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Discovery of Aryltrifluoroborates as Potent Sodium/Iodide Symporter (NIS) Inhibitors

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The sodium/iodide symporter (NIS) is an intrinsic glycoprotein that mediates the active accumulation of iodide into thyroid follicular cells, a fundamental step for the biosynthesis of iodinated hormones T4 and T3.[1] The driving force for I^- transport is the transmembrane Na^+ gradient maintained by the ouabain-sensitive Na^+/K^+ ATPase. A major step toward NIS characterization has been made by cloning the rat and human forms.[2] The human NIS is a 643-residue polypeptide with three N-glycosylation sites, and was proposed as a protein with 13 transmembrane segments.[3] The maturation, localization, and activity of NIS are regulated by thyrotropin (TSH), presumably by post-translational modifications.[4] NIS plays a key role in several thyroid diseases such as thyroid cancer, thyrotoxicosis, and congenital hypothyroidism.[5] Furthermore, the ability of NIS to take up iodide has provided the basis for cytoreductive gene strategy and cancer treatment with radioiodide.[6] Although many efforts have been made to characterize NIS at a molecular level, little is known concerning its structure, the origin of halogen selectivity, and the post-translational mechanisms responsible for NIS trafficking and function.

New inhibitors of NIS function were recently discovered by high-throughput screening.[7] These small organic molecules have opened new perspectives for the treatment of some thyroid dysfunctions such as Graves' disease and toxic nodules. Several anions such as ClO_4^-, SCN^-, BF_4^-, PF_6^-, ReO_4^-, and TcO_4^- (but not Cl^- and F^-) are also known to inhibit NIS-mediated iodide uptake.[8] Today it is well established that these anions alter NIS function because they share chemical attributes with iodide such as size and univalency.[8a] None of these anions are of pharmacological use because it is believed that chemical manipulations would abolish their activity. We aimed at taking advantage of the properties of these inorganic molecules to develop new mixed organic–inorganic inhibitors of NIS function. Among these anions, BF_4^- has retained our attention for several reasons. BF_4^- is transported by NIS, and the levels of accumulation are similar to that of I^-. Iodide uptake is efficiently and competitively inhibited by BF_4^- ($IC_{50} = 10^{-7}$–10^{-6} M). BF_4^- is neither readily metabolized nor very toxic.[1,8a,9] We speculated that the replacement of one fluoride atom of BF_4^- by an organic moiety would not be deleterious to its biological activity. It can also be expected that the presence of an organic element may provide new bonding possibilities and enhance the binding strength of such compounds. Organotrifluoroborates may fulfill these criteria. They were discovered in the 1960s and have been used as reagents in a wide variety of reactions, particularly palladium-catalyzed cross-coupling reactions.[10] To the best of our knowledge, none have been reported with biological activity.

We evaluated the inhibition potency of 20 potassium organotrifluoroborates (Figure 1) on radioiodide uptake (RAIU) in a rat-derived thyroid cell line (FRTL5). All compounds tested, except $MeBF_3K$ (**1**) were commercially available. $MeBF_3K$ was prepared from the reaction of trimethylboroxine and KHF_2 according to reported procedures.[11] The dose–response curves were established for compounds **1**–**20**. The inhibitory potencies were expressed as IC_{50} values and compared with those of reference compounds KBF_4 and $KClO_4$ (Table 1).[12] Most of the compounds (**1**–**17**) were found to have no effect on radioiodide uptake at concentrations up to 1 mM. This result shows that the substitution of a fluoride atom by simple phenyl, benzyl, cinnamyl, or methyl groups abolishes the activity of

