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Enhanced Iodide Sequestration by 3-Biphenyl-5,6-dihydroimidazo[2,1-*b*]thiazole in Sodium/Iodide Symporter (NIS)-Expressing Cells

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*The ability of the sodium/iodide symporter (NIS) to take up iodide has long provided the basis for cytoreductive gene therapy and cancer treatment with radioiodide. One of the major limitations of this approach is that radioiodide retention in NIS-expressing cells is not sufficient for their destruction. We identified and characterized a small organic molecule capable of increasing iodide retention in HEK293 cells permanently transfected with human NIS cDNA (hNIS-HEK293) and in the rat thyroid-derived cell line FRTL-5. In the presence of 3-biphenyl-4′-yl-5,6-dihydroimidazo[2,1-*b*]thiazole (ISA1), the transmembrane iodide concentration gradient was increased up to 4.5-fold. Our experiments indicate that the imidazothiazole derivative acts either by inhibiting anion efflux mechanisms, or by promoting the relocation of iodide into subcellular compartments. This new compound is not only an attractive chemical tool to investigate the mechanisms of iodide flux at the cellular level, but also opens promising perspectives in the treatment of cancer after NIS gene transfer.*

Introduction

The sodium/iodide symporter (NIS) is an intrinsic membrane glycoprotein that catalyzes the active accumulation of iodide from blood into thyroid follicular cells. This transport is the first step in the biosynthesis of thyroid hormones T4 and T3, which are essential for many biological processes.[1] NIS is located in the basolateral membrane of thyrocytes and concentrates iodide against a 20- to 40-fold chemical gradient. The Na^+ gradient maintained by the ouabain-sensitive Na^+/K^+ ATPase is the driving force for I^- transport. The molecular characterization of NIS was carried out after cloning the rat and human forms.[2,3] Human NIS is a 643-amino-acid polypeptide, and the secondary structure model proposes 13 transmembrane segments.[4] Subcellular localization, expression, and activity of NIS were also shown to be regulated by thyroid-stimulating hormone (TSH) through diverse control mechanisms including phosphorylation by regulatory proteins.[5,6] S43, T49, S227, T577, and S581 were identified as in vivo phosphorylation sites, and it has been proposed that the phosphorylation state of these residues is correlated with the functional activity of NIS.[7] These studies indicate that NIS maturation, localization, and activity are modulated at the post-translational level. NIS is not specific for iodide, and other monovalent anions such as ClO_4^-, SCN^-, BF_4^-, PF_6^-, ReO_4^-, and TcO_4^- (but not Br^- and Cl^-) are also transported.[8–10] Since the discovery of NIS, thorough biochemical analysis has elucidated the mechanism of iodide transport across the basolateral membrane, and revealed the key role of NIS in thyroid disorders such as thyroid cancer, autoimmune disease, and congenital hypothyroidism. However, the mechanism of iodide efflux is still not understood.

In addition to its key role in thyroid physiology, NIS-mediated accumulation in the thyroid gland has long provided the basis for selective cell destruction in benign and malignant thyroid diseases using radioiodide (^{131}I).[11] In light of this, it was proposed to extend this strategy to a wide range of cancer tissues that do not express functional NIS, especially to patients with a poor prognosis.[12–14] In recent years, the expression of functional NIS by gene transfer was successfully achieved in cases of hepatoma, pancreatic cancer, myeloma, ovarian cancer, prostate carcinoma, and colon cancer.[15–20] Unfortunately, radioiodine retention within NIS-expressing cells is generally low, and for this reason this strategy is not ready for clinical trial.[21] Not only is NIS expression necessary, but radioiodide uptake and retention are also required to ensure a cytotoxic effect. Several strategies were reported that efficiently increase iodide uptake. Tumor reduction was observed after increasing NIS expression using TSH,[22] or by inducing ectopic expression of the thyroperoxidase gene.[23] Another method is to use small organic molecules. Retinoic acid was reported to increase NIS mRNA levels and iodide uptake in several cancer cells,[24] and its efficacy was later confirmed in vivo using mouse breast cancer models.[25] The DNA-demethylating agent 5-azacytidine was shown to restore NIS mRNA expression and iodide uptake in three thyroid cancer cell lines.[26] However, none of these agents has been shown to consistently enhance radioiodide uptake in human clinical studies.[12] 4,4′-Diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) was found to significantly increase intracellular radioiodide retention in thyroid

