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Synthesis of *N*-cyano-substituted sulfilimine and sulfoximine derivatives of S0859 and their biological evaluation as sodium bicarbonate co-transport inhibitors†

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We synthesized two analogs of S0859 with modified sulfur substituents and evaluated their effects on Na⁺, HCO₃[−] co-transport activity in cancer cells and tissue. Substitution of the *N*-cyano sulfonylamido moiety in S0859 by an *N*-cyano sulfimidoyl or an *N*-cyano sulfoximidoyl group eliminated the inhibitory action against Na⁺, HCO₃[−] co-transport in human MCF-7 breast cancer cells. The analogs of S0859 also did not inhibit Na⁺, HCO₃[−] co-transport in epithelial organoids freshly isolated from breast cancer tissue of ErbB2-overexpressing mice. Consistent with previous suggestions of poor tissue penetration, S0859 inhibited Na⁺, HCO₃[−] co-transport in MCF-7 cells but had no effect in breast cancer organoids. The inactivity of the tested analogs and the ability of S0859 to inhibit Na⁺, HCO₃[−] co-transport only in isolated cells underscore the need for new approaches to selectively inhibit Na⁺, HCO₃[−] co-transport in tissue preparations and *in vivo*.

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1. Introduction

Sulfonamides are well-established building blocks in organic synthesis, and they have a wide range of medical applications due to pronounced anti-inflammatory,¹ anti-bacterial,² anti-cancer,³ anti-thyroid⁴ and diuretic properties.⁵ *N*-Cyanosulfonamide S0859 (**1**) is of particular interest for biochemical investigations,^{6,7} as it shows inhibitory activities against sodium-coupled bicarbonate transporters (NCBTs) that ubiquitously control the intracellular pH (pH_i) by transporting Na⁺ and HCO₃[−] ions across cell membranes. Inhibiting NCBTs is of importance in the case of pH dysregulations, which are associated with various diseases. Cellular acid–base transport is particularly relevant in cancer tissue where increased amounts of acidic waste products (*e.g.*, lactic acid and CO₂) generated by metabolism must be eliminated to protect the cancer cells against intracellular acidification.⁸ Multiple mechanisms of acid–base transport across cell membranes exist but Na⁺, HCO₃[−] co-transport dominates cellular net acid extrusion from human and murine breast cancer

tissue.⁹ Protein expression of the Na⁺, HCO₃[−] co-transporter NBCn1 (Slc4a7) is up-regulated in primary breast carcinomas and metastases relative to normal breast tissue⁹ and NBCn1 is responsible for the up-regulated acid extrusion following chemical breast carcinogenesis.^{9c} Enhanced acid extrusion from cancer cells also contributes to the characteristically low extracellular pH in solid cancer tissue, which can be up to 1 pH unit more acidic than normal tissue.¹⁰ The low extracellular pH in tumors and the ability of cancer cells to defend against intracellular acidification are thought to facilitate the malignant behavior of cancer cells including enhanced cell proliferation, migration, metabolism and invasiveness.^{9c,11} On this background, acid–base transporters are promising targets for cancer therapy, and indeed genetic disruption of NBCn1 delays murine breast cancer development and decelerates tumor growth after chemical induction.^{9c} Selective inhibitors of NBCn1 and other NCBTs are therefore desired both for experimental purposes and as putative anti-cancer therapeutics.

Being monoaza analogs of sulfones, sulfoximines¹² have extensively been applied in synthetic chemistry, *e.g.* as ligands in metal catalysis.¹³ Although bioactivities led to the discovery of sulfoximines¹⁴ and despite progress achieved in early studies,¹⁵ the use of sulfoximines in medicinal¹⁶ and crop protection¹⁷ chemistry has only recently blossomed. Those studies led to important observations with foreseeable consequences for future work. For example, Goldberg and

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co-workers at AstraZeneca reported that certain *N*-methylated sulfoximines, despite being isolipophilic, showed a largely higher solubility than their structurally analogous sulfonylic counterparts.¹⁸ This discovery proved important in the development of a sulfoximine-based ATR protein kinase inhibitor currently undergoing clinical trials.¹⁹ Lücking described related observations in the search for an effective pan-CDK inhibitor leading to sulfoximine BAY 1000394.^{16a} We found a range of sulfoximines with COX-2 inhibitory activities,²⁰ and prepared a sulfoximidoylic analog of Zolimidine.²¹

