Radiohalogenated neopentyl derivatives: A novel scaffold for radioiodinated and astatinated compounds of high stability to \textit{in vivo} dehalogenation

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• $^{211}$At is one of the most promising $\alpha$-emitters applicable to TAT.

• Conjugation methods of $^{211}$At with targeting molecules such as antibodies and peptides are limited.

• A conventional radioiodination reagent, SIB has been applied to an astatination reagent, SAB. However, the stability of SAB-labeled compounds is lower than SIB-labeled ones.

A scaffold for astatinated compounds of high stability against $\textit{in vivo}$ dehalogenation is strongly required.
Chemical design

- Radioiodination at the neopentyl C-19 position of cholesterol provides a radioiodinated cholesterol derivative of high *in vivo* stability.\(^1\) Neopentyl halides were evaluated as a novel scaffold for astatination.

- Recently, a hypoxia imaging agent that had neopentyl fluoride structure was developed.\(^2\)

\(^1\) Steroids, 16 317-328 (1970)
Chemical design

Before the evaluation of $^{211}\text{At}$-labeled compounds, $^{125}\text{I}$-labeled compounds (BHIN and DEIN) were used for preliminary evaluation studies.

$^{[18\text{F}]}\text{DiFA}$

$X = ^{211}\text{At}$: $[^{211}\text{At}]\text{BHAN}$

$X = ^{125}\text{I}$: $[^{125}\text{I}]\text{BHIN}$

$^{[125}\text{I}]\text{DEIN}$

Stability against

1. Nucleophilic attack
2. CYP-mediated metabolism
3. In vivo dehalogenation
Radiolabeling

The concentration of the precursor: 10 mM
68.8±0.9% (37°C, 1 h)

The concentration of the precursor: 10 mM
57.3±0.6% (80°C, 1 h)
1. The stability against nucleophilic substitution reaction

10 mM glutathione (GSH)
1 mM EDTA
in 0.1 M P.B. (pH 7.4)

Incubation at 37°C

In vitro studies

% Intact

![Graph showing % Intact over time for [125I]DEIN and [125I]BHIN](image)

- [125I]DEIN: 95.1 ± 0.7%
- [125I]BHIN: 96.6 ± 1.0%
In vitro studies

1. The stability against nucleophilic substitution reaction

- 10 mM glutathione (GSH)
- 1 mM EDTA
- in 0.1 M P.B. (pH 7.4)
- Incubation at 37°C

2. The stability against CYP-mediated metabolism

- Mouse liver Microsomes (0.2 mg/mL)
- NADPH regenerating system
- 10 mM MgCl₂
- in 0.1 M tris-HCl (pH 7.4)
- Incubation at 37°C for 30 min

 ![Graph showing the stability of [125I]DEIN and [125I]BHIN over time.](image)

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<thead>
<tr>
<th>%Intact</th>
<th>Time after incubation (h)</th>
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<tbody>
<tr>
<td>96.6 ±1.0 %</td>
<td>24 h</td>
</tr>
<tr>
<td>95.1 ±0.7 %</td>
<td>24 h</td>
</tr>
<tr>
<td>98.0 ±2.0 %</td>
<td>0 h</td>
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<td>2.2 ±0.1 %</td>
<td>0 h</td>
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CYP-mediated metabolism of $\text{[^{125}I]DEIN}$

Before incubation of $\text{[^{125}I]DEIN}$ with microsomes

After incubation of $\text{[^{125}I]DEIN}$ with microsomes

Radioactivity vs. Retention time (min)
CYP-mediated metabolism of $[^{125}\text{I}]$DEIN

**RP-HPLC analyses**

Before incubation of $[^{125}\text{I}]$DEIN with microsomes

After incubation of $[^{125}\text{I}]$DEIN with microsomes

This peak was further analyzed by HILIC

$[^{125}\text{I}]$DEIN was dehalogenated to liberate $[^{125}\text{I}]$I⁻
**In vivo studies**

**Liver**

- %ID/g vs. Time after injection (h)

**Stomach**

- %ID vs. Time after injection (h)

**Neck**

- %ID vs. Time after injection (h)

