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<th>Journal:</th>
<th>Canadian Journal of Chemistry</th>
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<tr>
<td>Manuscript ID</td>
<td>cjc-2016-0239.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
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<tr>
<td>Date Submitted by the Author:</td>
<td>28-Jun-2016</td>
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<tr>
<td>Complete List of Authors:</td>
<td>Rathmann, Stephanie; McMaster University, Chemistry and Chemical Biology Janzen, Nancy; McMaster University, Dept of Chemistry and Chemical Biology Valliant, John; McMaster University</td>
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<tr>
<td>Keyword:</td>
<td>radiiodine, melanoma, triazole, radionuclide therapy, SPECT</td>
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Synthesis, radiolabelling and biodistribution studies of triazole derivatives for targeting melanoma

Stephanie M. Rathmann, Nancy Janzen, and John F. Valliant*

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Title: Synthesis, radiolabelling and biodistribution studies of triazole derivatives for targeting melanoma

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Abstract: Molecular probes that target specific markers expressed in solid tumours are in demand for cancer imaging and radionuclide therapy applications. The synthesis, characterization, and in vivo evaluation of radioiodinated triazoles designed as probes to target melanoma is described here. Compounds were prepared using a thermal click reaction between ethynylstannane and methyl 2-azidoacetate resulting in preferential formation of the corresponding 1,4-tin triazole. The primary amine of various targeting vectors was then coupled to the resulting tin triazole methyl ester. These precursors were labelled with no carrier added $^{123}$I or $^{125}$I and purified by high performance liquid chromatography to give isolated radiochemical yields between 6% and 51%, and radiochemical purities of $>95\%$ in all cases. Among the evaluated compounds, $N$-(2-diethylamino-ethyl)-2-(4-iodo-[1,2,3]triazol-1-yl)acetamide (7a) and $N$-(1-benzylpiperidin-4-yl)-2-(4-iodo-1H-1,2,3-triazol-1-yl)acetamide (7d) showed the most promising in vivo data and their $^{123}$I-labelled forms were used in single photon emission computed tomography-computed tomography (SPECT-CT) imaging studies. The imaging data showed excellent tumour visualization with a very high signal to noise ratio.

Key words: radioiodine, melanoma, imaging, SPECT, triazole, radionuclide therapy
Introduction

Melanoma accounts for 4% of all cases of skin cancer and it is responsible for 80% of all skin cancer deaths.\(^1\) Until recently, there has been a limited number of effective treatment options for patients with malignant melanoma. Inhibitors of the B-Raf kinase have shown some improved results over conventional therapies, but are effective only with types of melanoma that have the specific BRAF gene mutation.\(^2,3\) In addition, tumour resistance to these drugs and the resulting recurrence of disease are frequent.\(^4,5\) More recently, exciting clinical trial results have been obtained with novel immunotherapies against melanoma, notably CTLA-4 and PD-1 targeting antibodies.\(^6-8\) While these antibody therapies appear to be effective, they often work for specific subpopulations of patients, and long term overall survival may require repeat treatments or combination therapies with other anti-cancer agents. There remains a need for new therapeutics to treat late stage and recurrent metastatic melanoma.

One option that has been explored for treating metastatic melanoma is radionuclide therapy (RT) using melanin as the biochemical target. Melanin is present in tumour lesions in over 90% of melanoma cases,\(^9\) and can be targeted using small molecule benzamides that contain a pendent tertiary amine.\(^10,11\) Potential melanin-targeted RT agents have been reported, the majority of which are developed around an iodinated benzamide core, most notably N-(2-diethylaminoethyl) 4-iodobenzamide (BZA) (Fig. 1).\(^12,13\) Iodine radionuclides are frequently chosen, because they allow the production of isostructural agents for both diagnosis and therapy, simply by changing the isotope used to prepare the agent.\(^14\) Radioiodinated benzamides show high tumour uptake, however the probes also demonstrate high non-specific binding \textit{in vivo} due to the lipophilicity of the iodophenyl group.\(^15,16\) Changing the prosthetic group from iodophenyl to polar heterocycles
has the potential to improve the target to non-target uptake of a probe by increasing hydrophilicity and decreasing retention in non-target tissue.\textsuperscript{17,18} By reducing non-specific binding it is possible to develop a targeted RT agent with low radiotoxicity and that provides maximum dose to the tissue of interest.

The Triazole Appending AGent (TAAG) is a new iodine containing synthon that was designed as a hydrophilic prosthetic group that can be used in place of the commonly employed iodophenyl group.\textsuperscript{19} TAAG, when iodinated, clears rapidly via the kidneys and shows low non-specific binding \textit{in vivo}. The first TAAG conjugate synthesized was a derivative designed to bind prostate specific membrane antigen (PSMA);\textsuperscript{19} a protein over-expressed in prostate cancer cells. This compound, when iodinated, showed high uptake in prostate cancer tumours and low non-specific binding to non-target tissues, making the TAAG group an attractive building block for preparing a melanin-targeted imaging probe and radiotherapeutic.\textsuperscript{19}

A method was developed to prepare analogues of BZA, which contained tin-triazoles as precursors for oxidative radiohalogenation reactions. Following radiolabelling, the products were compared to non-radioactive reference standards, and the impacts of different substituents on their localization in melanin-expressing tumours and non-target tissues were assessed \textit{in vivo} using a syngeneic tumour model. An iterative approach was applied using modifications to the core structure based on biodistribution results for each successive derivative in order to determine the functional groups required for optimal target binding and clearance. Single photon emission computed tomography-computed tomography (SPECT-CT) imaging studies were then performed on the lead compounds.
Results and Discussion

Synthesis of TAAG derivatives and iodinated non-radioactive reference standards

A series of TAAG derivatives was prepared as analogues of the previously reported iodinated BZA (Fig. 1). As the first step towards creating the radiolabelling precursor compounds, the fluorous tin methyl esters 1 and 2 were synthesized by combining the appropriate perfluorinated tin alkyne and methyl 2-azidoacetate via a thermal click reaction. Both the 1,4- and 1,5-isomers were present, where the former was isolated by column chromatography. The two resulting esters were then coupled to \( N,N \)-diethylethylene diamine to produce 6a-6b (Scheme 1). For example, ester 1 was coupled with \( N,N \)-diethylethylene diamine in methanol at 60 °C to give 6a in 88% yield. The identity of 6a was confirmed through mass spectrometry, HPLC and NMR, where the \(^1\)H NMR spectrum showed characteristic singlet peaks at 8.08 and 5.24 ppm representing the triazole and adjacent methylene protons respectively (see Supplementary Data). A multiplet at 1.13 ppm indicated the terminal CH\(_3\) group on the targeting vector further confirming that the coupling reaction was successful. The fluorous tin derivatives were prepared initially to enable purification of the radiolabelled compound by fluorous solid phase extraction. In select cases precursors were developed containing tributyl tin derivatives (Scheme 2) in place of the fluorous groups to address solubility issues. Here the tributyl tin methyl ester (3) was synthesized through the thermal click reaction of tributyl tin acetylene with methyl 2-azidoacetate following a literature procedure. Compound 3 was subsequently coupled to different amines, prepared via simple alkylation of Boc-protected 4-amino-piperidine, in methanol at 60 °C to produce 6d-6i in 7-64% yield (Scheme 2).
Radiochemistry

Each of the tin-triazoles (6a-6i) were initially labelled with non-radioactive iodine to generate the HPLC reference standards required to validate the identity of the radioiodinated compounds. A crystal structure of 7d (N-(1-benzylpiperidin-4-yl)-2-(4-iodo-1H-1,2,3-triazol-1-yl)acetamide was obtained (Fig. 2), which showed that the major product was the 1,4-isomer, in agreement with the NMR data (see Supplementary Data).