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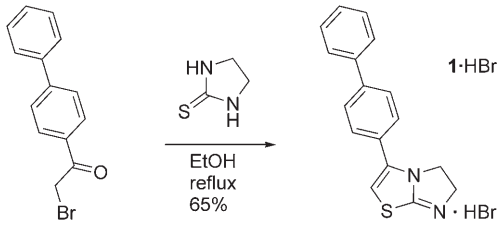
cancer cells by decreasing iodide efflux.[27] However, DIDS is a nonspecific inhibitor of anion exchange, and for this reason will probably not be a safe agent for clinical application.

Recently, the identification of several inhibitors of NIS function using high-throughput screening (HTS) was reported.[28] During the HTS campaign, a compound capable of increasing the level of iodide entrapment in NIS-expressing cells was also discovered. Herein we report the synthesis and biological characterization of 3-biphenyl-4′-yl-5,6-dihydroimidazo[2,1-*b*]thiazole (compound **1**) as a very potent iodide-sequestering agent. We used isotopic flux measurement in two NIS-expressing cell types to demonstrate its efficacy and formulate hypotheses for its mode of action at the cellular level.

Results and Discussion

Preparation of compound 1

3-Biphenyl-4′-yl-5,6-dihydro[2,1-*b*]thiazole hydrobromide **1**·HBr was prepared by condensing 2-imidazolidinethione with 2-bromo-4′-phenylacetophenone in boiling ethanol (Scheme 1).



Scheme 1. Chemical synthesis of target compound **1**.

This reaction is known as the Hantzsch cyclization reaction.[29,30] The compound was isolated in 65% yield after simple filtration procedures. The purity of **1**·HBr was confirmed by HPLC to exceed 99%, and ^{1}H and ^{13}C NMR showed the absence of by-products. Most of the biological studies described herein were performed on the hydrobromide salt **1**·HBr. The free base of **1** was also isolated after liquid–liquid extraction workup using a $CH_2Cl_2/NaHCO_3$ 5% biphasic system. HPLC analysis indicated 98% purity.

Compound 1 increases iodide entrapment in NIS-expressing cells

The concentration-dependent activity of compound **1** was measured in hNIS-HEK293 cells and in the rat thyroid-derived cell line FRTL-5 (Figure 1). A marked increase in iodide sequestration was observed for both cell lines in the presence of **1**. When hNIS-HEK293 cells were incubated for 120 min with NaI (10 μM), cell-trapped iodide was 32±3% of total iodide in the absence of **1**. In the presence of **1** (100 μM), iodide entrapment reached 74±4% of total iodide, indicating that the iodide concentration gradient was increased by a factor of 2.3 (Table 1). In FRTL-5 cells, this effect was even more pronounced (6±2% versus 27±5%), showing that compound **1** at 100 μM promoted a 4.5-fold increase in the iodide gradient. For both cell lines, the activity rapidly decreased when **1** was used at lower concentration; at 10 μM, the iodide gradient was only increased by a factor of 1.3 and 1.2 in hNIS-HEK293 and FRTL-5 cells, respectively. At concentrations greater than 100 μM, biological activity was also strongly reduced, probably due to cell toxicity. Reference compounds were tested under the same conditions. When all-*trans*-retinoic acid was present at a concentration of 100 μM, we observed that iodide uptake was slightly but consistently enhanced in hNIS-HEK293 cells (×1.07) and FRTL-5 cells (×1.11). DIDS (1 mM) increased iodide entrapment in FRTL-5 cells (×1.43), whereas no activity was observed

