



## Synthesis and biological evaluation of shikimic acid derivatives

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### ABSTRACT

From shikimic acid, new series of oseltamivir analogues containing either ether sulfur or ether oxygen at C-3 position were prepared and evaluated for their biological activities. This is the first report on synthesis of oseltamivir analogues with ether sulfur at C-3. Except for compounds **4d**, **4f**, **7c** and **7d**, the remaining compounds were found to be active against MCF-7, LU-1 and KB cell lines. As oseltamivir had no cytotoxicity against different cell lines such as MCF-7, LU-1, KB, MDCK, MRC-5, VERO, MK and 293, the modification of alkyl groups at C-3 of the oseltamivir ring framework could significantly increase the cytotoxicity for this class of compounds. Loss of neuraminidase inhibition activity of these synthetic oseltamivir analogues suggested that the alkyl ether groups at C-3 should be critical for their anti-neuraminidase activity.

### 1. Introduction

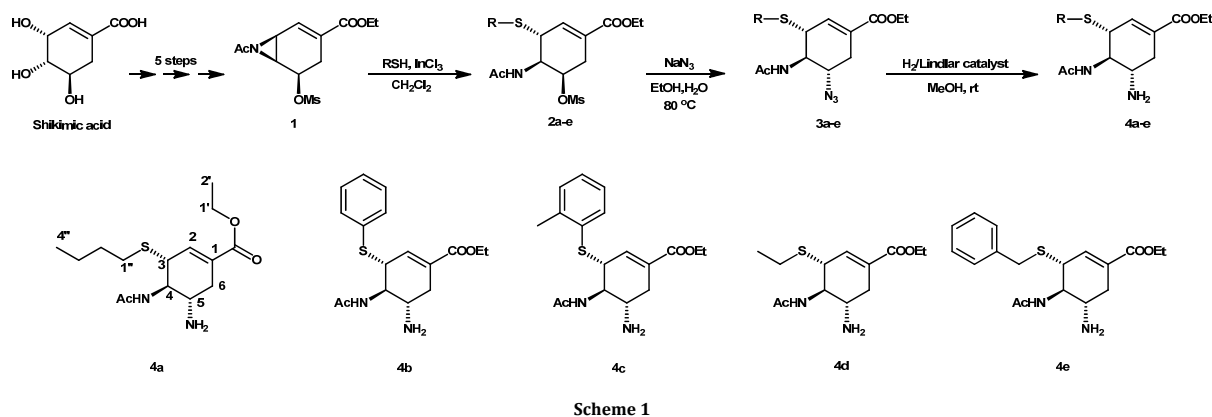
Shikimic acid is well known as starting material for production of oseltamivir phosphate (Tamiflu), an active neuraminidase inhibitor [1-5]. Although of some reports on total oseltamivir synthesis from other sources, shikimic acid remains the principal starting material for making oseltamivir. Shikimic acid is a key precursor for an enormous number of aromatic compounds, e.g. aromatic amino acids, vitamins and host of secondary metabolites [6-9], and is the ideal precursor for tracer studies because it is generally permeable to cells and is chemically stable. As shikimate pathway does not occur in mammals, much effort has been made to synthesize potential enzyme inhibitors in order to develop antimicrobial reagents. Many of these compounds, by virtue of their structural similarity to shikimic acid, have been targeted as likely inhibitor of enzyme on the shikimic acid pathway and are of biological interest as potential antifungal [10,11], antibacterial [11], antitumor [12,13], and antiparasitic agents [14]. Besides its bio-significance, shikimic acid, which is a highly functionalized carbocycle, is also an industrially useful chiral compound [15]. In our search of bioactive compounds based on shikimic acid, we reported here the synthesis and biological

activity of oseltamivir analogues containing either ether sulfur or ether oxygen at C-3 position. To our knowledge, the synthesis of oseltamivir analogues with ether sulfur at C-3 has not been reported yet. Also, there are a few reports on the cytotoxicity of compounds based on shikimic acid, whereas several shikimic acid's derivatives have been found to be potential antitumor agents such as pericosines [12,16]. Furthermore, the plant star anise (*Illicium verum*), widely distributing in the tropical and subtropical zones of Asia is considered as a rich source for extracting shikimic acid which should be used as starting material for different purposes [17].

### 2. Experimental

#### 2.1. Instrumentation

Chemical reagents were purchased from Aldrich Chemical Co. and used without further purification. Optical rotations were recorded on a Jasco P-2000 polarimeter in EtOH. Melting points were recorded on a MELTEMP3.0 instrument, and IR spectra were measured on a Nicolet Impact-410 FT-IR spectrometer. ESI-MS were recorded on an Agilent 1100 LC-MSD Trap spectrometer.



The NMR spectra were recorded on a Bruker spectrometer operating at 500.13 MHz.  $^1\text{H}$  chemical shifts were referenced to  $\text{CHCl}_3$  at 7.27, while the  $^{13}\text{C}$  chemical shifts were referenced to the central peak of  $\text{CDCl}_3$  at 77.0. Column chromatography was performed on Merck silica gel 60 (70-230 mesh).

## 2.2. Synthesis

### 2.2.1. General procedure for compounds 2a-e

To a solution of *N*-acetyl aziridine **1** (1.0 mmol) in dichloromethane (5 mL) was added  $\text{InCl}_3$  (20 mg, 0.1 mmol) and thiols (2.0 mmol). The reaction mixture was stirred at room temperature for 12 h. The solids were filtered off and washed with dichloromethane. The filtrate was evaporated under vacuum to give crude products **2a-e** which were used for the next step without purification (Scheme 1).

### 2.2.2. General procedure for compounds 3a-e

To a solution of compounds **2a-e** (0.5 mmol) in a mixture of ethanol (2 mL) and water (0.3 mL) was added sodium azide (10 eq). The reaction mixture was heated at 80 °C for 6 h. Water (20 mL) was added and the mixture was extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over sodium sulfate and concentrated under diminished pressure. The residue was purified over a silica gel column chromatography (*n*-hexane/EtOAc gradient) yielding compounds **3a-e** (Scheme 1).