The tributyl tin derivatives were radiolabelled using an iododestannylation reaction with iodogen as the oxidant. The fluorous compounds 6a-6c were labelled in methanol, while reactions with compounds 6d-6i were performed using a biphasic chloroform-water mixture. Reactions were complete within 10 min, and the products isolated by HPLC in radiochemical yields (RCY) between 6% and 51% (Table 1), with radiochemical purity >95% in all cases.

Biodistribution Studies

Biodistribution studies were performed for $^{[123/125]}$I-7a-7i in comparison with $^{[125]}$I-BZA in C57Bl/6 mice bearing melanin-expressing B16F1 tumours. Tissues, organs and fluids were collected and weighed 24 h after compound injection, and then radioactivity measured for each. The percentage injected dosage per gram (%ID/g) of selected tissues or fluids are shown in Figures 3 and 4, and key tumour to tissue ratios were calculated (Table 2). It is important to note that melanin binding can be assessed by evaluating the extent of localization to the tumour and the eyes; both of which express the target.

The first compound tested was $^{[123]}$I-7a, the TAAG analogue of BZA. $^{[123]}$I-7a showed low uptake in non-target tissues as well as low (< 0.1 %ID/g) tumour uptake at 24 h. However, uptake in the eyes was 1.50 %ID/g, suggesting that $^{[123]}$I-7a did indeed target melanin (Fig. 3). It...
is also important to note that although the tumour to blood ratio of $[^{123}\text{I}]$-7a was less than that of
$[^{125}\text{I}]-\text{BZA}$ (19:1 versus 183:1), $[^{123}\text{I}]-7a$ had a superior tumour to liver ratio compared to $[^{125}\text{I}]$-
BZA (97:1 versus 21:1). This result supports the hypothesis that non-specific binding can be
decreased through the use of an iodinated heterocycle in place of the iodo-aryl prosthetic group.

In an attempt to improve tumour uptake, substituents on the tertiary amine and triazole were
varied because altering the size of the amine and arene are known to impact melanin binding in
vivo.\textsuperscript{22,23} Compound 7b was synthesized by adding an aromatic group in the 5 position on the
triazole to enhance π stacking interactions with melanin.\textsuperscript{22} The biodistribution study of $[^{125}\text{I}]-7b$
showed a 10 fold increase in tumour uptake compared to $[^{123}\text{I}]-7a$ (Fig. 3). However, the ideal
clearance that was seen with $[^{123}\text{I}]-7a$ was somewhat compromised in $[^{125}\text{I}]-7b$, which showed
higher but not unreasonable (2.42 vs 0.004 %ID/g) accumulation in the liver.

In light of the results with $[^{125}\text{I}]-7b$, compound $[^{125}\text{I}]-7c$ was synthesized as a derivative with
intermediate polarity relative to $[^{123}\text{I}]-7a$ and $[^{125}\text{I}]-7b$. The biodistribution of $[^{125}\text{I}]-7c$
showed tumour and eye uptake of 0.77 and 3.17 %ID/g respectively at 24 h (Fig. 3). Tumour uptake was
eight-fold higher for $[^{125}\text{I}]-7c$ compared to $[^{123}\text{I}]-7a$. At the same time $[^{125}\text{I}]-7c$ displayed ideal
clearance with tumour-to-liver and tumour-to-blood ratios of 6:1 and 15:1 respectively, although
accumulation in the thyroid was somewhat elevated. Thyroid localization in biodistribution
studies is indicative of metabolic release of iodide. Compound 7d was then synthesized to
combine the structural features that produced the positive results from 7b and 7c, having both the
piperidine and phenyl rings within the molecule. The biodistribution study of $[^{125}\text{I}]-7d$ revealed
tumour and eye uptake of 2.21 and 6.59 %ID/g respectively at 24 h, which were the highest
values among all tested compounds (Fig. 3 and Supplementary Data). $[^{125}\text{I}]-7d$ had ten-fold
lower accumulation in the liver compared to $[^{125}\text{I}]-\text{BZA}$.
The results from these initial biodistribution studies showed that the clearance of this new class of compounds is not solely dependent on lipophilicity. For instance, the log $P$ values for $[{}^{125}\text{I}]\text{-7b}$ and $[{}^{125}\text{I}]\text{-7d}$ are 0.63 and 0.70 respectively (Table 1). However, despite having similar log $P$ values, the in vivo clearance of these probes was quite different. The tumour-to-liver ratios were 0.37:1 and 27:1 for $[{}^{125}\text{I}]\text{-7b}$ and $[{}^{125}\text{I}]\text{-7d}$ respectively; a large and significant difference for probes of similar polarity. This observation suggested that a mechanism other than the lipophilicity is guiding clearance, and that retaining the 1,4 triazole core was needed to promote clearance from non-target tissues.

Although $[{}^{125}\text{I}]\text{-7d}$ showed promising biodistribution results, higher uptake in the tumour might be necessary for use as a radiotherapeutic agent. Therefore further derivatization was explored to enhance tumour uptake. We first attempted to alter the electron density of the phenyl group through the use of electron donating and electron withdrawing substituents. The presence of the electron donating groups was expected to increase electron density in the ring and therefore provide a stronger interaction with melanin, which is a known π electron acceptor. The first derivative, $[{}^{125}\text{I}]\text{-7e}$, contained two methoxy groups and had a tumour and eye uptake of 1.32 and 7.36 %ID/g respectively, at 24 h (Fig. 4). The second derivative, $[{}^{125}\text{I}]\text{-7f}$, contained two electron withdrawing nitro groups and had a tumour and eye uptake of 1.15 and 8.06 %ID/g respectively at 24 h. These results show that the addition of electron donating and electron withdrawing substituents are overall deleterious to tumour and eye uptake, which was unexpected as the increased electron density should enhance interaction with melanin. Steric hindrance about the phenyl ring may be the cause of the decreased uptake.