2. Results and discussion

2.1. Chemistry

Realizing that modifications of the sulfur substituents could significantly alter the properties of given molecules, we wondered how a substitution of the *N*-cyano sulfonylamido moiety in S0859 (**1**) by an *N*-cyano sulfimidoyl and an *N*-cyano sulfoximidoyl group would affect the bioactivities.²² Of particular interest was the consequences of these substitutions for the inhibitory effect against $\text{Na}^+/\text{HCO}_3^-$ co-transport and for the plasma binding properties known to lower the activity of S0859 in isolated tissue preparations and, presumably, *in vivo*. In order to get first answers to these questions, sulfilimine **2** and sulfoximine **3** were prepared and studied (Fig. 1).

For approaching compounds **2** and **3**, the gram-scale synthesis of S0859 reported by Bunch and co-workers⁷ served as guideline. In contrast to their work, the reaction sequence was changed allowing late-stage modifications of the sulfur moiety. Dibenzylamine **6** was recognized as key component, and its synthesis was achieved by reductive amination starting from aldehyde **4** and benzylamine **5** in 85% yield (Scheme 1). From here, two routes to methylsulfide **11** were evaluated. The first (route A) involved a palladium-catalyzed Suzuki coupling of **6** with boronic acid **7** leading to **8** followed by acylation of **8** with acyl chloride **9**. While the second step proceeded well affording **11** in 89% yield, the low efficiency of the cross-coupling (first step) providing **8** in 50% yield led us to consider a synthetic alternative. Thus, route B started

Scheme 1 Synthesis of sulfide **11**. Reagents and conditions: (a) AcOH, NaBH_3CN , THF, MeOH, overnight (85%); (b) **7**, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , 70 °C, overnight; (c) **9**, NET_3 , THF, rt, overnight.

with an acylation of **8** with acyl chloride **9**, which gave amide **10** in 95% yield. Suzuki coupling of **10** with **7** afforded sulfide **11** in 85% yield proving that this strategy was superior to the former (route A).

Next, the oxidative conversions of sulfide **11** into sulfilimine **2** and sulfoximine **3** were investigated (Scheme 2). To our delight, sulfide **11** could easily be imidated under standard metal-free reaction conditions²³ providing the first target compound, *N*-cyanosulfilimine **2**, in 67% yield. Considering the significant steric hindrance of the sulfide induced by the *ortho* substituent of the arene, this moderate yield was considered satisfying,²⁴ and the subsequent oxidation of sulfilimine **2** towards sulfoximine **3** was set into the focus. To our surprise, *m*CPBA proved ineffective for this conversion. Using potassium permanganate,²⁵ in contrast, allowed us to finish the synthesis of *N*-cyanosulfoximine **3** providing it in 59% yield.† All new products were characterized by ^1H NMR,

Scheme 2 Oxidative transformations of sulfide **11**. Reagents and conditions: (a) $\text{PhI}(\text{OAc})_2$, NH_2CN , CH_3CN , rt, overnight (67%); (b) *m*CPBA, K_2CO_3 , EtOH, 0 °C–rt, overnight; (c) KMnO_4 , acetone, 50 °C, 44 h (59%).

† The current approach towards **2** and **3** provides racemic products. For the preparation of enantiomerically enriched derivatives, asymmetric imidations might be applied. For a guiding reference, see ref. 26.

Fig. 1 Structure of the $\text{Na}^+/\text{HCO}_3^-$ co-transport inhibitor S0859 (**1**) and two analogs: sulfilimine **2** and sulfoximine **3**.

^{13}C NMR, and IR spectroscopy as well as ESI mass spectrometry (MS and HRMS). The NMR analysis revealed the presence of rotamers for amides **2**, **3**, **10**, and **11**. Measurements at elevated temperature (70 °C) did not significantly reduce the observed broadening of the NMR signals.

2.2. Biology

The biological activity of the developed *N*-cyanosulfilimine **2** and *N*-cyanosulfoximine **3** was tested against $\text{Na}^+/\text{HCO}_3^-$ co-transport in MCF-7 human breast cancer cells and in multicellular epithelial organoids freshly isolated from breast cancer tissue of ErbB2-overexpressing mice. As shown in Fig. 2A, intracellular acidification was induced with the NH_4^+ prepulse technique. NH_4Cl was washed out to a Na^+ -free solution to initially inhibit Na^+ -dependent pH_i recovery *via* Na^+/H^+ exchange and $\text{Na}^+/\text{HCO}_3^-$ co-transport. Then, the rate of Na^+ -dependent pH_i recovery was assessed upon re-addition of bath Na^+ .