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(butylthio)cyclohex-1-ene-1-carboxylate (3a)*: Color: Yellow, amorphous solid. Yield: 46.2%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.90 (t,  $J = 7.2$  Hz, 3H,  $\text{CH}_3\text{-4}''$ ), 1.30 (t,  $J = 7.2$  Hz, 3H,  $\text{CH}_3\text{-2}'$ ), 1.38 (m, 2H,  $\text{CH}_2\text{-3}''$ ), 1.54 (m, 2H,  $\text{CH}_2\text{-2}''$ ), 2.07 (s, 3H,  $\text{CH}_3\text{-CO}$ ), 2.25 (m, 1H,  $\text{H}_a\text{-6}$ ), 2.52 (m, 2H,  $\text{CH}_2\text{-1}''$ ), 2.90 (dd,  $J = 5.5, 17.5$  Hz, 1H,  $\text{H}_b\text{-6}$ ), 3.58 (q,  $J = 9.0$  Hz, 1H,  $\text{H-4}$ ), 3.76 (br. d,  $J = 9.0$  Hz, 1H,  $\text{H-3}$ ), 4.04 (ddd,  $J = 5.5, 9.0, 10.0$  Hz, 1H,  $\text{H-5}$ ), 4.22 (q,  $J = 7.2$  Hz, 2H,  $\text{CH}_2\text{-1}'$ ), 5.72 (d,  $J = 9.0$  Hz, 1H, NH), 6.88 (br. s, 1H,  $\text{H-2}$ ).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 13.5 (1C,  $\text{CH}_3$ ), 14.1 (1C,  $\text{CH}_3$ ), 21.9 (1C,  $\text{CH}_3$ ), 23.4, 29.7, 29.8, 31.6, 45.6, 55.4, 58.9, 61.0, 127.9, 139.1, 165.5, 171.1.

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(phenylthio)cyclohex-1-ene-1-carboxylate (3b)*: Color: Yellow, amorphous solid. Yield: 42%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.27 (t,  $J = 7.3$  Hz, 3H,  $\text{CH}_3\text{-2}'$ ), 1.98 (s, 3H, Ac), 2.01 (m, 1H,  $\text{H}_a\text{-6}$ ), 2.80 (dd,  $J = 5.0, 17.5$  Hz, 1H,  $\text{H}_b\text{-6}$ ), 3.68 (q,  $J = 8.7$  Hz, 1H,  $\text{H-4}$ ), 3.93 (m, 1H,  $\text{H-5}$ ), 4.12 (br. d,  $J = 8.7$  Hz, 1H,  $\text{H-3}$ ), 4.21 (m, 2H,  $\text{CH}_2\text{-1}'$ ), 6.43 (d,  $J = 8.7$  Hz, 1H, NH), 6.90 (br. s, 1H,  $\text{H-2}$ ), 7.26-7.32 (m, 3H, Ar-H-2'', H-6'' and H-4''), 7.46 (m, 2H, Ar-H-3'' and H-5'').  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.1, 23.3, 29.7, 49.2, 55.6, 58.9, 61.0, 128.2, 128.4, 129.1, 131.8, 134.0, 138.1, 165.4, 171.1.

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(o-tolylthio)cyclohex-1-ene-1-carboxylate (3c)*: Color: Yellow, amorphous solid. Yield: 40%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.27 (t,  $J = 7.2$  Hz, 3H,  $\text{CH}_3\text{-2}'$ ), 1.93 (s, 3H, Ac), 2.16 (m, 1H,  $\text{H}_a\text{-6}$ ), 2.44 (s, 3H,  $\text{CH}_3\text{-7}''$ ), 2.85 (dd,  $J = 4.5, 17.5$  Hz, 1H,  $\text{H}_b\text{-6}$ ), 3.67 (q,  $J = 9.0$  Hz, 1H,  $\text{H-4}$ ), 4.04 (ddd,  $J = 5.0, 9.0, 9.0$  Hz, 1H,  $\text{H-5}$ ), 4.18 (m, 2H,  $\text{CH}_2\text{-1}'$ ), 4.19 (m, 1H,  $\text{H-3}$ ), 6.16 (br. s, 1H, NH), 6.84 (br. s, 1H,  $\text{H-2}$ ), 7.12-7.25 (m, 3H, Ar-H-4'', H-5'' and H-6'').  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.1, 20.9, 23.3, 29.8, 48.4, 56.5, 58.7, 61.0, 126.6, 127.7, 128.3, 130.7, 132.3, 133.6, 137.5, 140.7, 165.5, 171.1.

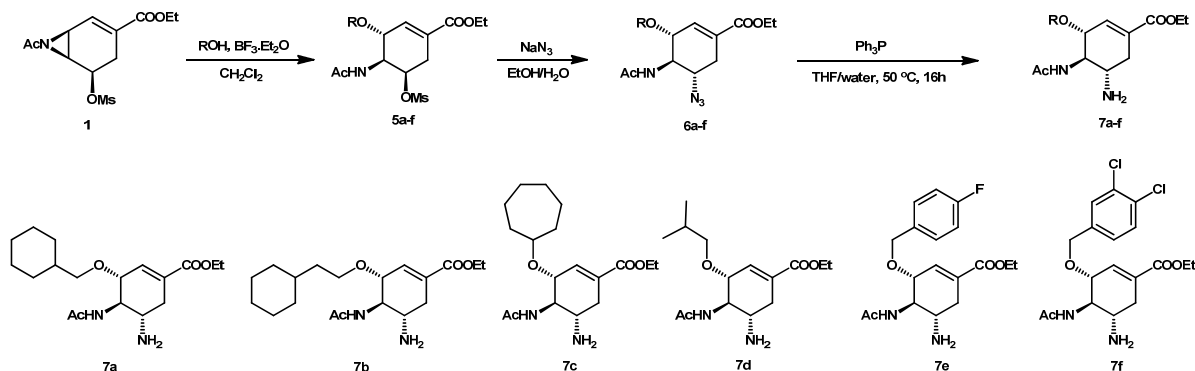
*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(ethylthio)cyclohex-1-ene-1-carboxylate (3d)*: Color: White, amorphous solid. Yield: 38%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.22 (t,  $J = 7.2$  Hz, 3H,  $\text{CH}_3\text{-2}''$ ), 1.29 (t,  $J = 7.2$  Hz, 3H,  $\text{H-2}$ ), 2.06 (s, 3H, Ac), 2.23 (m, 1H,  $\text{H}_a\text{-6}$ ), 2.53 (m, 2H,  $\text{CH}_2\text{-1}''$ ), 2.87 (dd,  $J = 5.0, 17.5$  Hz, 1H,  $\text{H}_b\text{-6}$ ), 3.67 (m, 1H,  $\text{H-4}$ ), 3.69 (m, 1H,  $\text{H-3}$ ), 3.95 (ddd,  $J = 5.0, 9.5, 9.5$  Hz, 1H,  $\text{H-5}$ ), 4.20 (q,  $J = 7.2$  Hz, 2H,  $\text{CH}_2\text{-1}'$ ), 6.16 (br. s, 1H, NH), 6.88 (br. s, 1H,  $\text{H-2}$ ).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.1, 14.6, 23.4, 24.1, 29.8, 45.5, 55.2, 58.9, 61.0, 127.9, 139.1, 165.5, 171.1.