A series of the fluorinated compounds (7g-7i) showed somewhat lower tumour uptake at 24 h compared to the parent compound $[{}^{125}\text{I}]\text{-7d}$ (Fig. 4). Although the 24 h tumour uptake of
fluorinated $[^{125}\text{I}]-7\text{h}$ and $[^{125}\text{I}]-7\text{i}$ were slightly lower than $[^{125}\text{I}]-7\text{d}$, they retained the excellent
tumour-to-non-tumour ratios seen with many of these TAAG constructs.\textsuperscript{24} Interestingly, the
fluorine in the para position of $[^{125}\text{I}]-7\text{i}$ resulted in lower thyroid localization than did $[^{125}\text{I}]-7\text{d}$
(2.2 vs. 7.7 %ID/g respectively). Compared to $[^{125}\text{I}]-\text{BZA}$, the equivalent TAAG derivative
$[^{123}\text{I}]-7\text{a}$ had similar thyroid uptake (Fig. 3), while uptake of $[^{125}\text{I}]-7\text{e}$ and $[^{125}\text{I}]-7\text{f}$ were
somewhat higher (Fig. 4). Overall, when compared to the established benzamide $[^{125}\text{I}]-\text{BZA}$
agent, TAAG derivatives $[^{123}\text{I}]-7\text{a}$ and $[^{125}\text{I}]-7\text{d}$ had lower tumour uptake, but had greatly
reduced uptake by non-target tissues (Fig. 4 and Supplementary Data).

SPECT-CT Imaging

Due to the rapid clearance of compound $[^{123}\text{I}]-7\text{a}$ and the high tumour uptake of $[^{125}\text{I}]-7\text{d}$, both
7\text{a} and 7\text{d} were then labelled with $^{123}\text{I}$ and used in a SPECT-CT imaging study. In both cases,
there was excellent tumour visualization and low non-specific binding (Fig. 5), which was
consistent with the biodistribution data at 24 h. In addition to the tumour, the eyes were also
visible due to the presence of melanin; an observation also in agreement with the biodistribution
data and literature reports for other benzamides.\textsuperscript{15}

Conclusions

A convenient synthetic method to prepare a new class of triazole-based radiiodinated
benzamide compounds capable of targeting melanin-expressing tumors \textit{in vivo} was developed.
Compounds were labelled in modest to high yields with $^{123}\text{I}$ or $^{125}\text{I}$, and obtained in $>95\%$
radiochemical purity prior to evaluation \textit{in vivo} using a murine melanoma tumour model. Two
promising compounds were identified, and SPECT-CT imaging studies showed clear tumour
uptake and clearance from non-target organs. As the lead compound, 7d represents a new melanin-binding radiopharmaceutical that can be used for treating metastatic melanoma alone or in combination with immunotherapies, or as a diagnostic agent to assess the impact of new treatments.

**Experimental**

**General Methods and Materials**

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker AV 600 spectrometer. $^1$H and $^{13}$C NMR chemical shifts are expressed in parts per million (ppm), while coupling constants are expressed in Hertz (Hz). High Resolution Mass Spectra (HRMS) were recorded on a Waters Micromass Q-TOF Global mass spectrometer using electrospray ionization (ESI). Infrared (IR) spectra were obtained on a Biorad FTS-40 FTIR spectrometer. High performance liquid chromatography (HPLC) was performed using a Varian ProStar Model 230 instrument, fitted with a Varian ProStar model 330 PDA detector, an IN/US $\gamma$-RAM gamma detector and a Star 800 analog interface module, and a Waters 2489 HPLC equipped with a Waters 2489 UV/Vis ($\lambda = 254$ nm) and/or a Bioscan glow count gamma detector (model 106). The radiolabelled compounds were isolated and analyzed using an C18 column (Waters X-Bridge 5 $\mu$m, 4.6 $\times$ 100 mm). Samples were eluted in HPLC grade water containing 0.1% TFA (eluent A) for 3 min, followed by gradient increase in acetonitrile containing 0.1% TFA (eluent B), from 10-100% over 13 min, at 1 mL/min.

SiliaFlash P60 Silica gel from SiliCycle (Quebec City, QC) was used for silica gel chromatography. Toluene and tetrahydrofuran were distilled using a PURE SOLV distillation system. FC-72 was purchased from 3M Canada (London, ON) and (CF$_3$(CF$_2$)$_5$(CH$_2$)$_2$)$_3$SnPh was
purchased from Fluorous Technologies (Ambridge, PA). All other reagents were purchased from Sigma-Aldrich (Oakville, ON) and AK Scientific (Union City, CA) and used without further purification. $^{123}$I was produced by MDS Nordion (Vancouver, BC) using the $^{124}$Xe(p, 2n) reaction and was delivered as a [$^{123}$I]Na in 0.1M NaOH solution. $^{125}$I was produced by the McMaster Nuclear Reactor (Hamilton, ON) and was delivered as a [$^{125}$I]Na in 0.1M NaOH solution.

**Synthesis**

**TAAG constructs.**

Compounds 1, 2 and 3 were synthesized according to literature procedures.\(^{19}\)

**General Procedure 1: Preparation of protected amines (4e-4i).**

To a solution of 4-(N-Boc-amino)piperidine (1 equiv.) in DCM, was added the appropriate benzylhalide (1.2 equiv.) and DIPEA (1.5 equiv.), and the reaction stirred at room temperature for 24 h. The reaction mixture was concentrated and purified through column chromatography on silica with 1:1 hexane/ethyl acetate containing 1% triethylamine.

4e  *tert-Butyl 1-(3,5-dimethoxybenzyl)piperidin-4-ylcarbamate.* Following general procedure 1, the product was isolated as a white powder (51%, 0.160 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$: 6.48 (d, 2H, $J = 1.8$ Hz), 6.35 (t, 1H, $J = 2.2$ Hz), 4.43 (s, 1H), 3.78 (s, 6H), 3.47 (s, 1H) 3.41 (s, 2H), 2.78 (m, 2H), 2.08 (m, 2H), 1.90 (m, 2H), 1.44 (s, 11H). $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$: 160.7, 155.1, 141.1, 106.8, 98.9, 79.2, 63.1, 55.3, 52.4, 47.8, 32.6, 28.4. HRMS-ESI (m/z): [M+H]$^+$ calcd for C$_{19}$H$_{30}$N$_2$O$_4$H: 351.2284; found 351.2278. Mp. 74-77 ºC (decomp.).
4f  *tert-Butyl 1-(3,5-dinitrobenzyl)piperidin-4-ylcarbamate*  Following general procedure 1, the product was isolated as a yellow solid (92%, 0.279 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$: 8.92 (s, 1H), 8.54 (s, 2H), 4.46 (s, 1H), 3.67 (s, 2H), 3.51 (m, 1H), 2.77 (m, 2H), 2.23 (m, 2H), 1.95 (m, 2H), 1.44 (s, 11H). $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$: 155.2, 148.6, 144.3, 128.6, 117.7, 79.4, 61.3, 52.5, 47.5, 32.5, 28.4. HRMS-ESI (m/z): [M+H]$^+$ calcd for C$_{17}$H$_{24}$N$_4$O$_6$H: 381.1774; found 381.1770. Mp. 145-148 °C (decomp.).

