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Synthesis and evaluation of photoreactive probes to elucidate iodide efflux in thyrocytes

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ABSTRACT

Four photoreactive analogues of 3-biphenyl-4′-yl-5,6-dihydroimidazo[2,1-*b*]thiazole were prepared and evaluated as iodide sequestering agents in sodium iodide symporter-expressing cells. One of these new photoactivatable compounds retained biological activity and was further radiolabeled with tritium. This compound may provide a useful tool for labeling, purification, and identification of target protein responsible for iodide efflux in thyrocytes.

The sodium iodide symporter (NIS) is an intrinsic membrane glycoprotein that catalyzes the active accumulation of iodide into thyroid follicular cells. NIS-mediated iodide uptake in the thyroid gland has long provided the basis for selective cell destruction in benign and malignant thyroid diseases using radioiodine (^{131}I).[1] In the light of this experience, it was proposed to extend this strategy to cancer tissues that do not take-up iodide by gene transfer of the sodium iodide symporter (NIS).[2] In recent years, the expression of functional NIS by gene transfer was successfully achieved in several type of cancer using rat or mice models.[3] Unfortunately, radioiodine retention within these non-thyroid cells was low due to a lack of iodide organification. For this reason, this strategy cannot be applied to humans. Several strategies were proposed to efficiently increase iodide retention in NIS-expressing cells,[4] one of which is to use small organic molecules. Recently, 3-biphenyl-4′-yl-5,6-dihydroimidazo[2,1-*b*]thiazole (**1**, Fig. 1) was reported to enhance iodide retention in NIS-expressing cells.[5] Using this compound, the intracellular iodide concentration is increased by a factor 4–5 in rat thyroid-derived cells (FRTL5). Isotopic flux experiments showed that compound **1** do not act by activating NIS function but rather by inhibiting the release of intracellular iodide. It was suggested that compound **1** may affect the function of an unidentified anion permeation mediator. We envisioned preparing photoreactive analogues of **1** to identify the protein(s) responsible for iodide efflux using photoaffinity labeling techniques.

Photoaffinity labeling is a powerful technique for the identification of target proteins and the characterization of ligand-binding sites.[6] Not only the probe must bear a photoactivatable group plus a chemical tag for the detection of labeled protein(s), but it has to be biologically active regarding the phenotype of interest. In this study we report the synthesis of four photoreactive analogues of compound **1** with a diazonium (**2**), an azido (**3**), a benzophenone (**4**), or a trifluoromethyldiazirine group (**5**) (Fig. 1). The compounds were evaluated for their ability to increase iodide retention in FRTL5 cells and the benzophenone **4** showed to retain biological activity. The preparation of tritium-labeled **4** is also described.

For the synthesis of compounds **2** and **3** (Scheme 1), commercially available 4′-iodoacetophenone, 2-imidazolidinethione and iodine were refluxed in EtOH to afford compound **6** in 78% yield. Subsequent Suzuki–Miyaura cross-coupling reaction with the

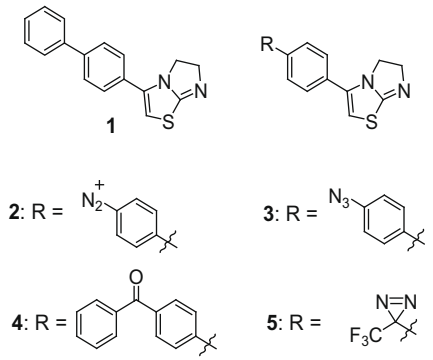

Figure 1. Structure of compound **1** and its photoreactive analogues **2**–**5**.

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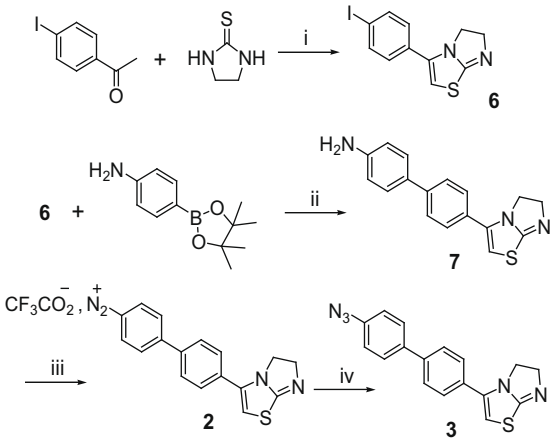
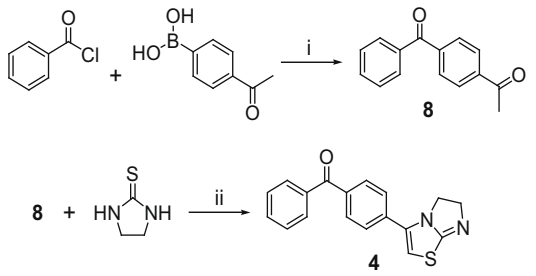