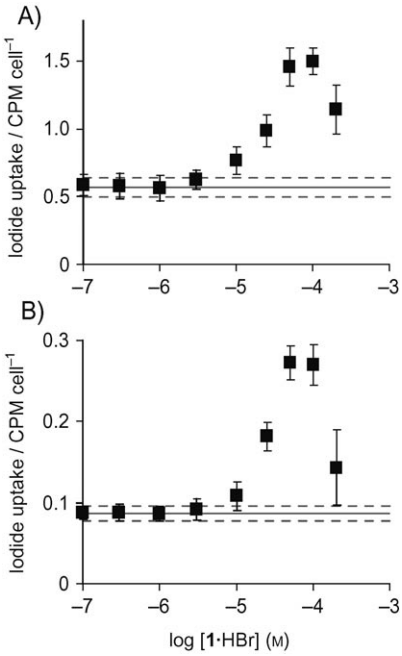


Figure 1. Dose–response curves of **1**·HBr in A) hNIS-HEK293 and B) FRTL-5 cells. hNIS-HEK293 and FRTL-5 cells in 96-well isoplates were incubated at 20 °C for 120 min with **1**·HBr (1×10^{-7}–2×10^{-4} M) and $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}). Cells were washed at +4 °C before EtOH (30 μL) was added. Cell-associated radioactivity was determined after addition of the scintillation cocktail followed by overnight shaking. Results are shown for one experiment representative of at least three independent experiments with mean values ±SD ($n=4$). The mean of 10 control wells (no compound added) are represented (——) ±SD (-----). $NaClO_4$-mediated inhibition of iodide uptake was tested as a control in each plate. The experimental data were validated when IC_{50} values were 5×10^{-7} M (±200%) in hNIS-HEK293, and 2×10^{-7} M (±200%) in FRTL-5 cells.

Table 1. Percentage of cell-trapped iodide in the presence or absence of **1**·HBr.[a]

	Intracellular I^- [%]		
	hNIS-HEK293	FRTL-5	HEK293 wild
− **1**·HBr	32±3	6±2	<0.6
+ **1**·HBr	74±4	27±5	<0.6

[a] Cells were incubated at 20 °C for 120 min with $Na^{125}I$ (0.2 μCi, 10 μM per well) in the absence or presence of **1**·HBr (100 μM) before cell-associated radioactivity was determined. Results are the means of three independent experiments ($n=4$).

in hNIS-HEK293 cells. These results show that compound **1** is more effective than known agents for increasing iodide entrapment in NIS-expressing cells. The free base of **1** was isolated after liquid–liquid extraction and tested on hNIS-HEK293 and FRTL-5 cells. The biological activity of this sample was identical to that of the hydrobromide salt (not shown). Compound **1** was named ISA1 for "iodide sequestering agent 1".

Toward the mechanism of action of compound 1

Compound **1** may act either by activating iodide influx, by inhibiting iodide efflux, or through chemically driven reactions, such as enhanced iodide organification, intracellular iodide complexation, and ionophore action. Several experiments were undertaken to establish the mechanism of action of compound **1** at the cellular level. We first tested radioiodide uptake in HEK293 cells that do not express the Na/I symporter (HEK293 wild). Iodide accumulation was not increased in the presence of **1**, showing that NIS is necessary for the biological activity of **1** (Table 1). This result strongly suggests that compound **1** does not act by facilitating the translocation of iodide across the lipid bilayer.

Iodide uptake was determined in the absence or presence of **1** (100 μM) using the Sandell–Kolthoff reaction.[31] This method uses the very specific catalytic effect of iodide on the reduction of the yellow-colored cerium ion (Ce^{4+}) to colorless Ce^{3+} by arsenious acid (As^{3+}). The cell-trapped iodide concentrations were similar to the level of iodide calculated from isotopic flux experiments, showing that iodide is not oxidized or organified in the presence of **1**.

To verify the stability and distribution of compound **1**, the assay mixtures were examined with HPLC. hNIS-HEK293 cells were incubated for 120 min at 20 °C with **1** (100 μM) and NaI (10 μM) before cells and supernatants were separated and analyzed. The results show that compound **1** was concentrated up to 8±2% (trapped relative to total) in the cellular compartment. The chromatograms showed that **1** is stable in both cellular and supernatant compartments during the time required for biological activity (0–180 min).