In MCF-7 cells, 30 μM S0859 reduced the overall Na^+ -dependent pH_i recovery rate by ~45% (Fig. 2A and B), which is very similar to previous findings and agrees well with the effect of siRNA-mediated knockdown of NBCn1.^{6d} Based on pharmacological inhibition and RNA interference, the S0859-insensitive pH_i recovery in MCF-7 cells is mediated *via* Na^+/H^+ exchange.^{6d} In contrast to the inhibition of pH_i recovery by S0859, no significant effect of the sulfoximine or sulfilimine analogs was seen when applied to MCF-7 cells at a concentration of 30 μM (Fig. 2A and B). In epithelial organoids from murine breast cancer tissue, we saw no effect of 30 μM S0859 or of its analogs (Fig. 2C) consistent with previous suggestions that S0859 penetrates poorly into tissue preparations due to strong (~98–99%) protein binding.⁷

3. Conclusion

In conclusion, we prepared analogs of S0859 where the *N*-cyano sulfonylamido moiety of the parent compound was modified to an *N*-cyano sulfimidoyl or an *N*-cyano sulfoximidoyl group. These compounds showed no inhibitory properties against $\text{Na}^+/\text{HCO}_3^-$ co-transport. Additionally, our findings reinforce that although S0859 is useful for $\text{Na}^+/\text{HCO}_3^-$ co-transport inhibition in isolated cells, it lacks inhibitory capacity in tissue preparations. New approaches for selective $\text{Na}^+/\text{HCO}_3^-$ co-transport inhibition in tissue preparations and *in vivo* are therefore still required.

§ Sulfilimine **2** and sulfoximine **3** were also submitted for screening within the Developmental Therapeutics Program of the Division of Cancer Treatment and Diagnosis [US National Cancer Institute (NCI)/National Institutes of Health (NIH); <http://dtp.cancer.gov>]. Applying the compounds at a single dose (1×10^{-5} molar) to the full NCI 60 cell panel, including leukemia, melanoma, and cancers of the lung, colon, central nervous system (CNS), prostate, and breast, as well as renal and ovarian cancer cell lines showed only minor growth inhibitions. For details, see ESI.†

Fig. 2 Sulfilimine **2** and sulfoximine **3** do not inhibit $\text{Na}^+/\text{HCO}_3^-$ co-transport in MCF-7 human breast cancer cells or in epithelial organoids freshly isolated from breast cancer tissue. A. Superimposed pH_i recordings from MCF-7 cells. To improve clarity, the pH_i recovery phase (marked by the dotted gray square) is displayed at twice the magnification of the initial part of the trace and only the control curve is shown for the period prior to addition of 30 μM inhibitor or DMSO (vehicle control). B + C. Relative initial rates of Na^+ -dependent pH_i recovery in MCF-7 cells (B, $n = 5$ –6) or epithelial organoids (C, $n = 4$ –5) in the presence of 30 μM inhibitor or vehicle. In panel B, data were compared by one-way ANOVA followed by Dunnett's post-test. In panel C, comparisons were performed by paired two-tailed student's *t*-test (S0859) or repeated measures one-way ANOVA followed by Dunnett's post-test (analogues). * $P < 0.05$, NS: not significantly different vs. control.

4. Experimental

4.1. General

Flash column chromatography (FCC) was performed with silica gel 60 (63–200 μm) purchased from Merck. For thin layer chromatography (TLC), aluminium sheets silica gel 60 F_{254} (Merck) were utilized, and the products were visualized by treatment with aqueous potassium permanganate or *n*-butanolic ninhydrin solutions.

Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent VNMR 400 (^1H NMR: 400 MHz, ^{13}C NMR: 101 MHz) or an Agilent VNMR 600 (^1H NMR: 600 MHz, ^{13}C NMR: 151 MHz) spectrometer. The chemical shifts δ are given in

parts per million (ppm) relative to tetramethylsilan (TMS) and the residual solvent peak of the non-deuterated solvent was used as reference (CHCl_3 : ^1H NMR: $\delta = 7.26$ ppm, $^{13}\text{C}\{^1\text{H}\}$ NMR: CDCl_3 : $\delta = 77.00$ ppm). The multiplicity was reported with the following abbreviations: br.s = broad singlet, s = singlet, d = doublet, m = multiplet, and coupling constants were given in Hertz (Hz).