Scheme 1. Reagents and conditions: (i) I_2 (2 equiv), EtOH, reflux, 16 h, 78% yield; (ii) $Pd(PPh_3)_4$ (0.1 equiv), K_3PO_4 (2 equiv), DMF, 80 °C, 16 h, 82% yield; (iii) $NaNO_2$ (1.2 equiv), TFA, +4 °C, 30 min, 90% yield; (iv) NaN_3 (10 equiv), TFA, +4 °C to rt, 2 h, 93% yield.

pinacol ester of 4-aminophenylboronic acid afforded the aniline derivative **7** in 82% yield. The diazonium salt **2** was obtained from this amine after reaction with sodium nitrite in trifluoroacetic acid.[7] Compound **2** was isolated after simple solvent evaporation (58% overall yield). Compound **2** was converted into the azido derivative **3** by the addition of sodium azide to the acidic diazonium salt solution. Compound **3** was isolated after DCM/$NaHCO_3$ extraction work-up (53% overall yield).[8] ^1H NMR and LC-MS showed that **2** and **3** purities were >90%. For biological assays and analytical purposes, **2** and **3** were further purified using preparative HPLC (X-bridge C18—5 μm—19 × 150 mm).

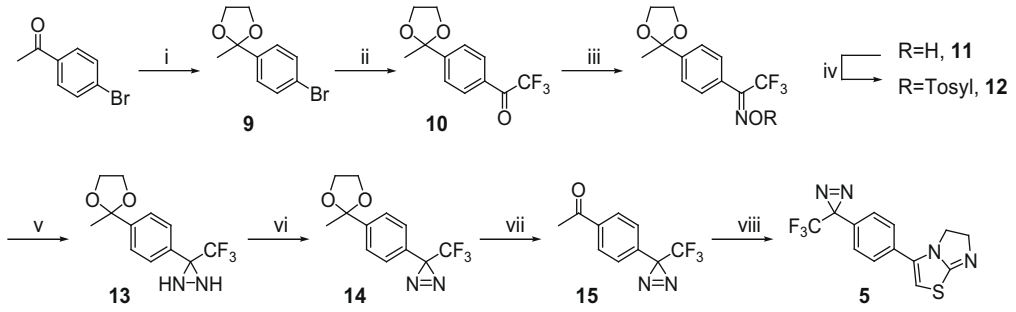


Scheme 2. Reagents and conditions: (i) $Pd(PPh_3)_4Cl_2$ (0.04 equiv), K_3PO_4 (2 equiv), toluene, 100 °C (2.5 h) then 75 °C (14 h), 83% yield; (ii) I_2 (2 equiv), EtOH, reflux, 16 h, 91% yield.

For the preparation photoprobe **4** (Scheme 2), 4-acetylbenzophenone (**8**) was first synthesized using a Suzuki–Miyaura type coupling reaction from benzoyl chloride and 4-acetylphenylboronic acid using a general procedure described elsewhere.[9] Compound **8** was then reacted with 2-imidazolidinethione and iodine in boiling EtOH to afford the desired product **4** in 75% overall yield after DCM/$Na_2S_2O_3$ and DCM/$NaHCO_3$ extraction work-up followed by flash chromatography (DCM then DCM/MeOH 95:5).[10]