Time-dependent iodide uptake in hNIS-HEK293 cells was established in the presence or absence of **1**. When the monolayer cell culture was incubated with $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) alone, iodide concentration gradually increased in the cell compartment until it reached a steady state after 90 min, indicating equal rates of efflux and influx (Figure 2a). When **1** (50 μM) and $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) were added together to the cell culture, the rates of iodide entrapment remained unchanged during the first 30 min. After 30 min, iodide fluxes had almost reached a steady state in the absence of **1**, whereas iodide uptake was prolonged in the presence of **1**. The steady-state was reached much later, and a higher iodide gradient was observed. Comparison of kinetic profiles at 0–30 min suggests that **1** does not directly activate iodide influx mechanisms. However, a delayed action cannot be excluded. To test this hypothesis, compound **1** was pre-incubated for 30 and 60 min before the addition of $Na^{125}I$ to hNIS-HEK293 cells. The time-dependent profile of iodide uptake was identical to that

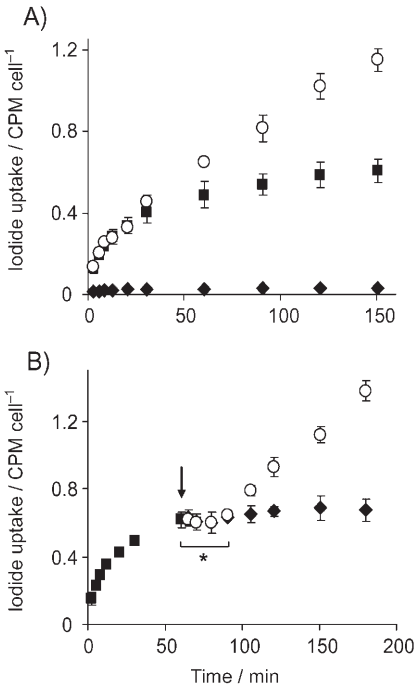


Figure 2. Time-dependent iodide uptake in hNIS-HEK293 cells in the presence of **1**·HBr. A) Iodide uptake was measured at 2, 5, 8, 12, 20, 30, 60, 90, 120, and 150 min in hNIS-HEK293 cells after incubation with $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) at 20 °C in the absence (■), or presence of **1**·HBr (○, 50 μM), $NaClO_4$ (◆, 50 μM). B) hNIS-HEK293 cells were incubated at 20 °C with $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) for 60 min before **1**·HBr (○, 50 μM) or buffer alone (◆) was added to the cells (the addition of **1**·HBr is indicated with an arrow). Radioiodide uptake was measured at 65, 70, 80, 90, 105, 120, 150, and 180 min after the addition of radioiodide. Results are shown for a representative experiment with mean values ±SD ($n=4$). The lag phase is indicated (*).

of the experiment performed with simultaneous addition of **1**·HBr and $Na^{125}I$ (not shown). This result argues against a delayed action on iodide uptake, thus reinforcing the theory that **1** reduces iodide efflux.

In another experiment, hNIS-HEK293 cells were incubated with $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) for 60 min before **1**·HBr (50 μM) was added. We observed a lag phase (20–30 min) before iodide entrapment resumed (Figure 2b), indicating that the activity of **1** is not immediate. The observed delay may correspond to the time necessary for **1** to cross the membrane bilayer. However, HPLC analysis of cell samples incubated with **1**·HBr (50 μM) for 2, 5, 10, 15, 20, and 60 min showed that the cellular compartment was saturated with **1** (11% trapped versus total) within the first two minutes. The imidazothiazole derivative had therefore probably reached its target before the biological activity was observed. It can therefore be proposed that the action of **1** on iodide efflux is not direct.

To confirm the action of **1**, an isotope dilution experiment was set up. hNIS-HEK293 cells were loaded with $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) for 120 min in the absence or presence of **1** (100 μM), before exposure to excess NaI (250 μM). Cell-associated radioactivity was monitored for 180 min. In the absence of **1**, radioiodide was >95% discharged in 45 min (Figure 3), illustrating the dynamic equilibrium between intra- and extracellu-

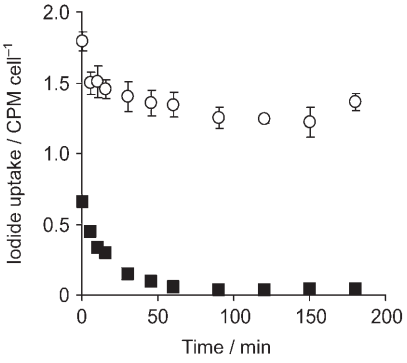


Figure 3. Time dependence of 'cold' iodide-mediated radioiodide discharge from hNIS-HEK293 cells in the presence of **1**·HBr. hNIS-HEK293 cells were incubated at 20 °C with $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) for 120 min in the presence (○) or absence (■) of **1**·HBr (100 μM). Cell-associated radioactivity was measured at 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 min after the addition of NaI (250 μM). The experiments are representative of three independent experiments with mean values ±SD ($n=4$).