4g  *tert-Butyl 1-(2-fluorobenzyl)piperidin-4-ylcarbamate.*  Following general procedure 1, the product was isolated as a white solid (93%, 0.296 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$: 7.38 (m, 1H), 7.24 (m, 1H), 7.11 (m, 1H), 7.02 (m, 1H), 4.42 (s, 1H), 3.59 (s, 2H), 3.47 (m, 1H), 2.84 (m, 2H), 2.19 (m, 2H), 1.92 (m, 2H), 1.43 (s, 11H). $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$: 161.4 (d, $J_{CF}$ = 247.6 Hz), 155.2, 131.7, 128.9, 123.5, 115.3 (d, $J_{CF}$ = 22.3 Hz), 79.3, 55.1, 52.0, 47.5, 32.4, 28.4.

4h  *tert-Butyl 1-(3-fluorobenzyl)piperidin-4-ylcarbamate.*  Following general procedure 1, the product was isolated as a white solid (77%, 0.180 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$: 7.25 (m, 1H), 7.05 (m, 2H), 6.92 (m, 1H), 4.47 (s, 1H), 3.46 (s, 3H), 2.77 (m, 2H), 2.09 (m, 2H), 1.90 (m, 2H), 1.44 (m, 11H). $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$: 163.1 (d, $J_{CF}$ = 245.0 Hz), 155.2, 141.4 (d, $J_{CF}$ = 5.4 Hz), 129.6 (d, $J_{CF}$ = 8.6 Hz), 124.4, 115.6 (d, $J_{CF}$ = 21.4 Hz), 113.9 (d, $J_{CF}$ = 21.4 Hz), 79.2, 62.4, 52.4, 47.7, 32.6, 28.4.

4i  *tert-Butyl 1-(4-fluorobenzyl)piperidin-4-ylcarbamate*  Following general procedure 1, the product was isolated as a white solid (65%, 0.209 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$: 7.27 (m, 2H), 6.99 (m, 2H), 4.43 (s, 1H), 3.48 (m, 1H), 3.45 (s, 2H), 2.78 (m, 2H), 2.09 (m, 2H), 1.91 (m, 2H), 1.14 (s, 11H). $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$: 162.0 (d, $J_{CF}$ = 243.5 Hz), 155.2, 130.6 (d, $J_{CF}$ = 7.0 Hz), 115.1 (d, $J_{CF}$ = 21.0 Hz), 79.3, 62.2, 52.2, 47.7, 32.5, 28.4.
General Procedure 2: Deprotection of protected amines (5e-i).

To a solution of Boc protected amine (1 equiv.) in DCM was added TFA (10 equiv.), and the reaction was stirred at room temperature overnight. The reaction mixture was concentrated, dissolved in 10 mL of saturated sodium bicarbonate and extracted three times with 10 mL of DCM (Scheme 2, part 2). The organic layer was dried over Na$_2$SO$_4$ and concentrated to give the desired free amine.

5e  1-(3,5-Dimethoxybenzyl)piperidin-4-amine. Following general procedure 2, the product was isolated as a yellow oil (93%, 0.072 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) δ: 6.48 (s, 2H), 6.34 (s, 1H), 3.77 (s, 6H), 3.42 (s, 2H), 2.82 (m, 2H), 2.64 (m, 1H), 2.01 (m, 2H), 1.77 (m, 2H), 1.47 (s, 2H), 1.38 (m, 2H). $^{13}$C NMR (CDCl$_3$, 150 MHz) δ: 160.7, 141.2, 106.8, 98.9, 63.1, 55.3, 52.5, 48.8, 36.0.

5f  1-(3,5-Dinitrobenzyl)piperidin-4-amine. Following general procedure 2, the product was isolated as an orange oil (89%, 0.178 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) δ: 8.93 (t, 1H, $J = 2.05$ Hz), 8.54 (d, 2H, $J = 1.98$ Hz), 3.68 (s, 2H), 2.80 (m, 3H), 2.18 (td, 2H, $J = 2.12, 11.76$ Hz), 1.87 (m, 6H), 1.50 (m, 2H). $^{13}$C NMR (CDCl$_3$, 150 MHz) δ: 148.6, 144.2, 128.6, 117.6, 61.3, 52.3, 48.4, 35.0. HRMS-ESI (m/z): [M+H]$^+$ calcd for C$_{12}$H$_{16}$N$_4$O$_4$H: 281.1250; found 281.1262.

5g  1-(2-Fluorobenzyl)piperidin-4-amine. Following general procedure 2, the product was isolated as a yellow oil (39%, 0.078 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) δ: 7.35 (td, 1H, $J = 1.6, 7.5$ Hz), 7.21 (m, 1H), 7.08 (td, 1H, $J = 0.9, 7.5$ Hz), 7.00 (m, 1H), 3.56 (s, 2H), 2.84 (m, 2H), 2.64 (m, 1H), 2.07 (td, 2H, $J = 2.0, 11.6$ Hz), 1.78 (m, 2H), 1.71 (s, 2H), 1.39 (m, 2H). $^{13}$C
NMR (CDCl₃, 150 MHz) δ: 161.4 (d, J_CF = 245.9 Hz), 131.5 (d, J_CF = 4.1 Hz), 128.6 (d, J_CF = 8.2 Hz), 125.0 (d, J_CF = 14.3 Hz), 123.8 (d, J_CF = 2.6 Hz), 115.2 (d, J_CF = 22.4 Hz), 55.2, 52.2, 48.6, 35.8.

5h 1-(3-Fluorobenzyl)piperidin-4-amine Following general procedure 2, the product was isolated as a yellow oil (35%, 0.032 g yield). ¹H NMR (CDCl₃, 600 MHz) δ: 7.24 (m, 1H), 7.05 (m, 2H), 6.91 (m, 1H), 3.46 (s, 2H), 2.79 (m, 2H), 2.66 (m, 1H), 2.01 (m, 2H), 1.78 (m, 2H), 1.59 (s, 2H), 1.38 (m, 2H). ¹³C NMR (CDCl₃, 150 MHz) δ: 162.9 (d, J_CF = 245.6 Hz), 141.5 (d, J_CF = 7.11 Hz) 129.5 (d, J_CF = 8.32 Hz), 124.4, 115.6 (d, J_CF = 21.27 Hz), 113.8 (d, J_CF = 21.27 Hz), 62.4, 52.4, 48.7, 35.9.