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(benzylthio)cyclohex-1-ene-1-carboxylate (3e)*: Color: Yellow, amorphous solid. Yield: 39%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.29 (t,  $J = 7.2$  Hz, 3H,  $\text{CH}_3\text{-2}'$ ), 2.00 (s, 3H, Ac), 2.20 (m, 1H,  $\text{H}_a\text{-6}$ ), 2.83 (dd,  $J = 5.0, 17.5$  Hz, 1H,  $\text{H}_b\text{-6}$ ), 3.62 (m, 1H,  $\text{H-3}$ ), 3.68 (m, 1H,  $\text{H-4}$ ), 3.74 (d,  $J = 13.0$  Hz, 1H,  $\text{H}_a\text{-1}''$ ), 3.80 (d,  $J = 13.0$  Hz, 1H,  $\text{H}_b\text{-1}''$ ), 3.91 (ddd,  $J = 4.5, 10.0, 10.0$  Hz, 1H,  $\text{H-5}$ ), 4.17 (q,  $J = 7.2$  Hz, 2H,  $\text{CH}_2\text{-1}'$ ), 5.56 (d,  $J = 7.0$  Hz, 1H, NH), 6.78 (br. s, 1H,  $\text{H-2}$ ), 7.22-7.31 (m, 5H, Ar-H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.1, 23.5, 29.7, 34.9, 45.4, 54.9, 58.7, 61.0, 127.4, 127.8, 128.6, 129.0, 137.6, 138.6, 165.4, 170.8.

### 2.2.3. General procedure for the synthesis of compound 4a-e

To a mixture of compounds **3a-e** (0.1 mmol) in methanol (2 mL) was added Lindlar's catalyst (10 mg). The reaction mixture was stirred under hydrogen atmosphere for 5 h. The solids were filtered off and washed with methanol. The filtrate was concentrated to dryness. The residue was purified by silica gel column chromatography eluted with  $\text{CH}_2\text{Cl}_2\text{:MeOH}$  (20:1, v:v) to provide compounds **4a-e** (Scheme 1).

*(3R,4R,5S)-ethyl 4-acetamido-5-amino-3-(butylthio)cyclohex-1-enecarboxylate (4a)*: Color: White, amorphous solid. Yield: 82%.  $[\alpha]_{\text{D}}^{25}$ : -30.8 (c, 0.15,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.88 (t,  $J = 7.5$  Hz, 3H,  $\text{CH}_3\text{-4}''$ ), 1.28 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3\text{-2}'$ ), 1.38 (m, 2H,  $\text{CH}_2\text{-3}''$ ), 1.52 (m, 2H,  $\text{CH}_2\text{-2}''$ ), 2.08 (s, 3H, Ac), 2.24 (m, 1H,  $\text{H}_a\text{-6}$ ), 2.46 (m, 1H,  $\text{H}_a\text{-1}''$ ), 2.54 (m, 1H,  $\text{H}_b\text{-1}''$ ), 2.88 (m, 1H,  $\text{H}_b\text{-6}$ ), 3.20 (m, 1H,  $\text{H-5}$ ), 3.47 (br. d,  $J = 9.7$  Hz, 1H,  $\text{H-3}$ ), 3.81 (q,  $J = 9.7$  Hz, 1H,  $\text{H-4}$ ), 4.17 (q,  $J = 7.0$  Hz, 2H,  $\text{CH}_2\text{-1}'$ ), 6.53 (br. s, 1H, NH), 6.86 (br. s, 1H,  $\text{H-2}$ ).



Scheme 2

$^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 13.6, 14.2, 21.9, 23.6, 29.4, 31.5, 31.9, 46.6, 50.8, 55.2, 60.9, 128.9, 139.3, 165.9, 171.9.

MS (ESI,  $m/z$ ): 315  $[\text{M}+\text{H}]^+$ .

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(phenylthio)cyclohex-1-ene-1-carboxylate (4b)*: Color: White, amorphous solid. Yield: 85%.  $[\alpha]_D^{25}$ : -9.2 (c 0.17,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.26 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 2.00 (s, 3H, Ac), 2.03 (m, 1H,  $\text{H}_a$ -6); 2.80 (dd,  $J = 4.5, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 3.17 (ddd,  $J = 4.5, 10.2, 10.2$  Hz, 1H, H-5), 3.75 (q,  $J = 10.2$  Hz, 1H, H-4), 3.90 (br. d,  $J = 10.5$  Hz, 1H, H-3), 4.16 (m, 2H,  $\text{CH}_2$ -1'), 6.27 (d,  $J = 10.2$  Hz, 1H, NH), 6.89 (br. s, 1H, H-2), 7.27-7.33 (m, 3ArH, H-3'', H-4'' and H-5''), 7.46 (dd,  $J = 1.5, 8.5$  Hz, 2ArH, H-2'' and H-6'').  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.2, 23.6, 32.1, 50.2, 50.9, 56.4, 60.9, 128.3, 129.0, 129.3, 132.0, 134.0, 138.1, 165.8, 171.6. MS (ESI,  $m/z$ ): 335  $[\text{M}+\text{H}]^+$ .

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(o-tolylthio)cyclohex-1-ene-1-carboxylate (4c)*: Color: Yellow, amorphous solid. Yield: 83%.  $[\alpha]_D^{25}$ : -42.9 (c 0.14,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.26 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.96 (s, 3H, Ac), 2.15 (m, 1H,  $\text{H}_a$ -6), 2.43 (s, 3H,  $\text{CH}_3$ -7''), 2.82 (dd,  $J = 4.5$  and 17.5 Hz, 1H,  $\text{H}_b$ -6), 3.14 (ddd,  $J = 4.5, 9.5$  and 9.5 Hz, 1H, H-5), 3.80 (q,  $J = 9.5$  Hz, 1H, H-4), 3.93 (br. d,  $J = 9.5$  Hz, 1H, H-3), 4.15 (m, 2H,  $\text{CH}_2$ -1'), 6.07 (br. s, 1H, NH), 6.82 (br. s, 1H, H-2), 7.15 (dt,  $J = 1.5, 8.0$  Hz, 1H, Ar-H-4''), 7.18 (dt,  $J = 1.5, 8.0$  Hz, 1H, Ar-H-5''), 7.21 (dd,  $J = 1.5, 8.0$  Hz, 1H, Ar-H-3''), 7.45 (dd,  $J = 1.5, 8.0$  Hz, 1H, Ar-H-6'').  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.1, 20.9, 23.5, 32.5, 49.6, 50.9, 57.4, 60.9, 126.6, 128.0, 129.0, 130.7, 132.7, 133.2, 137.4, 140.6, 166.0, 171.4. MS (ESI,  $m/z$ ): 349  $[\text{M}+\text{H}]^+$ .