Photoprobe **5** was prepared by means of standard procedures with slight modifications (Scheme 3).[11] Starting from the commercially available 4′-bromoacetophenone, carbonyl protection followed by Br/Li exchange and subsequent acylation by ethyl trifluoroacetate gave the expected intermediate **10**. The ketone was converted into its oxime **11** with hydroxylamine hydrochloride in pyridine and ethanol. This oxime was next reacted with tosyl chloride and triethylamine with catalytic DMAP in dichloromethane to afford the corresponding *O*-tosyl oxime **12**. Conversion of **12** to diaziridine **13** was brought about with ammonia under pressure in a sealed tube. Oxidation of the diaziridine to diazirine **14** was achieved with iodine and triethylamine in methanol. Finally ketone deprotection with 2 N HCl in THF/H_2O afforded **15** that was reacted with 2-imidazolidinethione and iodine in boiling EtOH to afford the desired photoprobe **5** after filtration and subsequent DCM/$Na_2S_2O_3$ and DCM/$NaHCO_3$ extraction work-up. Compound **5** was sufficiently pure (>98%) for biological assays.[12]

The biological properties of photoreactive dihydroimidazo [2,1-*b*]thiazole derivatives **2**–**5** and reference compound **1** were investigated for their ability to enhance iodide entrapment in rat thyroid-derived cells (FRTL5). Briefly, the monolayer cell culture was incubated in the dark in the presence of compounds **1**–**5** (0.1–200 μM) and $Na^{125}I$ (10 μM) for 120 min before cell-trapped iodide was measured by scintillation counting (a detailed experimental procedure is reported elsewhere).[5] The results showed that photoreactive probes **2**, **3**, and **5** were inactive, whereas the benzophenone-containing analogue **4** retained biological activity (Fig. 2). The increase of iodide entrapment was concentration-dependent with an EC_{50} value of 50 μM.[13] At this concentration iodide concentration was increased by a factor 1.8 and reached a factor 2.5 at 200 μM of **4**. For comparison EC_{50} value for compound **1** was calculated to be 20 μM, with an increase of intracellular [I^-] by a factor 2.5, whereas the maximum iodide entrapment was reached at 50 μM (×3.9). It has to be noted that even though compound **4** is less active than reference compound **1**, it can be used in photoaffinity labeling experiments. The bell-like shape of the curve corresponding to compound **1** was reported to be due to a cytotoxic effect when high compound concentrations are used (>100 μM).[5] The photodecomposition of **4** was tested in MeCN using a 100 W UV lamp (Black-Ray® B100AP, $\lambda = 365$ nm). The light-induced



Scheme 3. Reagents and conditions: (i) ethylene glycol (2.5 equiv), PTSA (0.05 equiv), benzene, reflux, 16 h, 39% yield; (ii) *n*-BuLi (1.5 equiv), THF, −78 °C, 3 h then CF_3CO_2Et (2 equiv), THF, −78 °C to rt, 14 h, 80% yield; (iii) $NH_2OH{\cdot}HCl$ (1.1 equiv), pyridine/EtOH 2:1, 60 °C, 14 h, quant; (iv) tosyl chloride (1.1 equiv), DMAP (0.05 equiv), TEA (2.5 equiv), DCM, +4 °C to rt, 1 h, 85% yield; (v) NH_3 liq., −78 °C to rt, 20 h, 91% yield; (vi) I_2 (1.3 equiv), TEA (3 equiv), MeOH, rt, 1 h, 53% yield; (vii) 2 N HCl, THF/H_2O 1:1, rt, 14 h, 95% yield; (viii) 2-imidazolidinethione (1.5 equiv), I_2 (1.5 equiv), EtOH, reflux, 8 h, 48% yield.

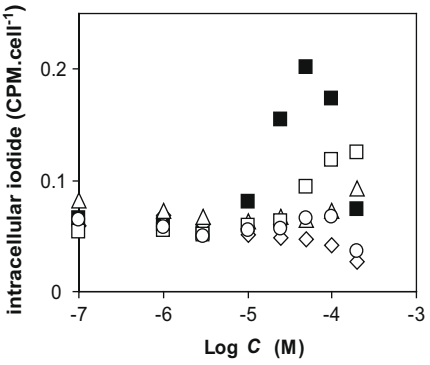
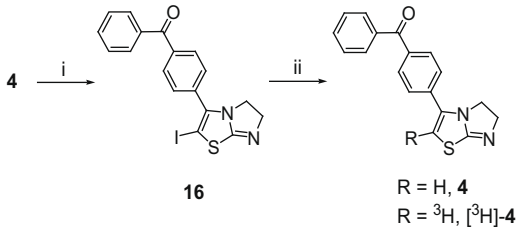