Infrared spectra (IR) were recorded on a PerkinElmer Spectrum 100 spectrometer using the attenuated total reflectance (ATR) technique. The wavenumber ν is given in cm^{-1} .

Mass spectra were recorded on a Finnigan SSQ 7000 spectrometer [electron ionisation (EI), 70 eV; chemical ionisation (CI), 100 eV]. For high resolution mass (HRMS) spectra, a Thermo Fisher Scientific LTQ Orbitrap XL spectrometer [electrospray ionisation (ESI) in positive ion mode] was used. The resulting signals are given according to their m/z values and their relative intensity is reported in parenthesis.

Anhydrous solvents were freshly distilled under moisture-free atmosphere with adequate drying reagents or purchased. If not mentioned differently, common Schlenk technique was applied. Purchased chemicals were used without further purification.

For compounds 2 and 3, published imination²³ and oxidation²⁵ protocols were applied. Compounds 6, 8, 10, and 11 were prepared in analogy to reported protocols by Bunch and co-workers.⁷

4.2. Preparative protocols

General procedure 1 (GP1). To a solution of the appropriate amine in THF, 2-(methylthio)benzeneboronic acid (7, 1.2 equiv.), $\text{Pd}(\text{PPh}_3)_4$ (0.03 equiv.) and Na_2CO_3 (aq., 2 M, 2.4 equiv.) were added. After reflux overnight, the mixture was cooled to room temperature and the solvent was evaporated. The residue was dissolved in ethyl acetate and the organic layer was washed with a saturated Na_2CO_3 solution and brine. The organic layer was dried over MgSO_4 and finally, products 8 and 11 were isolated after purification by FCC as described for all reactions in the following parts.

General procedure 2 (GP2). To a solution of the appropriate amine in THF, triethylamine (1.4 equiv.) was added. After cooling to 0 °C, the solution was treated dropwise with 2-chlorobenzoyl chloride (9, 1.3 equiv.) and the reaction mixture was left to stir overnight at room temperature. After evaporation, the residue was dissolved in ethyl acetate and washed with diluted HCl (1 M) and a saturated Na_2CO_3 solution. The organic layer was dried over MgSO_4 and finally, products 10 and 11 were isolated after purification by FCC as described for all reactions in the following parts.

2-Chloro-*N*-(4-methylbenzyl)-*N*-((2'-*N*-cyano-sulfilimidoyl-[1,1'-biphenyl]-4-yl)-methyl)benzamide (2). To a solution of sulfide 11 (173 mg, 0.37 mmol) in acetonitrile (7 mL), cyanoamide (23 mg, 0.55 mmol) and $\text{PhI}(\text{OAc})_2$ (130 mg, 0.403 mmol) were added. The reaction mixture was left to stir overnight and then, compound 2 was obtained as a white crystalline solid after purification by FCC (*n*-pentane/acetone = 2 : 1

to *n*-pentane/acetone 1 : 1). Yield: 67%; mp: 66–67 °C (decomposition). ^1H NMR (400 MHz, CDCl_3 , mixture of rotamers): $\delta = 8.27$ – 8.21 (m, 1H), 7.72 – 7.63 (m, 2H), 7.52 – 7.47 (m, 1H), 7.47 – 7.29 (m, 7H), 7.27 – 7.21 (m, 2H), 7.21 – 7.13 (m, 2H), 7.07 – 7.00 (m, 1H), 5.42 – 5.14 (m, 1H), 4.45 – 4.15 (m, 3H), 2.68 (2 s, 3H), 2.37 , 2.35 (2 s, 3H). ^{13}C NMR (101 MHz, CDCl_3 , mixture of several conformers): $\delta = 169.05$, 168.88 , 145.23 , 144.45 , 141.03 , 137.82 , 137.77 , 137.47 , 137.18 , 136.38 , 136.00 , 135.63 , 133.02 , 132.74 , 132.59 , 132.36 , 131.35 , 130.49 , 130.37 , 129.80 , 129.70 , 129.57 , 129.54 , 129.46 , 129.38 , 129.19 , 128.70 , 128.18 , 127.97 , 127.39 , 127.28 , 125.44 , 125.32 , 51.04 , 50.35 , 46.57 , 46.27 , 36.37 , 36.18 , 21.17 , 21.11 . MS (EI): $m/z = 471$ (11), 366 (48), 258 (50), 213 (32), 197 (34), 139 (100). MS (ESI): $m/z = 534$ ($[\text{M} + \text{Na}]^+$, 46), 512 ($[\text{M} + \text{H}]^+$, 100). IR (ATR): $\nu = 3056$, 3021 , 2922 , 2860 , 2323 , 2239 , 2146 , 1990 , 1732 , 1636 , 1514 , 1410 , 1360 , 1295 , 1243 , 1151 , 1111 , 1053 , 993 , 963 , 913 , 844 , 816 , 760 , 729 . HRMS (ESI): m/z calcd. for $\text{C}_{30}\text{H}_{27}\text{ON}_3\text{ClS}$: 512.1558 ; found: 512.1553 .

