at 37 °C in the atmosphere of 5% CO_2. On the following day, cells were washed twice with PBS. After washing, medium supplemented with 2.5% dialyzed FBS (SH3007903, HyClone) was added to a final volume of 50 μL per well. Test compounds in DMSO were added into the assay plate using a D300 digital dispenser. Plates were incubated for 6 days (144 h) at 37 °C/5% CO_2. Following incubation 2× diluted MTS/PMS mix was added to the plates and incubated for 3 h at 37 °C/5% CO_2. Absorbance measurement at 490 nm was performed using a Spark 20 M Tecan plate reader. Time 0 plates were used as a reference to identify the difference between cytostatic and cytotoxic effects of compounds.

1000 MC38 cells/well were seeded onto a black/clear flat 96-well plate (353219, Falcon) in 200 μL complete growth media and allowed for overnight recovery at 37 °C in the atmosphere of 10% CO_2. On the following day, cells were washed once with PBS (14190-094, Gibco), and 200 μL of fresh media was added. Test compounds dissolved in DMSO were added into the assay plate using a Tecan D300e Digital Dispenser, and the plate was incubated at 37 °C in the atmosphere of 10% CO_2 for 72 h. Viability was determined following manufacturer's instructions (CellTiter-Glo Luminescent Cell Viability Assay; G7573, Promega), and the luminescence was measured using the Varioscan Lux (Thermo Scientific) plate reader.

Fluorescent Labeling of Molecule 18t to Generate 18u. Molecule **18t** was dissolved and mixed in DMSO and labeled via the reactive amino group to PEG4-DY-647 (Dyomics). The mixture was incubated in the dark at RT for 2 h. The conjugate was purified by reversed-phase HPLC (HP-1100, Agilent, Palo Alto, USA) using a linear gradient of water and acetonitrile. Resulting **18u** was lyophilized, dissolved in DMSO, and stored at −20 °C prior to the use in a binding assay or in imaging experiments.

Expression of MCT4-eGFP. The full-length protein of MCT4 was cloned as C-terminal GFP fusion in a mammalian expression vector pCGTO (a derivative of pcDNA3.1, Invitrogen, Cat. no. V860-20) under the control of the TET-promoter and expressed in HEK293 cells. Correct expression of MCT4-eGFP was confirmed by fluorescence microscopy and western blotting. Typically, HEK293 cells (5×10^6) were transfected with 8 μg of plasmids encoding GFP-tagged protein using the Nanofectin transfection kit (PAA, Cat. no. Q051-005). Transfected cells were investigated microscopically or harvested 24 h after the transfection. During the harvest, the cells were washed twice with PBS, pelleted for 5 min at 1100 g, and then frozen at −80 °C.

Membrane Preparation and Receptor Solubilization. Membrane preparation from HEK293 cells expressing MCT4-eGFP was performed as described in the literature.[35] Membrane preparation was then diluted with TBS (50 mM Tris/HCl pH 7.4, 150 mM NaCl) supplemented with protease inhibitor cocktail (Roche #04906845001) (henceforth termed TBS/PI) to a concentration of 1 mg/mL (total membrane protein), and 0.25% Lauryl Maltose Neopentyl Glycol (LMNG) (Anatrace #NG310) was added at a final concentration of 0.25% (w/v). The membrane preparation was solubilized for 1 h at 4 °C with end-over-end rotation. Insoluble material was pelleted by centrifugation typically for 1 h at 100,000 g at 4 °C. The supernatant containing solubilized MCT4-eGFP was used in FCCS experiments, which were carried out at RT.

FCCS Measurements. All FCCS measurements were performed as described previously.[35,37] Briefly, saturation binding assays were first carried out to determine the equilibrium dissociation constants (KD) of MCT4-EGFP and **18u** interaction. Then, IC_{50}-values were determined from the inhibition binding data by using a previously described four-parameter logistic function.[35] All the experiments were carried out after incubating the samples for 1 h at RT while shaking. Data acquisition for samples in equilibrium typically required 20−60 s per sample.

Live Cell Imaging/Confocal Microscopy. Confocal laser scanning microscopy was conducted on an LSM510 confocal microscope as previously explained.[35] Cells were washed once with PBS, then compounds were added to the cells in a solution of PBS with or without 0.05% Triton to allow the permeabilization of the cell membrane. Images were acquired after 5 min incubation at RT.

Mice. SCID beige, nude, and C57/Bl6 mice were obtained from Charles River Laboratories. All mice used for experiments were 7 to 12 week old females. Mice were housed with ad libitum access to food and water in a pathogen-free facility. All procedures were performed in accordance with institutional protocols approved by the Institutional Animal Care and Use Committee of EMD Serono Research and Development Institute.