Figure 2. Dose-response curves of **1** (■), **2** (◇), **3** (Δ), **4** (□) and **5** (○) in FRTL5 cells. FRTL5 cells in 96-Isoplates (Perkin-Elmer) were incubated at 20 °C for 120 min with **1**–**5** (1×10^{-7} to 2×10^{-4} M) and $Na^{125}I$ (10 μM, 0.2 μCi per well). Cells were washed at +4 °C before ethanol (30 μL) was added. Cell-associated radioactivity was determined after addition of the scintillation cocktail. Results are shown for one experiment representative of at least three independent experiments with mean values ± standard deviation ($n = 3$). $NaClO_4$-mediated inhibition of iodide uptake was tested as a control in each plate ($IC_{50} = 1 \times 10^{-7}$ M, ±100%).



Scheme 4. Reagents and conditions: (i) *N*-iodosuccinimide (1.5 equiv), MeCN, rt, 2 h, 32% yield; (ii) 10% Pd/C, TEA, H_2 or $^3H_2$ (1.8 atm), MeOH, rt, 12 h.

breakdown of **4** was irreversible and time-dependent, with $t_{1/2}$ of 12 min at a distance of 5 cm from the mixture.

We envisioned preparing tritium-labeled photoprobe **4** by introducing the radiolabeled portion by selective tritium reduction of the corresponding iodoarene. Thus compound **4** was iodinated by treatment with N-iodosuccinimide in acetonitrile to provide the monoiodinated compound **16** in 32% yield (Scheme 4) after DCM/$Na_2S_2O_3$ and DCM/$NaHCO_3$ extraction work-up followed by flash chromatography (DCM/TEA 99:1 then DCM/MeOH/TEA 95:5:1). Catalytic dehalogenation of iodoarene **16** with H_2, 10% Pd/C and TEA in MeOH gave **4** in >98% yield (HPLC). The identical reaction with tritium gas provided the desired radiolabeled photoaffinity probe [^{3}H]-**4** after purification by semi-preparative HPLC using a Zorbax-C18 column.[14] Comparison of HPLC UV traces for [^{3}H]-**4** and **4** showed them to be identical. The radiochemical purity of [^{3}H]-**4** was >98% (analytical radio-HPLC and radio-TLC). The specific activity of [^{3}H]-**4** was determined by ESI-MS to be 350 mCi mmol^{-1} representing an isotopic enrichment of 1.2%. This is a surprisingly low value for this type of reaction. However, this may not be a major drawback in the context of photolabeling experiments since the effective concentration of **4** is in the range of 20–200 μM. All attempts to increase the specific activity of [^{3}H]-**4** failed.[15] Finally, [^{3}H]-**4** was stable at least 6 months when stored at +4 °C in MeCN/H_2O 1:1 at a concentration of 4 mCi mL^{-1}.

In conclusion, four new photoreactive probes were designed in order to target proteins responsible for iodide efflux in NIS-expressing cells. The benzophenone-based photoaffinity probe was found to be biologically active. This compound was further labeled with tritium, thus providing a tool for detection of the photolabeled targets.