2-Chloro-*N*-(4-methylbenzyl)-*N*-((2'-*N*-cyano-sulfoximidoyl-[1,1'-biphenyl]-4-yl)-methyl)benzamide (3). *N*-Cyano sulfilimine 2 (90.0 mg, 0.17 mmol) was dissolved in acetone (3.00 mL) and potassium permanganate (57.0 mg, 0.35 mmol) was added. The reaction mixture was heated for 44 h to 60 °C. Title compound 2 was obtained as a colorless crystalline solid after purification by FCC (*n*-pentane/ethyl acetate = 2 : 1 to *n*-pentane/ethyl acetate 1 : 1). Yield: 59%; mp: 63–64 °C (decomposition). ^1H NMR (600 MHz, CDCl_3 , mixture of rotamers): $\delta = 8.41$ – 8.30 (m, 1H), 7.88 – 7.82 (m, 1H), 7.78 – 7.72 (m, 1H), 7.61 – 7.19 (m, 12H), 7.10 (s, 1H), 5.48 – 5.21 (m, 1H), 4.52 – 4.20 (m, 3H), 2.99 (s, 3H), 2.44 , 2.42 (2 s, 3H). ^{13}C NMR NMR (151 MHz, CDCl_3 , mixture of several conformers): $\delta = 168.98$, 168.93 , 168.83 , 141.54 , 141.34 , 137.72 , 137.67 , 137.37 , 137.05 , 136.69 , 136.24 , 135.68 , 135.60 , 134.95 , 134.79 , 134.73 , 134.67 , 133.47 , 133.43 , 132.95 , 132.28 , 130.39 , 130.33 , 130.30 , 129.79 , 129.71 , 129.67 , 129.51 , 129.42 , 129.32 , 129.27 , 128.91 , 128.73 , 128.64 , 128.48 , 128.35 , 127.89 , 127.40 , 127.25 , 127.17 , 111.97 , 111.84 , 50.97 , 50.40 , 46.59 , 46.29 , 43.54 , 43.34 , 21.13 , 21.07 . MS (ESI): $m/z = 550$ ($[\text{M} + \text{Na}]^+$, 94), 528 ($[\text{M} + \text{H}]^+$, 100). IR (ATR): $\nu = 3463$, 3017 , 2926 , 2194 , 1732 , 1635 , 1425 , 1243 , 1192 , 1042 , 976 , 822 , 762 . HRMS (ESI): m/z calcd. for $\text{C}_{30}\text{H}_{27}\text{O}_2\text{N}_3\text{ClS}$: 528.1507 ; found: 528.1508 .

***N*-(4-Bromobenzyl)-*N*-(4-methylbenzyl)amin (6).** To a solution of 4-bromobenzaldehyde (4, 1.50 g, 8.15 mmol) in THF/methanol (18 mL/22 mL), 4-methylbenzylamine (5, 1.00 mL, 8.15 mmol) and acetic acid (1.00 mL, 16.3 mmol) were added. After stirring for 30 min at room temperature, NaBH_3CN (1.02 g, 16.3 mmol) in methanol (14 mL) was added within a minute to the reaction mixture, which was left to stir overnight at room temperature. The organic layer was washed with diluted HCl (1 M, 200 mL) and the aqueous phase was extracted with ethyl acetate (3×100 mL). The combined organic layers were dried over MgSO_4 . Title compound 6 was obtained as yellow crystalline solid after purification by FCC (*n*-pentane/ethyl acetate = 1 : 1). Yield: 85%; mp: 29–30 °C (decomposition). ^1H NMR (400 MHz, CDCl_3): $\delta = 7.47$ – 7.42

