Supplementary information

Synthesis and cytotoxic activity of novel C-23-modified asiatic acid derivatives

| Contents | Page |
|----------|------|
| H and C NMR spectra of 1 | 3 |
| H and C NMR spectra of 2 | 4 |
| H and C NMR spectra of 3 | 5 |
| H and C NMR spectra of 4 | 6 |
| H and C NMR spectra of 5 | 7 |
| H and C NMR spectra of 6 | 8 |
| H and C NMR spectra of 7 | 9 |
| H and C NMR spectra of 8 | 10 |
| H and C NMR spectra of 9 | 11 |
| H and C NMR spectra of 10 | 12 |
| H and C NMR spectra of 11 | 13 |
| H and C NMR spectra of 12 | 14 |
| H and C NMR spectra of 13a | 15 |
| H, C, DEPT, HSQC and HMBC NMR spectra of 13b | 16-18 |
| H and C NMR spectra of 13c | 19 |
| H and C NMR spectra of 13d | 20 |
| H and C NMR spectra of 13e | 21 |
| H and C NMR spectra of 13f | 22 |
| H and C NMR spectra of 13g | 23 |
| H and C NMR spectra of 13h | 24 |
| H and C NMR spectra of 13i | 25 |
| H and C NMR spectra of 13j | 26 |
| H and C NMR spectra of 13k | 27 |
| H and C NMR spectra of 14a | 28 |
| H, C, DEPT, HSQC and HMBC NMR spectra of 14b | 29-31 |
| H and C NMR spectra of 14c | 32 |
| H and C NMR spectra of 14d | 33 |
| H and C NMR spectra of 14e | 34 |
| H and C NMR spectra of 14f | 35 |
| H and C NMR spectra of 14g | 36 |
| H and C NMR spectra of 14h | 37 |
| H and C NMR spectra of 14i | 38 |
| H and C NMR spectra of 14j | 39 |
$^1$H and $^{13}$C NMR spectra of 14k

NMR titration of 13b and 14b

IC$_{50}$ curves of compounds 10, 13k, 14j and 14k against HL-60

$^1$H NMR Spectrum of $\alpha$-hydroxy-3$\beta$,23-isopropylenedioxy-urs-12-ene-28-oic acid (1) (400 MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of $\alpha$-hydroxy-3$\beta$,23-isopropylidenedioxy-urs-12-ene-28-oic acid (1) (101 MHz, CDCl$_3$)

$^1$H NMR Spectrum of $\alpha$-hydroxy-3$\beta$,23-isopropylidenedioxy-urs-12-ene-28-benzylamide (2) (400 MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of $\alpha$-hydroxy-3β,23-isopropylidenedioxy-urs-12-ene-28-benzylamide (2) (101 MHz, CDCl3)

$^1$H NMR Spectrum of $\alpha$-methoxy-3β,23-isopropylidenedioxy-urs-12-ene-28-benzylamide (3) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of $\alpha$-methoxy-3$\beta$,23-isopropylidenedioxy-urs-12-ene-28-benzylamide (3) (101 MHz, CDCl$_3$)

$^1$H NMR Spectrum of 2$\alpha$-methoxy-3$\beta$,23-dihydroxy-urs-12-ene-28-benzylamide (4) (400 MHz, CDCl$_3$)

5
$^{13}$C NMR Spectrum of 2α-methoxy-3β,23-dihydroxy-urs-12-ene-28-benzylamide (4) (101 MHz, CDCl₃)

$^1$H NMR Spectrum of 2α-methoxy-3,23-dioxo-urs-12-ene-28-benzylamide (5) (400 MHz, CDCl₃)
$^{13}$C NMR Spectrum of 2α-methoxy-3,23-dioxo-urs-12-ene-28-benzylamide (5) (101 MHz, CDCl₃)

$^1$H NMR Spectrum of 2α-methoxy-3β-hydroxy-23-oxo-urs-12-ene-28-benzylamide (6) (400 MHz, CDCl₃)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-hydroxy-23-oxo-urs-12-ene-28-benzylamide (6) (101 MHz, CDCl$_3$)

$^1$H NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oic acid (7) (400 MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oic acid (7) (101 MHz, CDCl3)