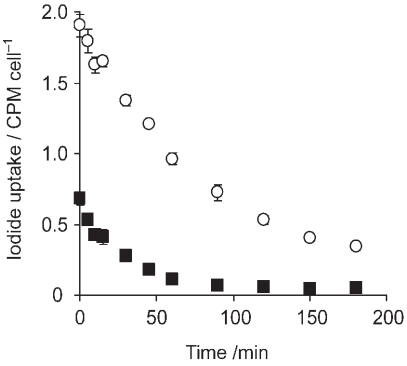


Figure 4. Time dependence of perchlorate-mediated iodide discharge from hNIS-HEK293 cells in the presence of **1**·HBr. hNIS-HEK293 cells were incubated at 20 °C with $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) for 120 min in the presence (○) or absence (■) of **1**·HBr (100 μM). Cell-associated radioactivity was measured at 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 min after the addition of $NaClO_4$ (100 μM). The experiments are representative of two independent experiments with mean values ±SD ($n=4$).

lar iodide. When the cells were loaded with $Na^{125}I$ in the presence of **1**, the addition of excess 'cold' NaI caused a remarkable inhibition of iodide exchange between intra- and extracellular compartments. Less than 30% of radioiodide was released within 20 min after NaI dilution before intracellular iodide concentration reached a constant level. Iodide permeation is thus greatly decreased in the presence of compound **1**, demonstrating that it acts by inhibiting the release of preloaded iodide. The effect was observed over 3 h, showing that **1** strongly increases the retention time of radioiodide within the cells. These experiments were repeated on FRTL-5 cells, and observations were less dramatic but similar (45 and 52% exchange at 20 and 180 min, respectively). In the light of these results, we can suggest two hypotheses on the mode of action of **1**. First, **1** may indirectly modulate the function of a transporter or a channel implicated in anion permeation. Second, iodide may no longer be available to efflux mechanisms and is relocalized to subcellular compartments.

We examined the action of **1** using the perchlorate discharge test. The perchlorate ion is a substrate of NIS.[8] It competitively inhibits the uptake of iodide, and promotes the release of intracellular free iodide with an IC_{50} value of $\sim 5\times 10^{-7}$ M.[28a] Monolayer cell cultures of hNIS-HEK293 were incubated with $Na^{125}I$ (10 μM, 0.2 μCi well^{-1}) for 120 min in the absence or presence of **1** (100 μM). Cell-trapped iodide concentrations were measured for 180 min at 20 °C after the addition of excess $NaClO_4$ (100 μM). Surprisingly, rapid iodide efflux was observed in the presence or absence of **1** immediately after the addition of perchlorate (Figure 4). Iodide discharge was almost total (>95%) after 60 min in the absence of **1**. In the presence of **1**, iodide discharge exceeded 85% after 180 min. Similar effects were observed in FRTL-5 cells (not shown). These results are in conflict with the isotopic dilution experiments described above. It is generally accepted that the inhibition of iodide uptake is due to competition between I^- and ClO_4^- for NIS-mediated transport. However, our results support a more complex action of perchlorate on iodide permeation.[32]

Limitations for the use of compound 1

The biological activity of **1** was examined as a function of iodide concentration in both hNIS-HEK293 and FRTL-5 cell lines. The results show that the effect of the imidazothiazole compound is diminished when lower concentrations of iodide are used (Table 2). In FRTL-5 cells, the gradient of iodide concentration was increased by factors of 4.0, 3.1, and 1.4 at iodide concentrations of 35, 10, and 3.5 μM, respectively. In hNIS-HEK293 cells, the iodide gradient was increased by factors of 2.7, 2.6, and 1.4 for the same range of iodide concentrations. For both cell lines, **1** was not active at sub-micromolar concentrations of iodide. Considering the concentration of iodide in blood (10^{-8}–10^{-7} M), our results indicate that the imidazothiazole derivative **1** is unlikely to be effective in vivo. However, the preparation of analogues is possible and may lead to the discovery of more active agents.