**Kidney**

- %ID/g vs. Time after injection (h)

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ICR, 6-week-old, male, n=4-5

- **[125I]BHIN**
- **[125I]DEIN**
In vivo studies

- $[^{125}\text{I}]\text{BHIN}$
  - ![Structure of BHIN](image1)

- $[^{125}\text{I}]\text{DEIN}$
  - ![Structure of DEIN](image2)

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<tr>
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<th>Urine</th>
<th>Feces</th>
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<tr>
<td>6h</td>
<td>60%</td>
<td>0%</td>
</tr>
<tr>
<td>24h</td>
<td>80%</td>
<td>20%</td>
</tr>
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</table>

ICR, 6-week-old, male, n=4-5
**In vivo studies**

- **[125I]BHIN**
- **[125I]DEIN**

The glucuronide conjugate of [125I]BHIN was identified by HILIC analysis. The fraction of intact [125I]DEIN was also analyzed. The radioactivity was measured over time in urine samples collected at 6h and 24h.

(Urine Analyses graphs showing peak retention times and radioactivity over time for [125I]BHIN and [125I]DEIN with annotations indicating the glucuronide conjugate and intact radioisotopes.)
[\(^{211}\text{At}\)]\text{BHAN} – 1: astatination reaction

The concentration of the precursor: 0.25 mM

Radiochemical yield: 14.6 ± 2.3%
Radiochemical purity: >98%

Retention time (min)

Radioactivity

\[ \frac{\text{120°C, 1 h}}{\text{H}_2\text{O}} \]

\[ \frac{\text{60°C, 0.5 h}}{\text{acetonitrile}} \]

\[ \text{[211At]NaAt} \]

\[ \text{[211At]BHAN} \]
[²¹¹At]BHAN – 2: stability against nucleophilic attack

[²¹¹At]BHAN (X=²¹¹At)
[¹²⁵I]BHIN (X=¹²⁵I)
[¹²⁵I]DEIN

[²¹¹At]BHAN

OH OH
X

[¹²⁵I]BHIN

[¹²⁵I]DEIN

10 mM glutathione (GSH)
1 mM EDTA
in 0.1 M P.B. (pH 7.4)
Incubation at 37ºC

TLC analyses

98.8 ± 1.7%

% Intact

0 95 90 85

[²¹¹At]BHAN [¹²⁵I]BHIN [¹²⁵I]DEIN
[²¹¹At]BHAN – 3: stability against CYP-mediated metabolism

[²¹¹At]BHAN (X=²¹¹At)
[¹²⁵I]BHIN (X=¹²⁵I)

[¹²⁵I]DEIN

Mouse liver Microsomes (0.2 mg/mL)
NADPH regenerating system
10 mM MgCl₂
in 0.1 M tris-HCl (pH 7.4)
Incubation at 37°C for 30 min

HPLC analyses
TLC analyses

Radioactivity

Retention time (min)

% Intact

96.9 ± 0.8%
In vivo studies

Liver

Stomach

Neck

Kidney

- [²¹¹At]BHAN (X=²¹¹At)
- [¹²⁵I]BHIN (X=¹²⁵I)
- [¹²⁵I]DEIN

ICR, 6-week-old, male, n=4-5

Time after injection (h)

%ID

OH

NO₂

OH

X

N

N

NO₂

125I
Conclusions

• $[^{125}\text{I}]$BHIN and $[^{125}\text{I}]$DEIN possessed high stability to the nucleophilic substitution.

• The presence of the hydroxyl groups in $[^{125}\text{I}]$BHIN provided further stabilization against CYP-mediated metabolism.

• $[^{211}\text{At}]$BHAN also showed high stability against both nucleophilic substitution reaction and CYP-mediated metabolism.

• $[^{125}\text{I}]$BHIN and $[^{211}\text{At}]$BHAN showed similar biodistribution profiles and low radioactivity levels in stomach and neck.

The neopentyl derivatives would serve as a useful scaffold to develop a radiotheranostic pair consisting of radiiodinated and $^{211}$At-labeled compounds.
Thank you for your kind attention!