(m, 2H), 7.25–7.19 (m, 4H), 7.17–7.12 (m, 2H), 3.75 (s, 4H), 2.35 (s, 3H), 1.65 (br.s, 1H). ^{13}C NMR (101 MHz, CDCl_3): δ = 139.35, 136.99, 136.61, 131.40, 129.84, 129.10, 128.07, 120.63, 52.78, 52.30, 21.09. MS (EI): m/z = 289 ($[\text{M}]^+$, 14), 183 (31), 168 (45), 120 (19), 105 (100), 89 (27). MS (CI): m/z = 290 ($[\text{M} + \text{H}]^+$, 100), 225 (16), 105 (11). IR (ATR): ν = 3018, 2917, 2822, 2326, 2096, 1995, 1897, 1743, 1589, 1450, 1358, 1199, 1103, 1009, 801. Elementary analysis: calcd. for $\text{C}_{15}\text{H}_{16}\text{BrN}$: 62.08 (C), 5.56 (H), 4.83 (N); found: 62.23 (C), 5.47 (H), 4.75 (N).

***N*-(4-Methylbenzyl)-1-(2'-(methylthio)-[1,1'-biphenyl]-4-yl)-methanamine (8).** Following GP 1, the cross coupling of amine 6 (106 mg, 0.36 mmol) and boronic acid 7 (71 mg, 0.42 mmol) catalyzed by $\text{Pd}(\text{PPh}_3)_4$ (12 mg, 0.010 mmol) and Na_2CO_3 (aq., 0.4 mL) in THF (3.00 mL) afforded title compound 8 as a colorless oil after purification by FCC (*n*-pentane/ethyl acetate = 2:1 to ethyl acetate 100%). Yield: 50%. ^1H NMR (600 MHz, CDCl_3): δ = 7.47–7.42 (m, 4H), 7.40–7.36 (m, 1H), 7.35–7.28 (m, 3H), 7.28–7.22 (m, 2H), 7.21–7.18 (m, 2H), 3.90 (s, 2H), 3.87 (s, 2H), 2.41 (s, 3H), 2.40 (s, 3H), 1.85 (br.s, 1H). ^{13}C NMR (101 MHz, CDCl_3): δ = 140.67, 139.59, 139.08, 137.16, 137.06, 136.47, 131.37, 129.95, 129.81, 129.28, 129.07, 129.04, 128.08, 128.04, 127.83, 127.81, 125.07, 124.64, 52.96, 52.83, 21.08, 15.94. MS (EI): m/z = 334 ($[\text{M} + 1]^+$, 100), 227 (46), 213 (84), 199 (51), 120 (37), 105 (50). MS (CI): m/z = 334 ($[\text{M} + \text{H}]^+$, 100), 212 (30), 197 (53), 167 (48), 149 (35), 98 (58). IR (ATR): ν = 3324, 3016, 2917, 2823, 2324, 2095, 1997, 1904, 1740, 1639, 1583, 1511, 1446, 1363, 1213, 1100, 1038, 1005, 957, 957, 810, 749. HRMS (ESI): m/z calcd. for $\text{C}_{22}\text{H}_{24}\text{NS}$: 334.1624; found: 334.1624.