Acknowledgment

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7. Characterization data for compound **2**: ^{1}H NMR (400 MHz, TFA-*d* at 11.50 ppm) δ 3.91 (s, 1H, NH), 4.41–4.49 (m, 4H), 6.89 (s, 1H), 7.61 (d, $J = 8.4$ Hz, 2H), 7.78 (d, $J = 8.4$ Hz, 2H), 8.07 (d, $J = 8.8$ Hz, 2H), 8.49 (d, $J = 8.8$ Hz, 2H); MS (ESI$^+$) *m*/*z* (%) 278 (100) $[M-N_2]^+$; IR (KBr) ν in cm^{-1} 3107, 2267, 1680, 1581, 1205, 1140, 802, 724; UV (EtOH) λ_{max} 272 nm; $R_f = 0.50$ (C_{18}—MeOH/H_2O 50:50).
8. Characterization data for compound **3**: ^{1}H NMR (400 MHz, $CDCl_3$ at 7.26 ppm) δ 3.92 (t, $J = 8.8$ Hz, 2H), 4.28 (t, $J = 8.8$ Hz, 2H), 5.78 (s, 1H), 7.12 (d, $J = 8.4$ Hz, 2H), 7.50 (d, $J = 8.4$ Hz, 2H), 7.59 (d, $J = 8.4$ Hz, 2H), 7.61 (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ at 77.0 ppm) δ 48.4, 60.1, 98.6, 119.5, 126.8, 127.2, 128.6, 129.6, 136.7, 136.9, 139.7, 140.6, 170.4; MS (ESI$^+$) *m*/*z* (%) 320 (100) [M+H]$^+$; IR (KBr) ν in cm^{-1} 3120, 2121, 1588, 1491, 1371, 1274, 837, 815; UV (EtOH) λ_{max} 254 nm; $R_f = 0.15$ (AcOEt/cHex/MeOH 7:2:0.5).
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10. Characterization data for compound **4**: ^{1}H NMR (400 MHz, DMSO-d_6 at 2.50 ppm) δ 3.93 (t, $J = 8.0$ Hz, 2H), 4.10 (t, $J = 8.0$ Hz, 2H), 6.39 (s, 1H), 7.57 (dd, $J = 8.4$ Hz, $J' = 8.0$ Hz, 2H), 7.69 (dd, $J = 8.0$ Hz, $J' = 1.8$ Hz, 1H), 7.74 (dd, $J = 8.4$ Hz, $J' = 1.8$ Hz, 2H), 7.75 (dd, $J = 8.4$ Hz, $J' = 1.8$ Hz, 2H), 7.79 (dd, $J = 8.4$ Hz, $J' = 1.8$ Hz, 2H); ^{13}C NMR (100 MHz DMSO-d_6 at 39.5 ppm) δ 48.2, 60.3, 100.7, 126.0, 128.6, 129.5, 130.3, 132.8, 134.0, 136.0, 136.7, 136.9, 168.2, 195.0; MS (ESI$^+$) *m*/*z* (%) 307 (100) [M+H]$^+$; IR (KBr) ν in cm^{-1} 3395, 2868, 1649, 1591, 1276, 931, 859, 700; UV (EtOH) λ_{max} 366 nm (4900 M^{-1} cm^{-1}); $R_f = 0.54$ (DCM/MeOH 9:1). ESI-HRMS calcd for $C_{18}H_{15}N_2OS$ ([M+H]$^+$) *m*/*z* 307.0900 found 307.0905.
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12. Characterization data for compound **5**: ^{1}H NMR (400 MHz, DMSO-d_6 at 2.50 ppm) δ 3.87 (t, $J = 8.8$ Hz, 2H), 4.07 (t, $J = 8.8$ Hz, 2H), 6.30 (s, 1H), 7.33 (d, $J = 8.0$ Hz, 2H), 7.69 (d, $J = 8.0$ Hz, 2H); ^{13}C NMR (100 MHz DMSO-d_6 at 39.5 ppm) δ 48.0, 60.3, 100.0, 107.8 (q, $J_{C\text{-}f} = 275$ Hz), 126.9, 127.0, 127.7, 132.1, 135.6, 168.1; MS (ESI$^+$) *m*/*z* (%) 311 (100) [M+H]$^+$; IR (KBr) ν in cm^{-1} 3433, 2939, 2085, 1609, 1514, 1178, 1138, 938, 828, 690; UV (EtOH) λ_{max} 349 nm (380 M^{-1} cm^{-1}); $R_f = 0.20$ (DCM/MeOH 9:1).
13. The term half-maximal effective concentration (EC_{50}) refers to the concentration of compound which induces a response halfway between the lower signal (i.e., signal at low compound concentrations) and maximum signal (50 μM for **1** and 200 μM for **4**). Experimental data were fitted by nonlinear regression to the four-parameter sigmoidal Hill equation.
14. For a general procedure of tritiation reactions see: Ambroise, Y.; Pillon, F.; Mioskowski, C.; Valleix, A.; Rousseau, B. *Eur. J. Org. Chem.* **2001**, *20*, 3961.
15. When other non protic solvents were tested (acetone, THF, AcOEt) no reaction occurred. The same reaction run with deuterium gas in MeOH afforded [^{2}H]-**4** with a moderate isotopic enrichment of 16%. For any unknown reasons, this reaction is subjected to strong isotopic effects.