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(ethylthio)cyclohex-1-ene-1-carboxylate (4d)*: Color: Yellow, amorphous solid. Yield: 82%.  $[\alpha]_D^{25}$ : -52.4 (c 0.55,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.22 (t,  $J = 7.5$  Hz, 3H,  $\text{CH}_3$ -2''), 1.29 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 2.09 (s, 3H, Ac), 2.24 (m, 1H,  $\text{H}_a$ -6), 2.50 (m, 1H,  $\text{H}_b$ -1''), 2.58 (m, 1H,  $\text{H}_b$ -1''), 2.85 (m, 1H,  $\text{H}_b$ -6), 3.19 (ddd,  $J = 5.0, 10.0, 10.0$  Hz, 1H, H-5), 3.47 (br. s,  $J = 10.0$  Hz, 1H, H-3), 3.83 (q,  $J = 10.0$  Hz, 1H, H-4), 4.20 (q,  $J = 7.0$  Hz, 2H,  $\text{CH}_2$ -1'), 6.40 (d,  $J = 10.0$  Hz, 1H, NH), 6.88 (br. s, 1H, H-2).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.2, 14.6, 23.6, 23.7, 32.0, 46.6, 50.9, 55.3, 60.9, 128.9, 139.2, 165.9, 171.9. MS (ESI,  $m/z$ ): 287  $[\text{M}+\text{H}]^+$ .

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(benzylthio)cyclohex-1-ene-1-carboxylate (4e)*: Color: White, amorphous solid. Yield: 83%.  $[\alpha]_D^{25}$ : -113.1 (c 0.53,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.27 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 2.05 (s, 3H, Ac), 2.21 (m, 1H,  $\text{H}_a$ -6), 2.84 (dd,  $J = 4.5, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 3.11 (ddd,  $J = 4.5, 10.0, 10.0$  Hz, 1H, H-5), 3.36 (br. d,  $J = 10.0$  Hz, 1H, H-3), 3.72 (d,  $J = 13.0$  Hz, 1H,  $\text{H}_a$ -1''), 3.78 (d,  $J = 13.0$  Hz, 1H,  $\text{H}_b$ -1''), 3.90 (q,  $J = 10.0$  Hz, 1H, H-4), 4.14 (m, 2H,  $\text{CH}_2$ -1'), 6.10 (br. s, 1H, NH), 6.76 (br. s, 1H, H-2), 7.21-7.31 (m, 5H, Ar-H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.1, 23.6, 32.0, 34.5, 46.7, 51.0,

54.9, 60.9, 127.2, 128.5, 128.7, 129.1, 137.7, 138.8, 165.8, 171.8. MS (ESI,  $m/z$ ): 349  $[\text{M}+\text{H}]^+$ .

#### 2.2.4. General procedure for compounds 5a-f

To a solution of compound **1** (0.6 mmol) and alcohols (4 eq.) in anhydrous  $\text{CH}_2\text{Cl}_2$  (0.5 mL) was added borontrifluoride diethyl etherate (1.5 eq) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) at  $-8^\circ\text{C}$ . The reaction mixture was stirred for 1 h. Aqueous solution of potassium carbonate 5% (15 mL) was added and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3  $\times$  15 mL). The organic layers were combined, washed with water (5 mL) and concentrated under reduced pressure. The residue was chromatographed (*n*-hexane:EtOAc gradient) to obtain compounds **5a-f** (Scheme 2).

*Ethyl (3R,4S,5R)-4-acetamido-3-(cyclohexylmethoxy)-5-((methylsulfonyl)oxy)cyclohex-1-ene-1-carboxylate (5a)*: Color: White, amorphous solid. Yield: 55%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.91-0.98 (m, 2H, cyclohexane), 1.15-1.30 (m, 3H, cyclohexane), 1.33 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.54-1.79 (m, 6H, cyclohexane), 2.05 (s, 3H, Ac), 2.75-2.87 (m, 2H,  $\text{CH}_2$ -6), 3.06 (s, 3H, mesyl), 3.26 (dd,  $J = 6.5, 8.5$  Hz, 1H,  $\text{H}_a$ -1''), 3.47 (dd,  $J = 6.5, 8.5$  Hz, 1H,  $\text{H}_b$ -2''), 4.11 (dd,  $J = 1.5, 9.0$  Hz, 1H, H-3), 4.23 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ -1'), 4.33 (ddd,  $J = 2.0, 8.0, 9.0$  Hz, 1H, H-4), 5.29 (m, 1H, H-5), 5.85 (d,  $J = 8.0$  Hz, 1H, NH), 6.96 (br. s, 1H, H-2).

*Ethyl (3R,4S,5R)-4-acetamido-3-(2-cyclohexylethoxy)-5-((methylsulfonyl)oxy)cyclohex-1-ene-1-carboxylate (5b)*: Color: White, amorphous solid. Yield: 48%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.86-0.93 (m, 2H, cyclohexane), 1.12-1.29 (m, 3H, cyclohexane), 1.31 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.36-1.69 (m, 8H, cyclohexane and  $\text{CH}_2$ -2''), 2.03 (s, 3H, Ac), 2.73-2.84 (m, 2H,  $\text{CH}_2$ -6), 3.03 (s, 3H, mesyl), 3.48 (m, 1H,  $\text{H}_a$ -1''), 3.66 (m, 1H,  $\text{H}_b$ -1''), 4.11 (dd,  $J = 1.8, 9.0$  Hz, 1H, H-3), 4.22 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ -1'), 4.30 (ddd,  $J = 2.0, 7.5, 9.0$  Hz, 1H, H-4), 5.28 (m, 1H, H-5), 5.84 (d,  $J = 7.5$  Hz, 1H, NH), 6.94 (s, 1H, H-2).

*Ethyl (3R,4S,5R)-4-acetamido-3-(cycloheptyloxy)-5-((methylsulfonyl)oxy)cyclohex-1-ene-1-carboxylate (5c)*: Color: White, Amorphous solid. Yield: 52%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.30 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.32-1.39 (m, 2H, cycloheptane), 1.49-1.56 (m, 4H, cycloheptane), 1.58-1.65 (m, 6H, cycloheptane), 2.02 (s, 3H, Ac), 2.69-2.74 (m, 1H,  $\text{H}_a$ -6), 2.80-2.85 (m, 1H,  $\text{H}_b$ -6), 3.04 (s, 3H, mesyl), 3.65 (m, 1H, H-1''), 4.07 (dd,  $J = 1.5, 8.5$  Hz, 1H, H-3), 4.21 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ -1'), 4.26 (m, 1H, H-4), 5.22 (m, 1H, H-5), 5.89 (br. s, 1H, NH), 6.96 (br. s, 1H, H-2).