2-Chloro-*N*-(4-methylbenzyl)-*N*-(2'-(methylthio)-[1,1'-biphenyl]-4-yl)methyl-benzamide (10). Following GP 2, the reaction of amine 6 (400 mg, 1.72 mmol), triethylamine (0.38 mL, 2.70 mmol) and acyl chloride 9 (400 mg, 2.23 mmol) in THF (8 mL) afforded title compound 10 as a white crystalline solid after purification by FCC (*n*-pentane/ethyl acetate = 1:1 to *n*-pentane/ethyl acetate = 1:3). Yield: 95%; mp: 111–113 °C (decomposition). ^1H NMR (600 MHz, CDCl_3 , mixture of rotamers): δ = 7.50–7.43 (m, 2H), 7.43–7.36 (m, 1.5H), 7.36–7.27 (m, 2.5H), 7.24–7.19 (m, 2H), 7.19–7.10 (m, 2H), 7.01–6.94 (m, 2H), 5.26–5.15 (m, 1H), 4.33–4.01 (m, 3H), 2.36, 2.34 (2 s, 3H). ^{13}C NMR (101 MHz, CDCl_3 , mixture of several conformers): δ = 168.94, 168.88, 137.72, 137.40, 135.70, 135.67, 135.49, 134.92, 133.18, 133.12, 132.96, 132.35, 131.89, 131.68, 130.47, 130.28, 129.82, 129.76, 129.51, 129.33, 129.07, 128.69, 127.92, 127.85, 127.38, 127.21, 127.19, 121.66, 121.52, 50.54, 50.03, 46.26, 45.75, 21.16, 21.09. MS (EI): m/z = 429 ($[\text{M}]^+$, 4), 324 (21), 258 (42), 139 (100). MS (CI): m/z = 430 ($[\text{M} + \text{H}]^+$, 9), 259 (5), 157 (100), 139 (80). IR (ATR): ν = 3268, 2919, 2647, 2323, 2105, 1899, 1780, 1637, 1477, 1435, 1239, 1160, 1102, 1066, 992, 802, 755. Elementary analysis: calcd. for $\text{C}_{22}\text{H}_{19}\text{BrClNO}$: 61.63 (C), 4.47 (H), 3.27 (N); found: 61.38 (C), 4.08 (H), 2.86 (N).

2-Chloro-*N*-(4-methylbenzyl)-*N*-(2'-(methylthio)-[1,1'-biphenyl]-4-yl)methyl-benzamide (11). Synthesis 1: following GP 1, the cross-coupling of amine 10 (251 mg, 0.58 mmol) and boronic acid 7 (71 mg, 0.72 mmol), catalyzed by $\text{Pd}(\text{PPh}_3)_4$

(19 mg, 0.020 mmol) and Na_2CO_3 (0.70 mL) in THF (5.00 mL), afforded title compound 11 as a colorless crystalline solid after purification by FCC (*n*-pentane/ethyl acetate = 5:1 to *n*-pentane/ethyl acetate = 2:1). Yield: 81%; mp: 72–73 °C (decomposition). Synthesis 2: following GP 2, the reaction of amine 6 (109 mg, 0.330 mmol), triethylamine (64 μL , 0.46 mmol) and acyl chloride 9 (76 mg, 0.43 mmol) in THF (3 mL) afforded title 10 as a white crystalline solid after purification by FCC (*n*-pentane/ethyl acetate = 3:1). Yield: 89%. ^1H NMR (600 MHz, CDCl_3 , mixture of rotamers): δ = 7.50–7.42 (m, 5H), 7.42–7.37 (m, 1H), 7.37–7.32 (m, 4H), 7.31–7.27 (m, 1H), 7.27–7.18 (m, 4H), 7.10–7.07 (m, 1H), 5.33 (d, J = 14.6 Hz, 1H), 4.34–4.24 (m, 2H), 2.43, 2.42, 2.40 (s, 6H). ^{13}C NMR (151 MHz, CDCl_3 , mixture of several conformers): δ = 168.85, 168.82, 140.40, 140.03, 139.96, 139.64, 137.46, 137.16, 136.98, 136.92, 135.84, 135.58, 135.07, 133.21, 132.59, 130.47, 130.44, 130.11, 129.93, 129.83, 129.69, 129.66, 129.63, 129.45, 129.39, 129.21, 128.72, 128.28, 128.03, 127.97, 127.91, 127.88, 127.35, 127.16, 127.12, 127.06, 125.09, 125.03, 124.66, 124.64, 50.58, 50.41, 46.26, 46.17, 21.12, 21.05, 15.87, 15.83. MS (EI): m/z = 366 (22), 258 (28), 139 (100), 105 (21). MS (CI): m/z = 472 ($[\text{M} + \text{H}]^+$, 30), 430 (100), 350 (70), 314 (28), 258 (28), 139 (45), 105 (78), 91 (28). IR (ATR): ν = 3022, 2923, 2321, 2100, 1983, 1915, 1787, 1736, 1534, 1419, 1308, 1226, 1185, 1139, 985, 815, 751. HRMS (ESI): m/z calcd. for $\text{C}_{29}\text{H}_{26}\text{ONClNaS}$: 494.1316; found: 494.1313.