$^1$H NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oic acid (8) (400 MHz, CDCl3)
\( ^{13} \text{C NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oic acid (8)} (101 \text{ MHz, CDCl}_3) \)

\( ^{1} \text{H NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-methly ester (9)} (400 \text{ MHz, CDCl}_3) \)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-methly ester (9) (101 MHz, CDCl₃)

$^1$H NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-methly ester (10) (400 MHz, CDCl₃)
\(^{13}\text{C}\) NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-methy ester (10) (101 MHz, CDCl\(_3\))

\(^1\text{H}\) NMR Spectrum of 2α-methoxy-3-oxo-urs-12-ene-28-benzyl amide-23-methy ester (11) (400 MHz, CDCl\(_3\))
$^{13}$C NMR Spectrum of 2α-methoxy-3-oxo-urs-12-ene-28-benzyl amide-23-methly ester (11) (101 MHz, CDCl3)

$^1$H NMR Spectrum of 2α-methoxy-3β-acetoxy-23-oxo-urs-12-ene-28-benzyl amide (12) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-acetoxy-23-o xo-urs-12-ene-28-benzyl amide (12) (101 MHz, CDCl₃)

$^{1}$H NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-amide (13a) (400 MHz, CDCl₃)
$^{13}$C NMR Spectrum of $2\alpha$-methoxy-$3\beta$-hydroxy-urs-12-ene-28-benzyl amide-23-amide (13a) (101 MHz, CDCl3)

$^1$H NMR Spectrum of $2\alpha$-methoxy-$3\beta$-hydroxy-urs-12-ene-28-benzyl amide-23-methlyamide (13b) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of $2\alpha$-methoxy-$3\beta$-hydroxy-urs-12-ene-28-benzyl amide-23-methlyamide (13b) (101 MHz, CDCl$_3$)

DEPT135 NMR Spectrum of $2\alpha$-methoxy-$3\beta$-hydroxy-urs-12-ene-28-benzyl amide-23-methlyamide (13b) (126 MHz, CDCl$_3$)
2-D $^1$H-$^{13}$C HSQC NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-methlyamide (13b) ($^1$H NMR 500 MHz, CDCl$_3$, $^{13}$C NMR 126 MHz, CDCl$_3$)

2-D $^1$H-$^{13}$C HMBC NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl
amide-23-methlyamide (13b) (\(^1\)H NMR 500 MHz, CDCl\(_3\), \(^{13}\)C NMR 126 MHz, CDCl\(_3\))

\(^1\)H NMR Spectrum of 2\(\alpha\)-methoxy-3\(\beta\)-hydroxy-urs-12-ene-28-benzyl amide-23-ethlyamide (13c) (400 MHz, CDCl\(_3\))
$^{13}$C NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-ethylamide (13c) (101 MHz, CDCl$_3$)

$^1$H NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-isopropylamide (13d) (400 MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-isopropylamide (13d) (101 MHz, CDCl3)

$^1$H NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-propargylamide (13e) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-propargylamide (13e) (101 MHz, CDCl3)

$^1$H NMR Spectrum of N-(2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oyl)-L-alanine methyl ester (13f) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of N-(2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oyl)-L-alanine methyl ester (13f) (101 MHz, CDCl3)

$^1$H NMR Spectrum of N-(2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oyl)-L-valine methyl ester (13g) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of N-(2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oyl)-L-valine methyl ester (13g) (101 MHz, CDCl3)

$^1$H NMR Spectrum of N-(2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oyl)-L-phenylalanine methyl ester (13h) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of N-(2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oyl)- L-phenylalanine methyl ester (13h) (101 MHz, CDCl3)

$^1$H NMR Spectrum of N-(2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oyl)- 4-aminobutyric ethyl ester (13i) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of N-(2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23-oyl)- 4-aminobutyric ethyl ester (13i) (101 MHz, CDCl3)

$^1$H NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-dimethylamide (13j) (400 MHz, CDCl3)
\(^{13}\)C NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzyl amide-23-dimethlyamide (13j) (101 MHz, CDCl3)

\(^{1}\)H NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23- (4-methyl-1-piperazinyl)-amide (13k) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-hydroxy-urs-12-ene-28-benzylamide-23- (4-methyl-1-piperazinyl)-amide (13k) (126 MHz, CDCl$_3$)