5i 1-(4-Fluorobenzyl)piperidin-4-amine. Following general procedure 2, the product was isolated as a yellow oil (41%, 0.055 g yield). ¹H NMR (CDCl₃, 600 MHz) δ: 7.26 (m, 2H), 6.98 (m, 2H), 3.44 (s, 2H), 2.79 (m, 2H), 2.66 (m, 1H), 1.99 (m, 2H), 1.77 (m, 4H), 1.38 (m, 2H). ¹³C NMR (CDCl₃, 150 MHz) δ: 161.9 (d, J_CF = 243.4 Hz), 134.3 (d, J_CF = 2.4 Hz), 130.5 (d, J_CF = 7.5 Hz), 114.9 (d, J_CF = 21.1 Hz), 62.2, 52.3, 48.7, 35.8, 29.7.

Procedures to prepare 6a, 6b and 6c from alternate TAAG constructs.

6a N-(2-(Diethylamino)ethyl)-2-(4-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)-1H-1,2,3-triazol-1-yl)acetamide. To a solution of I, the tin TAAG methyl ester (1 equiv.) in methanol (1 mL), was added N,N-diethylethylenediamine (3 equiv.) and DIPEA (3 equiv.), and the reaction was heated to 60 °C for 2 h. The reaction mixture was concentrated and purified through column chromatography on silica with 20:1 dichloromethane/methanol. The fractions containing the desired compound were concentrated and the product was isolated as a clear oil (88%, 0.052 g yield). ¹H NMR (MeOD, 600 MHz) δ: 8.08 (s, 1H), 5.24 (s, 2H), 3.44 (t, 2H, J = 6.8 Hz), 2.82
(m, 6H), 2.47 (m, 6H), 1.40 (t, 6H, $J = 8.4, 30.2$ Hz), 1.13 (m, 6H). $^{13}$C NMR (MeOD, 150 MHz) δ: 168.2, 143.3, 134.2, 52.4, 52.0, 48.0, 37.0, 28.3 (t, $J_{CSn} = 22.9$ Hz), 10.4, 0.0 (t, $J_{CSn} = 197.9$ Hz). IR: (KBr disc) $\tilde{\nu} = 3324, 2973, 1675$ cm$^{-1}$. HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{34}H_{30}N_{5}O_{39}Sn$: 1386.0935; found 1386.0883. HPLC Rt = 18.5 min.

6b  $N$-(2-Diethylamino-ethyl)-2-{$5$-phenyl-4-{$tris$-$\{3,3,4,4,5,5,6,6,7,7,8,8,8$-tridecafluoro-octyl$\}$-stannanyl$\}$-$[1,2,3]$triazol$1$-yl$}$-acetamide. To a solution of 2, the phenyl-tin TAAG methyl ester (1 equiv.) in methanol (1 mL) was added $N,N$-diethylethylenediamine (3 equiv.) and DIPEA (3 equiv.), the reaction was heated to 60 °C for 3 days. The reaction mixture was concentrated and purified through column chromatography on silica with 20:1 dichloromethane/methanol. The fractions containing the desired compound were concentrated and the product was isolated as a clear oil (19%, 0.012 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) δ:

7.49 (m, 3H), 7.28 (m, 2H), 4.92 (s, 2H), 3.36 (q, 2H, $J = 5.6, 11.1$ Hz), 2.60 (m, 6H), 2.18 (m, 6H), 1.18 (m, 6H), 1.01 (t, 6H, $J = 7.1$ Hz). $^{13}$C NMR (CDCl$_3$, 150 MHz) δ: 166.2, 146.8, 142.8, 131.2, 130.1, 129.9, 127.9, 51.9, 51.5, 47.5, 37.3, 28.1 (t, $J_{CSn} = 23.0$ Hz), 11.8, 0.0 (t, $J_{CSn} = 188.0$ Hz). IR: (KBr disc) $\tilde{\nu} = 3286, 2929, 1683$ cm$^{-1}$. HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{40}H_{34}N_{5}O_{39}SnH$: 1462.1250; found 1462.1219. HPLC Rt = 19.10 min.

6c  $N$-(2-(Piperidin-1-yl)ethyl)-2-{$4$-{$tributylstannyl$}$\}$-1H-$1,2,3$-$triazol$1$-yl$}$-acetamide. To a solution of 3, the tin TAAG methyl ester (1 equiv.) in methanol (1mL) was added $1$-{$2$-aminoethyl}$\}$piperidine (3 equiv.) and DIPEA (3 equiv.), the reaction was heated to 60 °C for 3 days. The reaction mixture was concentrated and purified through column chromatography on silica with 20:1 dichloromethane/methanol. The fractions containing the desired compound were concentrated and the product was isolated as a yellow solid (76%, 0.089 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) δ: 7.61 (s, 1H), 7.11 (s, 1H), 5.11 (s, 2H), 3.40 (q, 2H, $J = 5.9, 11.8$ Hz), 2.56
(t, 2H, J = 5.9 Hz), 2.50 (s, 4H), 1.60 (m, 4H), 1.53 (m, 6H), 1.46 (s, 2H), 1.33 (m, 6H), 1.11 (m, 6H, J = 8.23, 26.9 Hz), 0.88 (t, 9H, J = 7.4 Hz); \(^{13}\)C NMR (CDCl\(_3\), 150 MHz) \(\delta\) 165.9, 145.2, 131.4 (t, \(J_{C,Sn} = 37.0\) Hz), 56.4, 54.1, 52.4, 35.7, 29.0 (t, \(J_{C,Sn} = 10.2\) Hz), 27.2 (t, \(J_{C,Sn} = 29.9\) Hz), 25.1, 23.7, 13.6, 9.9 (t, \(J_{C,Sn} = 176.4\) Hz). IR: (KBr disc) \(\tilde{\nu}\) = 3301, 2931, 2871, 2853, 1684, 1559, 1540, 1457 cm\(^{-1}\). HRMS-ESI (m/z): [M+H]\(^+\) calcld for C\(_{23}\)H\(_46\)N\(_5\)OSn: 528.2729; found 528.2735.

**General Procedure 3: Coupling amines to TAAG (6d-i).**

To a solution of tin TAAG methyl ester (3) (1 equiv.) in methanol (1 mL) was added amine (1.2 equiv.) and DIPEA (3 equiv.), and the reaction was heated to \(60^\circ\)C for 3 days. The reaction mixture was concentrated and purified through column chromatography on silica with 20:1 dichloromethane/methanol. The fractions containing the desired compound were concentrated to give the desired radiolabelling precursor.