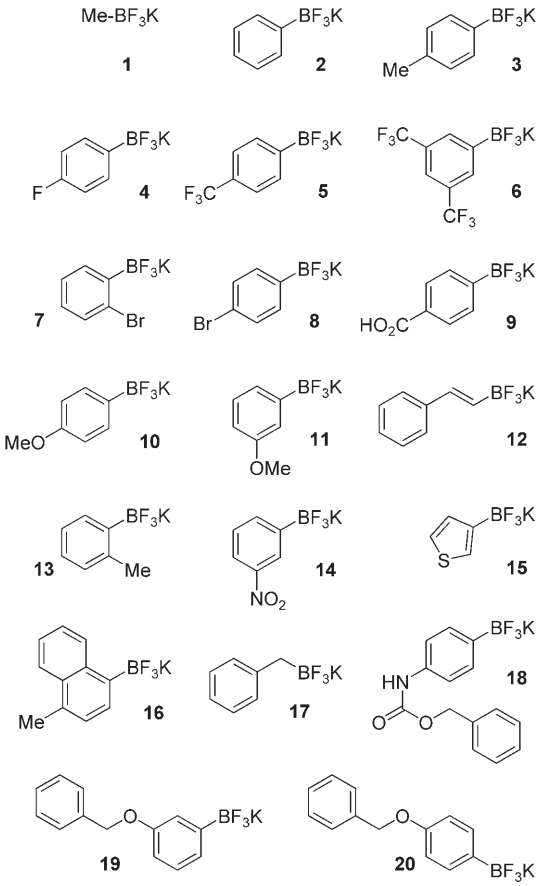

Figure 1. Compounds tested for the inhibition of iodide uptake in FRTL5 cells.

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Table 1. Inhibition potencies of compounds **1**–**20** on iodide uptake.[a]

Compound	IC_{50} [μM]
$KClO_4$	0.4
KBF_4	0.7
1–**17**	Not active
18	8
19	3
20	0.4

[a] For comparison, the IC_{50} values of reference compounds $KClO_4$ and KBF_4 are also shown; the results are representative of three independent experiments.

this series of compounds. However, compounds **18**, **19**, and **20** were found to be potent inhibitors of iodide uptake as illustrated by their respective IC_{50} values of 8×10^{-6}, 3×10^{-6}, and 4×10^{-7} M. Remarkably, compound **20** has an IC_{50} value similar to those of the reference compounds KBF_4 (7×10^{-7} M) and $KClO_4$ (4×10^{-7} M).

From an initial analysis, it can be concluded that at least two aromatic groups separated by two or four atoms are necessary for RAIU inhibition. The *para*-substituted compound **20** was found to be tenfold more potent than its *meta*-substituted isomer **19**. This observation shows that the substituent position on the phenyltrifluoroborate ion can influence biological activity. Inhibition is dose-dependent for compounds **18**, **19**, and **20**, and the Hill coefficients were close to 1, suggesting a stoichiometric binding mode (Figure 2).[13] Our results support the hypothesis that these compounds act through a drug–target interaction model. Whereas simple aryltrifluoroborates are inactive, appending an organic moiety onto the minimal recognition unit "BF_3^-" affords additional binding motifs that probably contribute to the stabilization of the drug–NIS complex. An important issue to address was to ensure that the observed biological activities were not due to the presence of free BF_4^- in the samples. ^{19}F NMR spectroscopic data confirmed that **18** and **20** were pure ($\delta=-136.8$ and -139.1 ppm, respectively) and no BF_4^- was detected ($\delta=-148.3$ ppm). In compound **19** ($\delta=-138.2$ ppm), traces of BF_4^- were detected (1.1% mol). However, such a low quantity can contribute only a small portion of the inhibition potency of sample **19**. These results show that the inhibition of iodide uptake observed in FRTL5 cells is mediated by the organotrifluoroborate compounds.

In conclusion, three organotrifluoroborates (**18**–**20**) were identified as iodide uptake inhibitors in NIS-expressing cells. Because they share structural similarities with the competitive inhibitor BF_4^-, it is reasonable to conclude that these molecules interact with NIS at the iodide binding site. Compound **20** has an IC_{50} value similar to that of BF_4^-, illustrating its efficacy. Organotrifluoroborates can be easily synthesized, and the BF_3^- function is compatible toward various chemical reactions, thus expanding the possibilities for preparing second-generation analogues. This discovery offers new opportunities for studying NIS structure and function. For example, solid-supported R–BF_3^- species designed for affinity chromatography may allow facile protein purification; fluorescent or photoaffinity labeling probes would allow investigating the amino acids involved in iodide binding and transport. This discovery is also of medical significance, as these molecules represent novel leads for the discovery of antithyroid drugs. However, further studies are necessary to evaluate the in vivo stability and toxicity of these compounds. Exploration of these novel agents is in progress.