*Ethyl (3R,4S,5R)-4-acetamido-3-isobutoxy-5-((methylsulfonyl)oxy)cyclohex-1-ene-1-carboxylate (5d)*: Color: White, amorphous solid. Yield: 60%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.90 (t,  $J = 6.5$  Hz, 6H,  $\text{CH}_3$ -3'' and  $\text{CH}_3$ -4''), 1.31 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.83 (m, 1H, H-2''), 2.03 (s, 3H, Ac), 2.73-2.85 (m, 2H,  $\text{CH}_2$ -6), 3.04 (s, 3H, mesyl), 3.19 (dd,  $J = 6.5, 8.5$  Hz, 1H,  $\text{H}_a$ -1''), 3.43 (dd,  $J = 6.5, 8.5$  Hz, 1H,  $\text{H}_b$ -1''), 4.11 (dd,  $J = 2.0, 9.5$  Hz, 1H, H-3), 4.23 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ -1'), 4.32 (m, 1H, H-4),

5.27 (m, 1H, H-5), 5.85 (d,  $J = 8.0$  Hz, 1H, NH), 6.94 (br. s, 1H, H-2).

**Ethyl (3R,4S,5R)-4-acetamido-3-(4-fluorophenoxy)-5-((methylsulfonyl)oxy)cyclohex-1-ene-1-carboxylate (5e):** Color: White, amorphous solid. Yield: 75%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.31 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 1.99 (s, 3H, Ac), 2.78-2.83 (m, 2H,  $\text{CH}_2$ -6), 3.02 (s, 3H, mesyl), 4.19-4.25 (m, 3H,  $\text{CH}_2$ -1' and H-3), 4.38 (ddd,  $J = 2.0, 8.5, 10.5$  Hz, 1H, H-4), 4.52 (d,  $J = 11.5$  Hz, 1H,  $\text{H}_a$ -1''), 4.67 (d,  $J = 11.5$  Hz, 1H,  $\text{H}_b$ -1''), 5.25 (m, 1H, H-5), 5.80 (d,  $J = 8.5$  Hz, 1H, NH), 6.95 (br. s, 1H, H-2), 7.04 (t,  $J = 8.5$  Hz, 2H, Ar-H-4'' and H-6''), 7.32 (dd,  $J = 5.5, 8.5$  Hz, 2H, Ar-H-3'' and H-7'').

**Ethyl (3R,4S,5R)-4-acetamido-3-(3,4-dichlorophenoxy)-5-((methylsulfonyl)oxy)cyclohex-1-ene-1-carboxylate (5f):** Color: White, amorphous solid. Yield: 45%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.31 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 2.02 (s, 3H, Ac), 2.76-2.83 (m, 2H,  $\text{CH}_2$ -6), 3.04 (s, 3H, mesyl), 4.21-4.25 (m, 3H,  $\text{CH}_2$ -1' and H-3), 4.42 (ddd,  $J = 2.5, 8.0, 10.0$  Hz, 1H, H-4), 4.50 (d,  $J = 12.0$  Hz, 1H,  $\text{H}_a$ -1''), 4.65 (d,  $J = 12.0$  Hz, 1H,  $\text{H}_b$ -1''), 5.23 (m, 1H, H-5), 5.99 (d,  $J = 8.0$  Hz, 1H, NH), 6.93 (br. s, 1H, H-2), 7.15 (dd,  $J = 2.0, 8.5$  Hz, 1H, Ar-H-7''), 7.41 (d,  $J = 8.5$  Hz, 1H, Ar-H-6''), 7.43 (d,  $J = 2.0$  Hz, 1H, Ar-H-3'').

### 2.2.5. General procedure for compounds 6a-f

To a solution of compounds **5a-f** (0.4 mmol) in a mixture of ethanol:H<sub>2</sub>O (5:1, v:v, 5 mL) was added sodium azide (3 eq.). The reaction solution was stirred for 8 h at reflux. The mixture was cooled to room temperature and water (15 mL) was added, then extracted with EtOAc (3 × 15 mL). The EtOAc solution was dried over sodium sulphate, concentrated under reduced pressure and the residue was purified by silica gel column chromatography (*n*-hexane:EtOAc gradient) to give compounds **6a-f** (Scheme 2).

**Ethyl (3R,4R,5S)-4-acetamido-5-azido-3-(cyclohexylmethoxy)cyclohex-1-ene-1-carboxylate (6a):** Color: White, amorphous solid. Yield: 71%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.90-0.97 (m, 2H, cyclohexane), 1.13-1.25 (m, 3H, cyclohexane), 1.31 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.54-1.76 (m, 6H, cyclohexane), 2.04 (s, 3H, Ac), 2.26 (m, 1H,  $\text{H}_a$ -6), 2.86 (dd,  $J = 5.0, 17.0$  Hz, 1H,  $\text{H}_b$ -6), 3.24 (dd,  $J = 6.5, 8.5$  Hz, 1H,  $\text{H}_a$ -1''), 3.45 (dd,  $J = 6.5, 8.5$  Hz, 1H,  $\text{H}_b$ -1''), 3.54 (m, 1H, H-3), 4.12 (m, 1H, H-4), 4.22 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ -1'), 4.43 (m, 1H, H-5), 5.61 (d,  $J = 8.0$  Hz, 1H, NH), 6.84 (s, 1H, H-2).

**Ethyl (3R,4R,5S)-4-acetamido-5-azido-3-(2-cyclohexylethoxy)cyclohex-1-ene-1-carboxylate (6b):** Color: Yellow, amorphous solid. Yield: 88%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.86-0.93 (m, 2H, cyclohexane), 1.12-1.27 (m, 3H, cyclohexane), 1.30 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.34-1.69 (m, 8H, cyclohexane and  $\text{CH}_2$ -2''), 2.05 (s, 3H, Ac), 2.26 (m, 1H,  $\text{H}_a$ -6), 2.86 (dd,  $J = 5.5, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 3.46-3.54 (m, 2H,  $\text{CH}_2$ -1''), 3.67 (m, 1H, H-3), 4.14 (m, 1H, H-4), 4.21 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ -1'), 4.45 (m, 1H, H-5), 5.67 (d,  $J = 8.0$  Hz, 1H, NH), 6.84 (br. s, 1H, H-2).