**6d**  
\(N\)-(1-Benzylpiperidin-4-yl)-2-(4-(tributylstannyl)-1H-1,2,3-triazol-1-yl)acetamide.

Following general procedure 3, the product was isolated as a white solid (64%, 0.165 g yield). \(^1\)H NMR (CDCl\(_3\), 600 MHz) \(\delta\): 7.57 (s, 1H), 7.29 (m, 4H), 7.23 (m, 1H), 6.08 (bs, 1H), 5.07 (s, 2H), 3.80 (m, 1H), 3.49 (s, 2H), 2.75 (m, 2H), 2.13 (m, 2H) 1.83 (m, 2H), 1.15 (m, 6H), 1.09 (t, 9H, J = 7.4 Hz). \(^{13}\)C NMR (CDCl\(_3\), 150 MHz) \(\delta\): 164.9, 145.7, 131.4, 129.1, 128.3, 127.2, 62.8, 52.7, 51.8, 46.8, 31.6, 29.0 (t, \(J_{C,Sn} = 10.9\) Hz), 27.2 (t, \(J_{C,Sn} = 29.8\) Hz), 13.7, 10.01 (t, \(J_{C,Sn} = 177.1\) Hz). IR: (KBr disc) \(\tilde{\nu}\) = 3297, 3092, 2954, 2927, 2871, 2851, 2754, 1653, 1558 cm\(^{-1}\). HRMS-ESI (m/z): [M+H]\(^+\) calcld for C\(_{28}\)H\(_{47}\)N\(_5\)OSnH: 590.2886; found 590.2877.
**6e**  
*N-(1-(3,5-Dimethoxybenzyl)piperidin-4-yl)-2-(4-(tributylstannyl)-1H-1,2,3-triazol-1-yl)acetamide.* Following general procedure 3, the product was isolated as an orange solid (7%, 0.014 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$: 7.58 (s, 1H), 6.48 (m, 2H), 6.35 (t, 1H, $J = 2.2$ Hz), 6.21 (s, 1H), 5.07 (s, 2H), 3.77 (s, 7H), 3.44 (s, 2H), 2.77 (m, 2H), 2.15 (m, 2H), 1.84 (m, 2H), 1.54 (m, 6H), 1.48 (m, 2H), 1.32 (m, 6H), 1.12 (m, 6H), 0.87 (t, 9H, $J = 7.3$ Hz). $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$: 164.8, 160.8, 145.6, 131.5, 99.3, 62.8, 55.3, 52.7, 51.9, 46.8, 31.4, 29.0 (t, $J_{CSn} = 10.7$ Hz), 27.2 (t, $J_{CSn} = 29.1$ Hz), 13.6, 10.0 (t, $J_{CSn} = 176.8$ Hz). IR: (KBr disc) $\nu = 3291$, 2955, 2851, 1669, 1597, 1552, 1463, 1366, 1341 cm$^{-1}$. HRMS-ESI (m/z): [M+H]$^+ \text{calcd for C}_{30}\text{H}_{51}\text{N}_{5}\text{O}_{3}\text{SnH}: 650.3098; \text{found 650.3111.}$

**6f**  
*N-(1-(3,5-Dinitrobenzyl)piperidin-4-yl)-2-(4-(tributylstannyl)-1H-1,2,3-triazol-1-yl)acetamide.* Following general procedure 3, the product was isolated as an orange solid (9%, 0.036 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$: 8.92 (s, 1H), 8.51 (s, 2H), 7.57 (s, 1H), 6.09 (m, 1H), 5.06 (s, 2H), 3.82 (m, 1H), 3.65 (s, 2H), 2.70 (m, 2H), 2.23 (t, 2H, $J = 10.9$ Hz), 1.87 (m, 2H), 1.55 (m, 6H), 1.45 (m, 2H), 1.32 (m, 6H), 1.14 (m, 6H), 0.88 (t, 9H, $J = 7.34$ Hz). $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$: 165.0, 148.6, 145.8, 144.0, 131.5, 128.5, 117.7, 61.3, 52.7, 52.1, 46.6, 31.6, 29.0 (t, $J_{CSn} = 10.3$ Hz), 27.2 (t, $J_{CSn} = 29.4$ Hz), 13.7, 10.0 (t, $J_{CSn} = 176.7$ Hz). IR: (KBr disc) $\nu = 3263$, 2954, 2852, 1656, 1539, 1459, 1344 cm$^{-1}$. HRMS-ESI (m/z): [M+H]$^+$ calcd for C$_{28}$H$_{45}$N$_5$O$_3$SnFH: 680.2588; found 680.2592.

**6g**  
*N-(1-(2-Fluorobenzyl)piperidin-4-yl)-2-(4-(tributylstannyl)-1H-1,2,3-triazol-1-yl)acetamide.* Following general procedure 3, the product was isolated as an orange oil (32%, 0.056 g yield). $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$: 7.33 (m, 1H), 7.21 (m, 1H), 7.07 (m, 1H), 7.00 (m, 1H), 6.04 (s, 1H), 5.05 (s, 2H), 3.76 (m, 1H), 3.53 (s, 2H), 2.74 (m, 2H), 2.15 (t, 2H, $J = 10.9$ Hz), 1.82 (m, 2H), 1.54 (m, 6H), 1.39 (m, 2H), 1.32 (m, 6H), 1.12 (m, 6H), 0.87 (t, 9H, $J = 7.4$ Hz).
13C NMR (CDCl₃, 150 MHz) δ: 164.9, 161.3 (d, J_CF = 246.5 Hz), 145.7, 131.4 (t, J_CSn = 36.9 Hz), 128.8 (d, J_CF = 7.6 Hz), 123.8, 115.2 (d, J_CF = 22.2 Hz), 55.1, 52.7, 51.7, 46.8, 31.7, 29.0 (t, J_CSn = 10.4), 27.2 (t, J_CSn = 29.2 Hz), 13.6, 10.0 (t, J_CSn = 178.3 Hz). IR: (KBr disc) ν = 3279, 3092, 2955, 2927, 2871, 2852, 1653, 1556, 1485, 1454, 1293, 1255. HRMS-ESI (m/z): [M+H]^+ calcd for C₂₈H₄₆N₅OSnFH: 608.2792; found 608.2781. R_f (10:1 dichloromethane/methanol) = 0.56.