$^1$H NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-amide (14a) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of $\alpha$-methoxy-3$\beta$-acetoxy-urs-12-ene-28-benzyl amide-23-amide (14a) (101 MHz, CDCl3)

$^1$H NMR Spectrum of 2$\alpha$-methoxy-3$\beta$-acetoxy-urs-12-ene-28-benzyl amide-23-methlyamide (14b) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-methlyamide (14b) (101 MHz, CDCl3)

DEPT135 NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-methlyamide (14b) ($^{13}$C NMR 126 MHz)
2-D $^1$H-$^{13}$C HSQC NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-methylamide (14b) ($^1$H NMR 500 MHz, CDCl$_3$, $^{13}$C NMR 126 MHz, CDCl$_3$)

2-D $^1$H-$^{13}$C HMBC NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-methylamide (14b)
amide-23-methylamide (14b) (\(^1\)H NMR 500 MHz, CDCl\(_3\), \(^13\)C NMR 126 MHz, CDCl\(_3\))

\(^1\)H NMR Spectrum of 2\(\alpha\)-methoxy-3\(\beta\)-acetoxy-urs-12-ene-28-benzyl amide-23-
ethlyamide (14c) (400 MHz, CDCl3)

\[ \text{\textsuperscript{13}C NMR Spectrum of 2\(\alpha\)-methoxy-3\(\beta\)-acetoxy-urs-12-ene-28-benzyl amide-23-ethlyamide (14c) (101 MHz, CDCl3) } \]

\[ \text{\textsuperscript{1}H NMR Spectrum of 2\(\alpha\)-methoxy-3\(\beta\)-acetoxy-urs-12-ene-28-benzyl amide-23-isopropylamide (14d) (400 MHz, CDCl3) } \]
$^{13}$C NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-isopropylamide (14d) (101 MHz, CDCl3)

$^1$H NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-propargylamide (14e) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of 2$\alpha$-methoxy-3$\beta$-acetoxy-urs-12-ene-28-benzyl amide-23-propargylamide (14e) (101 MHz, CDCl$_3$)

$^1$H NMR Spectrum of N-(2$\alpha$-methoxy-3$\beta$-acetoxy-urs-12-ene-28-benzylamide-23-oyl)-L-alanine methyl ester (14f) (400 MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of N-(2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oyl)-L-alanine methyl ester (14f) (101 MHz, CDCl$_3$)

$^1$H NMR Spectrum of N-(2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oyl)-L-valine methyl ester (14g) (400 MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of N-(2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oyl)-L-valine methyl ester (14g) (101 MHz, CDCl₃)

$^1$H NMR Spectrum of N-(2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oyl)-L-phenylalanine methyl ester (14h) (400 MHz, CDCl₃)
$^{13}$C NMR Spectrum of N-(2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oyl)- L-phenylalanine methyl ester (14h) (101 MHz, CDCl$_3$)

$^1$H NMR Spectrum of N-(2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oyl)-4-aminobutyric ethyl ester (14i) (400 MHz, CDCl$_3$)
$^{13}$C NMR Spectrum of N-(2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-oyl)-4-aminobutyric ethyl ester (14i) (101 MHz, CDCl3)

$^1$H NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-dimethylamide (14j) (400 MHz, CDCl3)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzyl amide-23-dimethlyamide (14j) (101 MHz, MeOD)

$^1$H NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-(4-methyl-1-piperazinyl)-amide (14k) (400 MHz, CDCl₃)
$^{13}$C NMR Spectrum of 2α-methoxy-3β-acetoxy-urs-12-ene-28-benzylamide-23-(4-methyl-1-piperazinyl)-amide (14k) (101 MHz, CDCl$_3$)

Fig. 1. NMR titration curves for compounds 13b and 14b.
Fig. 2. IC\textsubscript{50} curve of compound \textbf{10} against HL-60.
Fig. 3. IC₅₀ curve of compound 13k against HL-60.

Fig. 4. IC₅₀ curve of compound 14j against HL-60.
Fig. 5. IC$_{50}$ curve of compound 14k against HL-60.