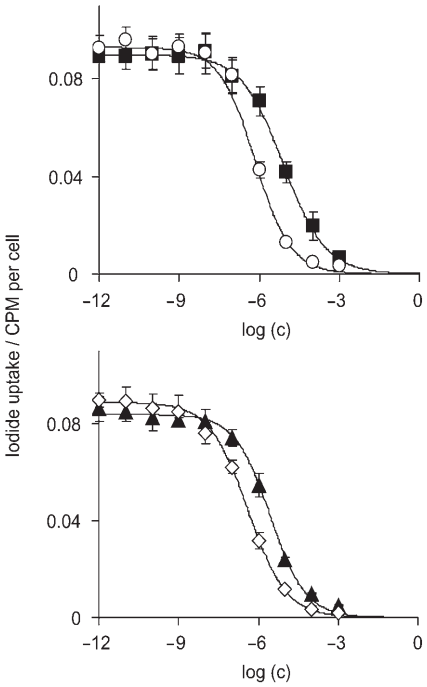

Figure 2. Dose–response curves of KBF_4 (○), **18** (■), **19** (▲) and **20** (◇). FRTL5 cells were incubated at 20 °C for 45 min with $Na^{125}I$ (10 μM, 0.2 μCi per well) and compounds (10^{-3}–10^{-12} M). Supernatants were discarded, and EtOH (30 μL) was added. Cell-trapped radioactivity was measured after the addition of scintillation cocktail. Experimental data were fitted by nonlinear regression to the Hill equation. Each compound was tested in triplicate.

Experimental Section

General. Compounds **2**–**15** and **17** were from Sigma–Aldrich, and **16** and **18**–**20** were from Combi Block, Inc. Carrier-free $Na^{125}I$ was from GE Healthcare. ^{19}F NMR spectra were recorded in [D_6]DMSO on a Bruker Avance DPX 400 spectrometer operating at 376 MHz. Chemical shifts for ^{19}F ($\delta=-131.0$, -136.8, -138.2, -139.1, and -148.3 ppm for **1**, **18**, **19**, **20**, and KBF_4, respectively) were referenced to external CF_3CO_2H ($\delta=-76.5$ ppm).

Cell lines. FRTL5 cells were cultured in Coon's modified F12 medium (Biochrom) supplemented with 5% heat-inactivated fetal bovine serum (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, FRTL5 cells were plated (4×10^4 cells per well) in 96-well microtiter 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 monolayer cell culture.

IC_{50} determination. Confluent culture cell microplates were washed (Power washer PW384, Tecan) with uptake buffer (HBSS/HEPES 10 mM) and allowed to stand at room temperature for 30 min. The supernatants were discarded, and the solutions of compounds (90 μL per well, 10^{-3}–10^{-12} M final) and NaI/$Na^{125}I$ (0.2 μCi per well final, 10 μL per well) in uptake buffer were immediately distributed in the microplates. The cells were allowed to stand at 20 °C for 45 min, washed with cold (4 °C) uptake buffer (PW384), and the remaining supernatants were immediately discarded. Ethanol (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 each plate $KClO_4$, KBF_4, or both were tested as reference compounds in the concentration range of 10^{-12}–10^{-3} M. IC_{50} values were calculated with nonlinear regression analysis by using an in-house application developed in Visual Basic for Excel (Microsoft).

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[12] $NaBF_4$ and $NaClO_4$ were also tested. As expected, we found that the nature of the cation had no influence on RAIU inhibition.

[13] Hill coefficients were found to be 0.98, 1.05, 0.83, 0.87, and 0.94 for KBF_4, $KClO_4$, **18**, **19**, and **20**, respectively.

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