6h N-(1-(3-Fluorobenzyl)piperidin-4-yl)-2-(4-(tributylstannyl)-1H-1,2,3-triazol-1-yl)acetamide. Following general procedure 3, the product was isolated as a yellow solid (20%, 0.014 g yield). ^1H NMR (CDCl₃, 600 MHz) δ: 7.58 (s, 1H), 7.23 (m, 1H), 7.02 (m, 2H), 6.91 (td, 1H, J = 2.1, 8.7 Hz), 6.05 (s, 1H), 5.06 (s, 2H), 3.78 (m, 1H), 3.44 (s, 2H), 2.69 (m, 2H), 2.10 (t, 2H, J = 10.6 Hz), 1.82 (m, 2H), 1.54 (m, 6H), 1.40 (m, 2H), 1.32 (m, 6H), 1.13 (m, 6H), 0.88 (t, 9H, J = 7.4 Hz). 13C NMR (CDCl₃, 150 MHz) δ: 164.9, 163.0 (J_CF = 246.1 Hz), 145.7, 141.2, 131.4 (J_CF = 36.2 Hz), 129.6 (J_CF = 8.0 Hz), 124.3, 115.5 (J_CF = 21.4 Hz), 113.9 (J_CF = 20.9 Hz), 62.3, 52.7, 51.9, 46.9, 31.7, 29.0 (J_CSn = 10.7 Hz), 27.2 (J_CSn = 29.6 Hz), 13.6, 10.0 (J_CSn = 176.8 Hz). IR: (KBr disc) ν = 3279, 3092, 2955, 2927, 2871, 2852, 1653, 1556, 1485, 1454, 1293, 1255. HRMS-ESI (m/z): [M+H]^+ calcd for C₂₈H₄₆N₅OSnFH: 608.2792; found 608.2781. R_f (10:1 dichloromethane/methanol) = 0.48.

6i N-(1-(4-Fluorobenzyl)piperidin-4-yl)-2-(4-(tributylstannyl)-1H-1,2,3-triazol-1-yl)acetamide. Following general procedure 3, the product was isolated as a white solid (18%, 0.036 g yield). ^1H NMR (CDCl₃, 600 MHz) δ: 7.58 (s, 1H), 7.26 (s, 2H), 5.08 (s, 2H), 3.81 (m, 1H), 3.51 (s, 2H), 2.79 (m, 2H), 2.16 (s, 2H), 1.85 (m, 2H), 1.54 (m, 6H), 1.49 (m, 2H), 1.32 (m, 6H), 1.13 (m, 6H), 0.87 (t, 9H, J = 7.1 Hz). 13C NMR (CDCl₃, 150 MHz) δ: 165.0, 162.1 (J_CF = 21.3 Hz), 145.6, 133.1, 131.6 (J_CSn = 35.4 Hz), 130.7,
115.1 \((J_{CF} = 21.3 \text{ Hz})\), 61.9, 52.6, 51.7, 46.7, 31.3, 29.0 \((J_{CSn} = 10.5 \text{ Hz})\), 27.2 \((J_{CSn} = 29.3 \text{ Hz})\), 13.6, 10.0 \((J_{CSn} = 176.3 \text{ Hz})\).

IR: (KBr disc) \(\bar{\nu} = 3292, 2955, 2924, 2852, 1669, 1603, 1551, 1510, 1464 \text{ cm}^{-1}\). HRMS-ESI (m/z): [M+H]+ calcd for C\(_{28}\)H\(_{46}\)N\(_5\)OSnFH: 608.2792; found 608.2794. R\(_f\) (10:1 dichloromethane/methanol) = 0.46.

**Radiolabelling**

**General radio-iodination procedure 1: Labelling of 6a-6c**

To a solution of a tin precursor (100 µg, 0.068-0.190 µmol) in methanol (100 µL) was added iodogen (25 µg) and acetic acid (5 µL), in the presence of no carrier added \[^{123}I/^{125}I\]-iodine (2.9 MBq - 196.5 MBq). The reaction was placed on the shaker for 10 min. at which point it was quenched with Na\(_2\)S\(_2\)O\(_3\) (0.1 M, 100 µL). The desired product was isolated by HPLC, concentrated to dryness and formulated in PBS for biological studies.

**General radio-iodination procedure 2: Labelling of 6d-6i,**

To a solution of a tin precursor (100 µg, 0.147-0.170 µmol) in chloroform (100 µL) was added iodogen (25 µg) in chloroform (25 µL) and acetic acid (5 µL), in the presence of no carrier added \[^{123}I/^{125}I\]iodine (4.4 MBq – 81.4 MBq). The reaction solution was placed on the shaker for 10 min, at which point it was quenched with Na\(_2\)S\(_2\)O\(_5\) (0.1 M, 100 µL). The aqueous layer of the reaction mixture was separate and the desired product was isolated by HPLC, concentrated to dryness and formulated in PBS for biological studies.

**Lipophilicity measurements.**

The lipophilicity (log \(P\)) values were determined by the shake flask method at pH 7.4.\(^{26}\) The results are summarized in Table 1.
Mouse melanoma tumour model

B16F1 cells derived from mouse melanoma were purchased from ATCC (CRL-6323). Cells were propagated using DMEM media, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and grown at 37 °C with 5% CO₂. Cells were used between passage numbers 7 to 28. Female C57Bl/6 mice were purchased from Charles River (Senneville, QC) and housed under SPF conditions in an established animal facility, with 12 h light/dark cycles and with food and water ad libitum. Mice were injected with $4.5 \times 10^5$ B16F1 cells in DPBS subcutaneously into the flank to generate syngeneic melanoma tumours. Animal studies were approved by the Animal Research Ethics Board at McMaster University in accordance with Canadian Council on Animal Care guidelines.

Biodistribution Studies

Biodistribution studies were performed on C57Bl/6 mice bearing B16F1 tumours at 10 days post inoculation ($n = 5$ for $^{123}$I-7a, $n = 4$ for $^{125}$I-7b, $n = 3$ for the remaining 7 compounds). Mice were injected with 0.48 MBq $^{123}$I-7a, 0.098 MBq $^{125}$I-7b, 0.15 MBq $^{125}$I-7c, 0.22 MBq $^{125}$I-7d, 0.11 MBq $^{125}$I-7e, 0.22 MBq $^{125}$I-7f, 0.25 MBq $^{125}$I-7g, 0.24 MBq $^{125}$I-7h, or 0.25 MBq $^{125}$I-7i. At 24 h post-injection, animals were anesthetized with 3% isoflurane and euthanized by cervical dislocation. Blood, adipose, adrenals, bone, brain, eyes, gall bladder, heart, kidneys, large intestine and caecum (with contents), liver, lungs, skeletal muscle, small intestine (with contents), spleen, stomach (with contents), thyroid/trachea, B16F1 tumour, urine + bladder, and tail were collected, weighed and counted in a Perkin Elmer Wizard 1470 Automatic Gamma Counter. Decay correction was used to normalize organ activity.
measurements to the time of dose preparation for final data calculations of percent injected dose per gram of tissue/fluid (%ID/g).