**Ethyl (3R,4R,5S)-4-acetamido-5-azido-3-(cycloheptyloxy)cyclohex-1-ene-1-carboxylate (6c):** Color: White, amorphous solid. Yield: 53%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.30 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.31-1.41 (m, 2H, cycloheptane), 1.49-1.66 (m, 8H, cycloheptane), 1.79-1.90 (m, 2H, cycloheptane), 2.03 (s, 3H, Ac), 2.22 (m, 1H,  $\text{H}_a$ -6), 2.85 (dd,  $J = 5.7, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 3.26 (m, 1H, H-3), 3.62 (m, 1H, H-1''), 4.21 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ -1'), 4.32 (m, 1H, H-4), 4.56 (m, 1H, H-5), 5.71 (d,  $J = 7.0$  Hz, 1H, NH), 6.77 (s, 1H, H-2).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.1, 22.5, 23.2, 23.9, 28.2, 29.8, 34.2, 34.9, 38.5, 51.6, 61.1, 72.2, 78.0, 80.0, 127.4, 137.0, 165.7, 170.6.

**Ethyl (3R,4R,5S)-4-acetamido-5-azido-3-isobutoxycyclohex-1-ene-1-carboxylate (6d):** Color: Yellow, amorphous solid. Yield: 57%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.88 (d,  $J = 6.5$  Hz, 3H,  $\text{CH}_3$ -3''), 0.89 (d,  $J = 6.5$  Hz, 3H,  $\text{CH}_3$ -4''), 1.29 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ -2'), 1.79 (m, 1H, H-2''), 2.03 (s, 3H, Ac), 2.25 (m, 1H,  $\text{H}_a$ -6), 2.85 (dd,  $J = 5.5, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 3.17 (dd,  $J = 6.5, 9.0$  Hz, 1H,

$\text{H}_a$ -1''), 3.42 (dd,  $J = 6.5, 9.0$  Hz, 1H,  $\text{H}_b$ -1''), 3.60 (m, 1H, H-3), 4.05 (m, 1H, H-4), 4.20 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ -1'), 4.39 (m, 1H, H-5), 5.93 (d,  $J = 8.0$  Hz, 1H, NH), 6.83 (t,  $J = 2.0$  Hz, 1H, H-2).

**Ethyl (3R,4R,5S)-4-acetamido-5-azido-3-(4-fluorophenoxy)cyclohex-1-ene-1-carboxylate (6e):** Color: Yellow, amorphous solid. Yield: 56%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.30 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 1.99 (s, 3H, Ac), 2.28 (m, 1H,  $\text{H}_a$ -6), 2.88 (dd,  $J = 5.5, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 3.62 (m, 1H, H-3), 4.08 (m, 1H, H-4), 4.22 (q,  $J = 7.0$  Hz, 2H,  $\text{CH}_2$ -1'), 4.49 (d,  $J = 11.5$  Hz, 1H,  $\text{H}_a$ -1''), 4.54 (m, 1H, H-5), 4.67 (d,  $J = 11.5$  Hz, 1H,  $\text{H}_b$ -1''), 5.60 (d,  $J = 7.5$  Hz, 1H, NH), 6.86 (d,  $J = 2.0$  Hz, 1H, H-2), 7.02 (t,  $J = 8.5$  Hz, 2H, Ar-H-4'' and H-6''), 7.29 (dd,  $J = 5.5, 8.5$  Hz, 2H, Ar-H-3'' and H-7'').

**Ethyl (3R,4R,5S)-4-acetamido-5-azido-3-(3,4-dichlorophenoxy)cyclohex-1-ene-1-carboxylate (6f):** Color: Yellow, amorphous solid. Yield: 47%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.31 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 2.02 (s, 3H, Ac), 2.30 (m, 1H,  $\text{H}_a$ -6), 2.90 (dd,  $J = 5.5, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 3.62 (m, 1H, H-3), 4.10 (m, 1H, H-4), 4.22 (q,  $J = 7.0$  Hz, 2H,  $\text{CH}_2$ -1'), 4.48 (d,  $J = 12.0$  Hz, 1H,  $\text{H}_a$ -1''), 4.56 (m, 1H, H-5), 4.67 (d,  $J = 12.0$  Hz, 1H,  $\text{H}_b$ -1''), 5.60 (d,  $J = 8.0$  Hz, 1H, NH), 6.86 (t,  $J = 2.0$  Hz, 1H, H-2), 7.15 (dd,  $J = 1.7, 8.2$  Hz, 1H, Ar-H-7''), 7.42 (d,  $J = 8.2$  Hz, 1H, Ar-H-6''), 7.44 (d,  $J = 1.7$  Hz, 1H, Ar-H-3'').

### 2.2.6. General procedure for synthesis of compound 7a-f

A mixture of compounds **6a-f** (0.1 mmol) and  $\text{Ph}_3\text{P}$  (2 eq.) in THF:H<sub>2</sub>O (10:1, v:v) was heated at 50 °C for 16 h. The mixture solution was concentrated to dryness and the residue was purified by column chromatography on silica gel, eluted with *n*-hexane:EtOAc gradient, affording compounds **7a-f** (Scheme 2).

**Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(cyclohexylmethoxy)cyclohex-1-ene-1-carboxylate (7a):** Color: Yellow, amorphous solid. Yield: 89%.  $[\alpha]_{\text{D}}^{25}$ : -74.8 (c 1.4,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.78-0.85 (m, 2H, cyclohexane), 1.03-1.14 (m, 3H, cyclohexane), 1.20 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 1.39-1.45 (m, 1H, cyclohexane), 1.66 (m, 5H, cyclohexane), 1.93 (s, 3H, Ac), 2.05 (m, 1H,  $\text{H}_a$ -6), 2.67 (dd,  $J = 5.0, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 2.83 (m, 1H, H-5), 3.09 (dd,  $J = 6.5, 8.5$  Hz, 1H,  $\text{H}_a$ -1''), 3.33 (dd,  $J = 6.5, 8.5$  Hz, 1H,  $\text{H}_b$ -1''), 3.67 (m, 1H, H-4), 3.95 (m, 1H, H-3), 4.11 (q,  $J = 7.0$  Hz, 2H,  $\text{CH}_2$ -1'), 6.23 (br. s, 1H, NH), 6.71 (br. s, 1H, H-2). MS (ESI,  $m/z$ ): 339 [M+H]<sup>+</sup>.

**Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(2-cyclohexylethoxy)cyclohex-1-ene-1-carboxylate (7b):** Color: Yellow, amorphous solid. Yield: 92%.  $[\alpha]_{\text{D}}^{25}$ : -26.4 (c 0.83,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 0.80-0.87 (m, 2H, cyclohexane), 1.07-1.20 (m, 3H, cyclohexane), 1.35 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 1.30-1.36 (m, 2H,  $\text{CH}_2$ -2''), 1.37 (m, 1H, cyclohexane), 1.57-1.64 (m, 5H, cyclohexane), 1.99 (s, 3H, Ac), 2.09 (m, 1H,  $\text{H}_a$ -6), 2.21 (br. s, 2H, NH<sub>2</sub>), 2.74 (dd,  $J = 5.0, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 2.99 (m, 1H, H-5), 3.39 (m, 1H,  $\text{H}_a$ -1''), 3.58 (m, 1H,  $\text{H}_b$ -1''), 3.70 (m, 1H, H-4), 4.05 (m, 1H, H-3), 4.15 (q,  $J = 7.0$  Hz, 2H,  $\text{CH}_2$ -1'), 6.20 (br. s, 1H, NH), 6.76 (br. s, 1H, H-2).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 13.9, 22.7, 25.6, 25.6, 26.3, 29.6, 29.7, 33.0, 38.0, 49.2, 55.8, 60.9, 74.5, 77.1, 129.7, 137.1, 166.1, 172.2. MS (ESI,  $m/z$ ): 353 [M+H]<sup>+</sup>.

**Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(cycloheptyloxy)cyclohex-1-ene-1-carboxylate (7c):** Color: Yellow, amorphous solid. Yield: 93%.  $[\alpha]_{\text{D}}^{25}$ : -88.8 (c 1.25,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 1.26 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ -2'), 1.32 (m, 2H, cycloheptane), 1.49-1.59 (m, 8H, cycloheptane), 1.76-1.92 (m, 2H, cycloheptane), 2.00 (s, 3H, Ac), 2.10 (m, 1H,  $\text{H}_a$ -6), 2.71 (dd,  $J = 5.0, 17.5$  Hz, 1H,  $\text{H}_b$ -6), 3.16 (m, 1H, H-1''), 3.48 (m, 1H, H-5), 3.58 (m, 1H, H-3), 4.17-4.19 (m, 3H, H-4 and  $\text{CH}_2$ -1'), 6.02 (br. s, 1H, NH), 6.72 (br. s, 1H, H-2).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm): 14.1, 22.6, 22.6, 23.5, 28.3, 28.3, 33.4, 33.9, 34.9, 35.0, 49.0, 60.8, 74.6, 79.4, 129.4, 137.6, 166.2, 172.3. MS (ESI,  $m/z$ ): 339 [M+H]<sup>+</sup>.

**Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-isobutoxycyclohex-1-ene-1-carboxylate (7d):** Color: Yellow, amorphous solid. Yield: 89%.  $[\alpha]_{\text{D}}^{25}$ : -4.8 (c 0.67,  $\text{CHCl}_3$ ).

**Table 1.** Cytotoxic activity of compounds **4a-f** and **7a-f**.

Compounds	IC <sub>50</sub> (µg/mL)			Compounds	IC <sub>50</sub> (µg/mL)		
	KB	MCF-7	LU-1		KB	MCF-7	LU-1
4a	72.0	38.2	40.1	7c	98.7	97.6	99.8
4b	9.6	8.0	8.8	7d	>100	>100	>100
4c	39.7	42.9	45.0	7e	84.6	72.7	74.1
4d	>100	>100	>100	7f	15.7	15.0	15.3
4e	79.7	62.5	82.9	Ellipticine	1.1	0.9	0.9
7a	84.9	80.0	79.7	Oseltamivir phosphate	>100	>100	>100
7b	27.6	24.1	25.8				

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ, ppm): 0.84 (d, J = 6.5 Hz, 3H, CH<sub>3</sub>-3''), 0.88 (d, J = 6.5 Hz, 3H, CH<sub>3</sub>-4''), 1.27 (t, J = 7.0 Hz, 3H, CH<sub>3</sub>-2'), 1.77 (m, 1H, H-2''), 2.01 (s, 3H, Ac), 2.12 (m, 1H, H<sub>a</sub>-6), 2.75 (dd, J = 5.0, 17.5 Hz, 1H, H<sub>b</sub>-6), 2.99 (m, 1H, H-5), 3.10 (dd, J = 8.0, 15.0 Hz, 1H, H<sub>a</sub>-1''), 3.36 (dd, J = 8.0, 15.0 Hz, 1H, H<sub>b</sub>-1''), 3.74 (q, J = 9.0 Hz, 1H, H-4), 4.06 (m, 1H, H-3), 4.17 (q, J = 6.5 Hz, 2H, CH<sub>2</sub>-1'), 6.03 (br. s, 1H, NH), 6.79 (br. s, 1H, H-2). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, δ, ppm): 14.0, 19.0, 19.2, 23.0, 28.6, 33.3, 49.2, 55.9, 60.9, 75.2, 80.8, 129.9, 137.1, 166.1, 172.3. MS (ESI, m/z): 298 [M+H]<sup>+</sup>.

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(4-fluorophenoxy)cyclohex-1-ene-1-carboxylate (7e)*: Color: Yellow, amorphous solid. Yield: 88%. [α]<sub>D</sub><sup>25</sup>: -38.75 (c 0.5, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ, ppm): 1.16 (t, J = 7.0 Hz, 3H, CH<sub>3</sub>-2'), 1.86 (s, 3H, Ac), 2.03 (m, 1H, H<sub>a</sub>-6), 2.65 (dd, J = 5.0, 17.5 Hz, 1H, H<sub>b</sub>-6), 2.75 (m, 1H, H-5), 3.71 (q, J = 9.0 Hz, 1H, H-4), 4.03 (m, 1H, H-3), 4.08 (q, J = 7.0 Hz, 2H, CH<sub>2</sub>-1'), 4.34 (d, J = 11.5 Hz, 1H, H<sub>a</sub>-1''), 4.50 (d, J = 11.5 Hz, 1H, H<sub>b</sub>-1''), 6.63 (br. s, 1H, NH), 6.72 (br. s, 1H, H-2), 6.89 (t, J = 8.5 Hz, 2H, Ar-H-4'' and H-6''), 7.17 (dd, J = 5.5, 8.5 Hz, 2H, Ar-H-3'' and H-7''). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, δ, ppm): 14.1, 23.5, 33.8, 49.8, 56.7, 60.9, 69.4, 76.4, 115.2, 115.3, 129.7, 129.8, 130.9, 133.6, 136.1, 161.4 and 163.3 (C-F), 165.9, 171.0. MS (ESI, m/z): 351.0 [M+H]<sup>+</sup>.

*Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(3,4-dichlorophenoxy)cyclohex-1-ene-1-carboxylate (7f)*: Color: White, amorphous solid. Yield: 68%. [α]<sub>D</sub><sup>25</sup>: -50.8 (c 1.0, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ, ppm): 1.30 (t, J = 7 Hz, 3H, CH<sub>3</sub>-2'), 2.04 (s, 3H, Ac), 2.18 (m, 1H, H<sub>a</sub>-6), 2.81 (dd, J = 5.0, 17.5 Hz, 1H, H<sub>b</sub>-6), 3.04 (m, 1H, H-5), 3.85 (q, J = 9.0 Hz, 1H, H-4), 4.22 (m, 3H, CH<sub>2</sub>-1' and H-3), 4.44 (d, J = 12.0 Hz, 1H, H<sub>a</sub>-1''), 4.65 (d, J = 12.0 Hz, 1H, H<sub>b</sub>-1''), 5.41 (d, J = 9.0 Hz, 1H, NH), 6.85 (br. s, 1H, H-2), 7.15 (dd, J = 1.5, 8.0 Hz, 1H, Ar-H-7''), 7.41 (d, J = 8.0 Hz, 1H, Ar-H-6''), 7.45 (d, J = 1.5 Hz, 1H, Ar-H-3''). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, δ, ppm): 14.1, 23.5, 33.9, 49.7, 56.3, 61.0, 68.5, 76.8, 126.9, 129.6, 130.3, 131.2, 131.7, 132.5, 135.6, 138.2, 165.9, 171.0. MS (ESI, m/z): 401 [M+H]<sup>+</sup>.

### 2.3. Cytotoxicity assay

The cancer cells (KB, MCF-7 and LU-1) were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL), streptomycin (100 µg/mL) and gentamicin (10 µg/mL). Stock solutions of compounds were prepared in DMSO/H<sub>2</sub>O (1/9), and cytotoxicity assays were carried out in 96-well microtiter plates against KB, MCF-7 and LU-1 cancer cell lines (3 × 10<sup>3</sup> cells/mL) using a modification of the published method [18]. After 72 h incubation at 37 °C in air/CO<sub>2</sub> (95:5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC<sub>50</sub> value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control. Ellipticine was used as a reference compound.

### 3. Results and discussion

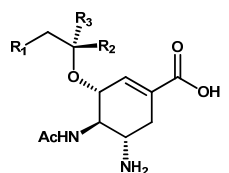
The synthesis of oseltamivir was previously described starting from shikimic acid, in which the synthesis via aziridine

intermediate was considered to be an effective method [19,20]. The approach in this work is preparation of ether sulfur or ether oxygen at C-3 position of oseltamivir analogues based on ring opening of aziridine compound by different thiols or alcohols. Accordingly, aziridine **1** was obtained from shikimic acid through five steps in high yields using previous reported methods [19,20].

Aziridines are highly strained, three-membered, nitrogen containing heterocycles that is easily opened by nucleophiles in order to relieve the strain. Yadav and co-workers demonstrated that the InCl<sub>3</sub> could promote the ring opening of aziridines by nucleophiles including thiols under mild reaction conditions with high regioselectivity [21]. Consequently, aziridine **1** was successfully reacted with different thiols (ethylthiol, *n*-butylthiol, benzylthiol, thiophenol and *o*-methyl thiophenol) in the presence of InCl<sub>3</sub> at room temperature to afford compounds **2a-e** in high yields (63-91%) with high stereoselectivity and regioselectivity. The next step was nucleophilic replacement of OM group at the C-5 position by the N<sub>3</sub> group. Treatment of compounds **2a-e** with NaN<sub>3</sub> afforded the intermediates **3a-e**. The inversion of configuration at C-5 was observed for compounds **3a-e** as indicated by the coupling constants of H-5 which, had two *anti* (J = 8.7-9.0 Hz) and a *gauche* (J = 0.0-1.0 Hz) coupling constants. Thus, a *trans* relationship between H-4 and H-5 was revealed for compounds **3a-e**. Finally, compounds **3a-e** were reduced by hydrogenation in the presence of Lindlar's catalyst to provide **4a-e**, respectively (Scheme 1) [19].

In the second approach, the analogues containing ether oxygen at C-3 were also prepared starting from **1** by aziridine ring opening with alcohols in the presence of BF<sub>3</sub>·Et<sub>2</sub>O to yield compounds **5a-f** which were then converted into the corresponding azide derivatives **6a-f**. The series of alkyl or aryl ethers at C-3 **7a-f** was obtained from the corresponding intermediates **6a-f** by reduction with Ph<sub>3</sub>P (Scheme 2) [22].

The synthetic compounds were evaluated for their cytotoxicity against three human cancer cell lines, KB (mouth epidermal carcinoma cells), LU-1 (human lung cancer cells) and MCF-7 (human breast cancer cells). Except for **4d**, **7c** and **7d**, the remaining compounds were found to be active against three tested cancer cell lines (Table 1). It was found that oseltamivir has no cytotoxicity against different cell lines such as MDCK, MRC-5, VERO, MK and 293 cells [23]. Also, when tested against KB, LU-1 and MCF-7 cell lines, oseltamivir phosphate had no inhibition even at the concentration of 100 µg/mL. Thus, the modification of alkyl groups at C-3 of the cyclohexene ring significantly increased the cytotoxicity for this class of compounds. In order to compare the neuraminidase inhibition activity with oseltamivir phosphate, the synthetic compounds **4a-e** and **7a-f** were also evaluated for their *in vitro* influenza A neuraminidase inhibitory activity by an enzymatic assay. However, these compounds had no inhibition activity even at the concentration of 50 µM. This observation suggested that the 3-*o*-pentyl group at C-3 of oseltamivir played an important role for the neuraminidase inhibitory activity of oseltamivir. This is in agreement with the previous reports on several oseltamivir analogues such as compounds **8-11** which are less active than oseltamivir (Figure 1) [24].



- 8: R<sub>1</sub> = COOH; R<sub>2</sub> = Et; R<sub>3</sub> = H  
 9: R<sub>1</sub> = COOH; R<sub>2</sub> = H; R<sub>3</sub> = Et  
 10: R<sub>1</sub> = CH<sub>2</sub>OH; R<sub>2</sub> = Et; R<sub>3</sub> = H  
 11: R<sub>1</sub> = CH<sub>2</sub>OH; R<sub>2</sub> = H; R<sub>3</sub> = Et

Figure 1. Compounds taken from the ref. [24].

#### 4. Conclusion

We have described the synthesis and biological evaluation of oseltamivir analogues containing either ether sulfur or ether oxygen at C-3 position for cytotoxic activity. Many of the synthetic derivatives exhibited cytotoxicity against the three tumor cell lines (KB, LU-1 and MCF-7). This is the first synthesis of oseltamivir analogues with ether sulfur at C-3.

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