Finding the target of compound 1

The literature shows that the most closely related biologically active compound is levamisole [(−)-tetramisole, (*S*)-(−)-6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole]. Levamisole

Table 2. Percentage of cell-trapped iodide in the presence or absence of **1**·HBr as a function of [I^-].[a]

[I^-] (μM)	FRTL-5		hNIS-HEK293	
	− **1**·HBr	+ **1**·HBr	− **1**·HBr	+ **1**·HBr
0.01	13	14	61	58
0.1	14	14	59	59
1	14	13	64	70
3.5	11	15	47	63
10	7	22	26	67
35	2	8	7	19

[a] Cells were incubated at 20 °C for 120 min with $Na^{125}I$ (0.2 μCi per well) in the absence or presence of **1**·HBr (100 μM) before cell-associated radioactivity was determined. Experiments were run in quadruplicate; standard deviations were <±4%.

was originally used as an antihelminthic agent, and was found later to have immunostimulant activity.[33] This property has been the basis for use of levamisole as an adjuvant in chemotherapy for colon cancer.[34] At a molecular level, levamisole efficiently inhibits the function of several alkaline phosphatase isoenzymes.[35] Levamisole was also found to inhibit intestinal chloride secretion,[36] and increase the Cl^-/HCO_3^- exchanger activity in intrahepatic biliary epithelium by down-regulating alkaline phosphatase function.[37] In light of these findings, it can be proposed that compound **1** targets an alkaline phosphatase isoenzyme that may play a role in iodide permeation. We preliminarily tested the effect of levamisole on iodide uptake and found it inactive in the concentration range of 10^{-9}–5×10^{-4} M. Alkaline phosphatase from bovine kidney (Sigma–Aldrich, P4653) was then assayed in the presence of compound **1** and levamisole. Whereas the antihelminthic drug inhibited the dephosphorylation of 4-nitrophenylphosphate with an IC_{50} value similar to published data,[35] compound **1** had no effect on enzyme activity. Taken together, these results suggest that compound **1** does not target the bovine kidney alkaline phosphatase isoenzyme. However, we cannot exclude the presence of other phosphatases that may be linked to anion permeation mechanisms.

Conclusions

In summary, 3-biphenyl-4′-yl-5,6-dihydroimidazo[2,1-*b*]thiazole (ISA1) was identified as a very potent iodide-sequestering agent in two cell lines that express the sodium/iodide symporter. Isotopic flux experiments showed that the imidazothiazole derivative acts by inhibiting the release of intracellular iodide rather than by activating NIS function. Our experiments suggest the intervention of two possible mechanisms: 1) compound **1** may affect the function of an unidentified anion permeation mediator, or 2) it may trigger the relocation of cytoplasmic iodide into subcellular compartments. The identification of compound **1** opens new perspectives for cancer cell destruction by radioiodide. Preparation of radioactive biotinylated photoaffinity probes designed for target identification may provide a useful tool for the discovery of cellular pathways implicated in iodide flux. Compound **1** was prepared in one step and no chromatography was necessary. This simple procedure should facilitate the synthesis and evaluation of second-generation analogues for the identification of compounds with improved activities and druglike properties.

Experimental Section

General methods for the synthesis and chemical analysis of 1·HBr and 1: All reagents and solvents were from Sigma–Aldrich. Melting points were determined using a B-540 apparatus (Büchi) and are uncorrected. HPLC analysis was performed on a system equipped with a high-pressure gradient solvent delivery system (LC-20AB, Shimadzu), and a UV detector (SPD-10A, Shimadzu). Compound (4 μg) was applied to a 4.6×250 mm Zorbax 5 μm SB-C_{18} column (Agilent) equilibrated with CH_3CN/H_2O (35:65) and 0.1% TFA at a flow rate of 1 mL min^{-1}. Samples were eluted by increasing CH_3CN to 75% (0–12 min) and detected at 270 nm. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 K in [D_6]DMSO or $CDCl_3$ on a Bruker Avance DPX 400 spectrometer operating at 400 and 100 MHz, respectively. IR spectra were recorded on a PerkinElmer 2000 FTIR system. MS analyses were recorded on an electrospray ionization mass spectrometer (Micromass-ZQ, Waters).