SPECT-CT Imaging

Imaging of $^{123}\text{I}}-\text{7a}$ and $^{123}\text{I}}-\text{7d}$ was completed using female C57Bl/6 mice bearing B16F1 tumours, 10 days post-inoculation. The mice were administered 200 µl of PBS containing $^{123}\text{I}}-\text{7a}$ (~24.1 MBq) or $^{123}\text{I}}-\text{7d}$ (~24.9 MBq) via tail vein injection. For $^{123}\text{I}}-\text{7a}$ the total amount of activity in the animal was low so it was euthanized prior to imaging to allow for longer acquisition times. Prior to imaging for $^{123}\text{I}}-\text{7d}$, the mouse was anaesthetized with 2.5% isoflurane and maintained under the same conditions for the length of the SPECT and CT scans. SPECT acquisitions were completed for 32 frames (300 sec/frame for $^{123}\text{I}}-\text{7a}$ and 180 sec/frame for $^{123}\text{I}}-\text{7d}$) on a GammaMedica Ideas X-SPECT system (North Ridge, California). CT acquisition consisted of 512 projections acquired over 360° with 75Kvp, 205mA cone beam CT system. Cobra Exxim software (Feldkamp filtered back-projection cone beam reconstruction software) was used to reconstruct the images at a voxel size of 155 microns and a matrix size of $512^3$. An OS-EM interactive reconstructed method (2 iterations/8 subsets) was used to reconstruct the SPECT data, which was fused to the CT data using in house software. AMIDE software was used to analyze the images.

Acknowledgements
This work was supported by grants from the Natural Sciences and Engineering Council of Canada, and the Ontario Institute for Cancer Research. X-ray crystallography support was provided by Dr. Hilary Jenkins, McMaster University, who acquired data and solved the crystal structure. The authors acknowledge the contributions of Dr. Denis Snider towards the preparation and editing of this manuscript.

References


Figure Captions:

Fig. 1. Comparison of the structures of iodinated benzamide (BZA) and 7a, the iodinated TAAG analogue.

Fig. 2. ORTEP drawing of 7d. The thermal ellipsoid probability was 50%. Hydrogen atoms are omitted for clarity.

Fig. 3. Biodistribution data (%ID/g) for selected tissues and fluids comparing radioiodinated BZA to radioiodinated compounds 7a-7d at 24 h post injection. Error bars are SEM (n = 3-5). Tissues and fluids are indicated on x-axis. Complete biodistribution data can be found in Supplementary Data.

Fig. 4. Biodistribution data (%ID/g) for selected tissues and fluids comparing radioiodinated BZA to radioiodinated compounds 7d-7i at 24 h post injection. Error bars are SEM (n = 3). Tissues and fluids are indicated on x-axis. Complete biodistribution data can be found in Supplementary Data.

Fig. 5. SPECT-CT images of mice given $[^{123}\text{I}]-7\text{a}$ (A) or $[^{123}\text{I}]-7\text{d}$ (B) at 24 h post-injection. Tumours are indicated by yellow arrows. For $[^{123}\text{I}]-7\text{d}$, uptake in the eyes is indicated by the white arrow. NOTE: images are presented at different intensities. To compare relative uptake, refer to the corresponding biodistribution data (see Supplementary Data).
Fig. 1. Comparison of the structures of iodinated benzamide (BZA) and 7a, the iodinated TAAG analogue.

25x5mm (600 x 600 DPI)
Fig. 2. ORTEP drawing of 7d. The thermal ellipsoid probability was 50%. Hydrogen atoms are omitted for clarity.
Figure 4
Fig. 5. SPECT-CT images of mice given [123I]-7a (A) or [123I]-7d (B) at 24 h post-injection. Tumours are indicated by yellow arrows. For [123I]-7d, uptake in the eyes is indicated by the white arrow. NOTE: images are presented at different intensities. To compare relative uptake, refer to the corresponding biodistribution data (see Supplementary Data).
Tables

Table 1. Log $P$ and radiochemical yields for compounds $[^{123}\text{I}]$-7a and $[^{125}\text{I}]$-7b-7i

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log $P_{7.4}$</th>
<th>RCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZA</td>
<td>1.34</td>
<td>42%</td>
</tr>
<tr>
<td>$[^{123}\text{I}]$-7a</td>
<td>-0.64 ± 0.08</td>
<td>18%</td>
</tr>
<tr>
<td>$[^{125}\text{I}]$-7b</td>
<td>0.63 ± 0.05</td>
<td>19%</td>
</tr>
<tr>
<td>$[^{125}\text{I}]$-7c</td>
<td>-0.12 ± 0.02</td>
<td>33%</td>
</tr>
<tr>
<td>$[^{125}\text{I}]$-7d</td>
<td>0.70 ± 0.19</td>
<td>51%</td>
</tr>
<tr>
<td>$[^{125}\text{I}]$-7e</td>
<td>1.02 ± 0.37</td>
<td>6%</td>
</tr>
<tr>
<td>$[^{125}\text{I}]$-7f</td>
<td>1.33 ± 0.27</td>
<td>13%</td>
</tr>
<tr>
<td>$[^{123}\text{I}]$-7g</td>
<td>1.14 ± 0.19</td>
<td>40%</td>
</tr>
<tr>
<td>$[^{125}\text{I}]$-7h</td>
<td>1.39 ± 0.25</td>
<td>38%</td>
</tr>
<tr>
<td>$[^{122}\text{I}]$-7i</td>
<td>0.84 ± 0.11</td>
<td>27%</td>
</tr>
</tbody>
</table>

Note: Log $P$ data for BZA were from previously reported work.\textsuperscript{23}
Table 2. Tumour-to-blood/tissue ratios of $[^{123/125}\text{I}]-\text{TAAG}$ derivatives from biodistribution data, using C57BL/6 mice bearing B16F1 tumours (24 h post injection).

<table>
<thead>
<tr>
<th>$[^{123/125}\text{I}]$ Compound</th>
<th>Tumour: Blood</th>
<th>Tumour: Liver</th>
<th>Tumour: Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZA</td>
<td>162.5</td>
<td>6.6</td>
<td>0.5</td>
</tr>
<tr>
<td>7a</td>
<td>19.0</td>
<td>9.0</td>
<td>0.1</td>
</tr>
<tr>
<td>7b</td>
<td>89.0</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>7c</td>
<td>15.0</td>
<td>6.4</td>
<td>0.2</td>
</tr>
<tr>
<td>7d</td>
<td>221.0</td>
<td>27.6</td>
<td>0.3</td>
</tr>
<tr>
<td>7e</td>
<td>66.0</td>
<td>10.2</td>
<td>0.2</td>
</tr>
<tr>
<td>7f</td>
<td>115.0</td>
<td>8.2</td>
<td>0.1</td>
</tr>
<tr>
<td>7g</td>
<td>103.0</td>
<td>11.4</td>
<td>0.2</td>
</tr>
<tr>
<td>7h</td>
<td>189.0</td>
<td>63.0</td>
<td>0.4</td>
</tr>
<tr>
<td>7i</td>
<td>191.0</td>
<td>17.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Scheme 1: Synthesis of compounds 6a-6c and 7a-7c

63x22mm (600 x 600 DPI)
Scheme 2: Synthesis of compounds 6d-6i and 7d-7i

78x32mm (600 x 600 DPI)