4.3. Evaluation of inhibitory activity against $\text{Na}^+/\text{HCO}_3^-$ co-transport

S0859 and the developed sulfilimine 2 and sulfoximine 3 analogs were tested as inhibitors of $\text{Na}^+/\text{HCO}_3^-$ co-transport in MCF-7 human breast cancer cells and in epithelial organoids isolated from breast cancer tissue of ErbB2-overexpressing mice (FVB/N-Tg(MMTVneu)202Mul/J, Jackson Laboratories, USA). Animal experiments were approved by the Danish Animal Experiments Inspectorate.

MCF-7 cells were grown in RPMI-1640 medium (#61870-010, Gibco) supplemented with 6% fetal bovine serum (FBS, #S0115, Biochrom AG), 1% penicillin/streptomycin (#15140-122, Gibco), 1 $\mu\text{g mL}^{-1}$ puromycin (#P9620, Sigma), and 200 $\mu\text{g/mL}$ G418 (#G8168, Sigma). Cells were maintained at 37 °C in an atmosphere of 5% CO_2 and used at passages 15–21. MCF-7 cells were seeded on cover glasses coated for 2 hours with 0.2% autoclaved gelatin (#G2625, Sigma) and investigated after 2–3 days.

Breast epithelial organoids were isolated as previously described:^{9b,c} breast cancer samples were cut into 1 mm pieces in phosphate-buffered saline (PBS, in mM: 154.2 Na^+ , 4.1 K^+ , 140.6 Cl^- , 8.1 HPO_4^{2-} , 1.5 H_2PO_4^- , pH 7.4) and transferred to Tissue Culture Flat Tubes (#91243, Techno Plastic Products AG) containing advanced DMEM/F12 culture medium (Life Technologies, Denmark) supplemented with 1% GlutaMAX (#35050-038, Gibco), 10% FBS and a final concentration of 450 IU mL^{-1} collagenase type 3 (Worthington

Biochemical Corporation). Culture flasks were kept in a shaking incubator (~60 rpm, 5% CO₂, 37 °C) for 4 hours before organoids were allowed to sediment for 20 minutes without shaking.

MCF-7 cells or freshly isolated cancer organoids were loaded with 3 μM BCECF-AM (Invitrogen) for 20 minutes at 37 °C. Mounted on the stage of an Olympus IX70 microscope, the preparations were alternately excited at 485 and 440 nm and emission light collected at 510 nm with a CCD-based EasyRatioPro fluorescence imaging system (Photon Technology International). The 485/440 BCECF fluorescence ratio was taken as a measure of pH_i and recovery rates expressed relative to control conditions without inhibitor present.

Intracellular acidification was induced by the NH₄⁺ prepulse technique:²⁷ 20 mM NH₄Cl was added to the bath and then washed out to a Na⁺-free solution to determine the rate of Na⁺-independent net acid extrusion. Then, Na⁺ was returned to the bath solution and the increase in pH_i recovery rate determined. The initial abrupt alkalinization caused by application of NH₄Cl can be explained by fast influx of NH₃ and was followed by the expected gradual recovery due to NH₄⁺ uptake and activation of base extrusion mechanisms. Following washout of NH₄Cl, NH₃ rapidly leaves the cells shifting the NH₄⁺ ⇌ NH₃ + H⁺ equilibrium to the right, liberating H⁺ and causing the desired intracellular acidification. The tested analogs were diluted in DMSO (final [DMSO] in the bath <0.1%) and added to the bath solution at a concentration of 30 μM at the time point of NH₄Cl washout.

The physiological saline solution used for pH_i recordings contained (in mM): 140 Na⁺, 4 K⁺, 1.6 Ca²⁺, 1.2 Mg²⁺, 122 Cl⁻, 24 HCO₃⁻, 1.2 SO₄²⁻, 1.18 H₂PO₄⁻, 10 HEPES, 5.5 glucose, 0.03 EDTA. In Na⁺-free solutions, Na⁺ was replaced with equimolar amounts of *N*-methyl-D-glucammonium. All solutions were aerated with 5% CO₂/balance air; pH was adjusted to 7.4 at 37 °C. Solutions contained 5 mM probenecid to inhibit BCECF extrusion by the organic anion transporter.

Statistics. Data are expressed as mean ± SEM, *n* equals number of mice or culture flasks. Data from MCF-7 cells were compared by one-way ANOVA followed by Dunnett's post-test. Organoids isolated from the same mouse were matched and the resultant data compared using paired two-tailed Student's *t*-test (two groups) or repeated measures one-way ANOVA followed by Dunnett's post-test (>2 groups). Statistical analyses were performed using GraphPad Prism 5.02 software.

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