Synthesis of 3-biphenyl-4′-yl-5,6-dihydroimidazo[2,1-*b*]thiazole hydrobromide (1·HBr): A mixture of 2-imidazolidinethione (511 mg, 5 mmol) and 2-bromo-4′-acetophenone (1.38 g, 5 mmol) in absolute EtOH (12.5 mL) was held at reflux with stirring for 5 h. The resulting suspension was allowed to cool to room temperature and was filtered. The solid was resuspended in EtOH (12.5 mL) and filtered again to yield **1**·HBr (1.17 g, 65% yield) as a yellow solid; mp: >240 °C (dec.); ^{1}H NMR ([D_6]DMSO): $\delta=9.63$ (br s, 1H, NH^+), 7.84 (d, 2H, $J=8.2$ Hz, H_{phenyl}), 7.71–7.75 (m, 4H, H_{phenyl}), 7.50 (t, 2H, $J=7.4$ Hz, H_{phenyl}), 7.42 (t, 1H, $J=7.4$ Hz, H_{phenyl}), 7.00 (s, 1H, $H_{thiazole}$), 4.52 (t, 2H, $J=9.2$ Hz, $H_{imidazole}$), 4.29 ppm (t, 2H, $J=9.2$ Hz, $H_{imidazole}$); ^{13}C NMR ([D_6]DMSO): $\delta=171.0$, 141.5, 138.9, 136.8, 129.1, 128.1, 127.7, 127.3, 127.0, 126.7, 106.6, 51.9, 47.8 ppm; IR (KBr): $\tilde{\nu}=$ 3300–3600 (br), 3072, 2997, 2897, 2360, 1585 cm^{-1}; MS (*m/z*) 279 ([*M*+1 (−HBr)]$^+$, 100); HPLC $t_R=6.5$ min (>99%).

Synthesis of 3-biphenyl-4′-yl-5,6-dihydroimidazo[2,1-*b*]thiazole (1): 1·HBr (600 mg) was resuspended in $NaHCO_3$ (5%, 50 mL) and extracted with CH_2Cl_2 (50 mL). The organic solution was dried using Na_2SO_4, filtered, and concentrated. The solid was purified by chromatography on silica (EtOAc/MeOH/NEt_3 85:15:0.5) to give compound **1** (427 mg, 92% yield) as a yellow powder. Alternatively, the crude material can be recrystallized from EtOH at +4 °C to afford yellow needles; mp: 152–153 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.63$ (d, 2H, $J=8.2$ Hz, H_{phenyl}), 7.60 (d, 2H, $J=7.8$ Hz, H_{phenyl}), 7.49 (d, 2H, $J=8.2$ Hz, H_{phenyl}), 7.46 (t, 2H, $J=7.8$ Hz, H_{phenyl}), 7.37 (t, 1H, $J=$ 7.8 Hz, H_{phenyl}), 5.76 (s, 1H, $H_{thiazole}$), 4.27 (t, 2H, $J=9.2$ Hz, $H_{imidazole}$), 3.90 ppm (t, 2H, $J=9.2$ Hz, $H_{imidazole}$); ^{13}C NMR ([D_6]DMSO): $\delta=$ 168.8, 140.8, 139.6, 136.9, 129.7, 129.4, 128.2, 127.5, 127.1, 127.0, 98.7, 60.3, 48.4 ppm; MS (*m/z*) 279 ([*M*+1]$^+$, 100); HPLC $t_R=$ 6.5 min (>98%).

Chemicals and stock solutions for biology: Carrier-free $Na^{125}I$ (GE Healthcare) was diluted with water to a concentration of 5 mCi mL^{-1}, and stored at room temperature for no more than two months. Solutions of 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), levamisole, all-*trans*-retinoic acid (Sigma–Aldrich), **1**, and **1**·HBr were prepared at 50 mM in DMSO, and stored at +4 °C for two weeks. $NaClO_4$ and NaI (Sigma–Aldrich) were diluted in water (1 M), and stored at room temperature. These stock solutions were further diluted in uptake buffer (HBSS/HEPES 10 mM) at 10× final concentration, as required, daily. The concentration of DMSO was maintained <0.4%, a concentration that was confirmed to have no effect on radioiodide uptake and cell viability.