Mouse Tumor Models. Cell lines were cultured as described above, harvested during the log phase of growth and injected subcutaneously above the right foreleg of each animal within 1 h of collection. MDA-MB-231 cells were harvested by trypsinization and resuspended to a concentration 5×10^6/mouse in 200 μL of PBS: Matrigel to be implanted in female, Nu/Nu mice. About 14 days after cell implantation, when the tumors reached 100−200 mm^3, tumor-bearing mice were divided into the treatment groups using a rank sort method that produces a starting mean tumor volume in each group that is similar. 1×10^6 MC38 cells in PBS were subcutaneously implanted into C57/BL6 mice. Mice were randomized into treatment groups on the day of treatment initiation, when tumor volumes reached 50−80 mm^3 for the efficacy study, and 300−400 mm^3 for the biomarker (lactate) study.

The tumor volume was calculated using tumor length (l) and width (w) measurements with the equation $l \times w^2/2$. Using electronic calipers, the length was measured along the longest axis of the tumor, and the width was measured perpendicular to the length.

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PHARMACOKINETICS AND PHARMACODYNAMICS

Animal Treatment. To examine the PK and pharmacodynamic (PD) relationships of **18i** and **18k** in MDA-MB-231 or **18n** in MC38, tumors were established as described above. For the analysis of compound concentrations in plasma and tumor and intratumoral lactate accumulation, animals were treated with vehicle or indicated concentrations of the compounds and terminal plasma and tumor samples were collected upon euthanasia at 2 h after the treatment. **1** was dissolved in vehicle (0.5% methocel, 0.25% tween20 in water) and administered at 150 mg/kg at a volume of 10 mL/kg for administration.

Determination of Compound Concentrations. **18i**, **k**, and **n** in plasma and tumor tissue were measured by HPLC-MS/MS analysis. The compound concentration was determined using a standard curve (standard curve fitting was accomplished with Analyst 1.7 software from AB Sciex). Pharmacokinetic analyses were performed by non-compartmental methods using Phoenix WinNonlin version 6.4 (Pharsight Corporation, Mountain view, CA).

Tumoral Intracellular Lactate Quantification. Tumor samples (50−80 mg) were placed on ice in 400 μL of RIPA buffer with benzonase, followed by homogenization by homogenization stick and incubation for 20 min on ice. Next, samples were sonicated and centrifuged for 20 min at 4 °C. Samples were diluted in RIPA buffer, and the lactate concentration was measured with Lactate Reagent (M Dialysis AB; ref#P000024) according to the manufacturer's protocol.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00448.

HPLC traces of all target compounds; viability effect of target compounds; SAR for acid isosteres; acid isosteres revisited; bridge SAR revisited; SAR for middle ring B; preferred conformation; off-target selectivity; MCT isoform selectivity, structure of FFCS fluorescent probe molecule **18u**; binding of **18n** to the mouse MCT4 protein; in vivo activity of **18k** and **18i** in xenograft tumor models; inhibition of both MCT4 and MCT1 transporters; safety assessment; in silico field analysis; supplement compounds; and HPLC traces for supplement compounds (PDF)

Compound smiles codes, MDA-MB-231 IC50 with standard deviation, SNU-398, RT4, MiaPaCa-2 IC50, FCCS Ki (CSV)

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C.H. was supervising the project, together with M.N. and M.G.; A.S.-H., S.R., A.B., J.S., J.M., E.S., M.M., and F.C. did all

in vitro investigations, screening method development, and data acquisition; R.F. designed and oversaw all in vivo studies; L.C. and P.W. performed the in vivo studies; C.P. was responsible for pharmacokinetics; A.W., S.M., and F.B. did all biophysical considerations; A.G., H.S., P.B., L.D., M.L., P.N., K.O., H.P., L.W., K.Z., and J.R.S.A. synthesized all compounds; Mireille Krierr and Marcin Król did the in silico work; and S.J. was in charge of safety.

Notes

The authors declare the following competing financial interest(s): The authors declare no competing interest. SM and FB are current employees of Intana Bioscience GmbH. Merck Healthcare KGaA sponsored the part of the study performed at Intana Bioscience GmbH.

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■ ABBREVIATIONS

FaSSIF, fasted state simulated intestinal fluid; FeSSIF, fed state simulated intestinal fluid; FCCS, fluorescence cross-correlation spectroscopy; GFP, green fluorescent protein; LMNG, lauryl maltose neopentyl glycol; MCT, monocarboxylate transporter; PBS, phosphate buffered saline; SAR, structure activity relationship; SGF, simulated gastric fluid; SLC, solute carrier; QD, once per day; p.o., per os, orally

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