Cell lines: HEK293 cells stably expressing the human Na^+/I^- symporter (hNIS) were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen), 2 mM L-glutamine (Sigma), 100 U mL^{-1} penicillin (Sigma), and 0.1 mg mL^{-1} streptomycin (Sigma) at 37 °C and 5% CO_2. FRTL-5 cells were cultured as described elsewhere with slight modifications.[38] Briefly, FRTL-5 cells were cultured in Coon's modified F12 medium (Biochrom) supplemented with 5% heat-inactivated FBS (Invitrogen), 2 mM L-glutamine (Sigma), 100 U mL^{-1} penicillin (Sigma), 0.1 mg mL^{-1} streptomycin (Sigma), 10 μg mL^{-1} insulin (Sigma), 10 nM hydrocortisone (Sigma), 10 ng mL^{-1} Gly-His-Lys acetate (Sigma), 1 mU mL^{-1} TSH (Sigma), and 5 μg mL^{-1} transferrin (Sigma) at 37 °C and 5% CO_2. For iodide uptake assays, hNIS-HEK293 cells (3.5×10^4 cells per well) and FRTL-5 cells (4×10^4 cells per well) were plated in 96-well mi-

crotiter plates (isoplate-96, PerkinElmer) using a Multidrop 384 instrument (Thermo Labsystems) and cultured at 37 °C and 5% CO_2 for three days to reach a confluent cell monolayer. For the HEK293 cell line, microtiter plates were preliminarily treated with poly-L-lysine (Sigma) to prevent cell wash-out.

Radioiodide uptake measurements: Confluent cell culture microplates were washed (Power washer PW384, Tecan) with uptake buffer (HBSS/HEPES 10 mM) such that 80 μL per well of fresh buffer remained at the end of the cycle, and allowed to stand at room temperature for 30 min. Solutions of compounds (10 μL per well), and NaI/Na^{125}I (0.2 μCi well^{-1} final, 10 μL per well) were distributed such that the specified incubation time was reached when the entire plate was washed (cold uptake buffer, PW384). The remaining supernatants were immediately discarded. EtOH (30 μL per well) and scintillation cocktail (160 μL per well, Analytic Unisafe 1, Zinsser) were successively added. The plates were shaken overnight at room temperature before the radioactivity was measured (Microbeta Trilux). In some cases, the supernatants and the cells were collected separately, and the radioactivity measured in 20-mL scintillation vials (Liquid Scintillation Counter 1409, Wallac) after the addition of scintillation cocktail (Analytic Unisafe 1, Zinsser). In each plate, $NaClO_4$-mediated inhibition of NIS was measured as a control. For dose–response studies, $NaClO_4$ was used in the range of 10^{-12}–10^{-3} M, and IC_{50} values were calculated using nonlinear regression analysis. For time-dependent iodide uptake studies, a single concentration of 50 μM was used.

Iodide determination by the modified Sandell–Kolthoff method:[31] Solutions of 'cold' NaI (10 μM, 10 μL per well), and **1**·HBr (10 μL per well) or buffer alone (as a control) were distributed onto a confluent cell monolayer of hNIS-HEK293. The cells were incubated for 120 min at room temperature. The plates were washed with cold uptake buffer (PW384), and the remaining supernatants were immediately discarded. The cells were treated with EtOH (200 μL) for 15 min at room temperature, and the resulting alcoholic mixtures were diluted in water (×5000). Iodide concentrations were assayed in 96-well clear polystyrene microplates by diluting the samples (1 μL) in 4 g L^{-1} NaCl, 8 mM sodium arsenite (prepared from As_2O_3 and NaOH) in total volumes of 229 μL per well. The reaction was started after the addition of 20 μL of a solution consisting of 40 mM ammonium cerium(IV) sulfate in sulfuric acid (3.6 N). The absorbance at 420 nm was recorded on a SpectraMax Plus 384 instrument (Molecular Devices) after 20 min and compared with that of NaI standards (0, 5, 10, 15, 20, 25, 30 μg L^{-1}).

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