Addition of bis(trimethylsilyl)ketene acetals to activated 2-(pyridin-3-yl)-1,3-benzothiazole: Synthesis and cytotoxic activity of novel carboxylic acids and δ-bromolactone derivatives


Abstract

The nucleophilic addition of the alkyl substituted bis(trimethylsilyl)ketene acetals to 2-(pyridin-3-yl)-1,3-benzothiazole in the presence of triflic anhydride has been examined. The behavior of the chosen substrate in these transformations is peculiar due to an exclusive activation of its pyridine fragment, resulting in the exclusive formation of 3-benzothiazolyl-1,4-dihydropyridine carboxylic acids, as confirmed by single crystal X-ray crystallography. These isolated acids have been efficiently turned into novel δ-bromolactones by the regioselective ring closing procedure promoted by NBS. In vitro cytotoxic activities of the synthetized carboxylic acids and lactones have shown moderate anticancer activity against six human tumor cell lines; the derivatives bearing a cyclobutyl group present the highest activity.

Keywords: Benzothiazole, ketene acetals, chemoselectivity, carboxylic acids, lactones, cytotoxic activity
Introduction

Nitrogen-containing heterocycles play an important role not only in the life sciences, but also in many industrial fields related to fine chemistry. Among nitrogen-containing heterocycles, benzothiazoles and pyridines stand out as two classes of bioactive and industrially important organic compounds. Molecules with a benzothiazole ring are attractive targets for synthesis since they often exhibit diverse and important biological properties. These compounds possess potential application as anticancer, antimicrobial, anti-convulsant, antiviral, anthelmintic, antifungal, analgesic, anti-inflammatory, antidiabetic, etc. Thereby, the benzothiazole ring system is considered as a privileged structure.

Likewise, pyridine derivatives have occupied a privileged position in the field of medicinal chemistry. The incorporation of the pyridine moiety into other organic structures is an important synthetic strategy in drug discovery. The high therapeutic properties of the pyridine-containing drugs have encouraged the organic chemists to synthesize a large number of novel pyridine compounds with important biologically active structures. Similarly, 1,4-dihydropyridine (1,4-DHP) nucleus is a privileged scaffold that, if appropriately substituted, exerts potent and selective action at diverse receptors, ion channels and enzymes. The ability of 1,4-DHP to revert multi drug resistance continues to be of great interest in medicinal chemistry.

Among the synthetic methods to obtain 1,4-DHPs, the addition of nucleophiles to N-activated pyridines stands out from others, as it allows access to derivatives with high regio- and stereoselectivity. In this context, our group have developed new methods based upon the inherent reactivity of bis(trimethylsilyl)ketene acetals to act as 1,3-carbon-oxygen dinucleophiles to allow access to novel carboxylic acid substituted 1,4-DHP and other dihydro(aza-aromatic) compounds using pyridine or other wide-ranging aza-aromatic substrates. These transformations occur by activation of nitrogen atoms via the formation of the corresponding salts with triflic anhydride or by interaction with alkyl chloroformates. The isolable carboxylic acid intermediates could be turned into novel functionalized γ- and δ-lactones through a subsequent lactonization step, and these often show a broad range of biological activities.

As was early reported by Ito, the addition of silyl enol ethers to N-alkoxycarbonyl quaternary salts of thiazole or benzothiazole in the presence of triethylamine affords the corresponding 2-substituted 4-thiazolines or 2,3-dihydrobenzothiazoles in good yields. Thus, one might expect that either the pyridine or the benzothiazole (or both) components of 2-(pyridin-3-yl)-1,3-benzothiazole would be involved in the reaction with bis(trimethylsilyl)ketene acetals to furnish novel carboxylic acid or lactone scaffolds which may exhibit interesting biological properties.

In continuation of our recent research, we report herein the synthesis of novel carboxylic acid substituted 1,4-dihydropyridines and bicyclic δ-lactones bearing a benzothiazole ring from activated 2-(pyridin-3-yl)-1,3-benzothiazole and different bis(trimethylsilyl)ketene acetals. The anticancer properties of the compounds were evaluated in vitro against a panel of human tumor cell lines.

Results and Discussion

Synthesis

Firstly, we were interested in evaluating the reactivity of N-triflyl quaternary salt of benzothiazole towards the nucleophilic attack of bis(trimethylsilyl)ketene acetals. When benzothiazole was treated with triflic anhydride during 30 min at -78 °C followed by the addition of bis(trimethylsilyl)ketene acetals, 2-substituted 2,3-dihydrobenzothiazoles were obtained in moderate to good yields (Table 1, entries 1-4).
Table 1. Synthesis of 2,3-dihydrobenzothiazoles 3a-e using different bis(trimethylsilyl)ketene acetals

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acetal</th>
<th>R1 + R2</th>
<th>Product</th>
<th>Yieldb %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2a</td>
<td>CH₃</td>
<td>3a</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>2b</td>
<td>-(CH₂)₃⁻</td>
<td>3b</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>2c</td>
<td>-(CH₂)₄⁻</td>
<td>3c</td>
<td>59</td>
</tr>
<tr>
<td>4</td>
<td>2d</td>
<td>-(CH₂)₅⁻</td>
<td>3d</td>
<td>65</td>
</tr>
</tbody>
</table>

a Reaction conditions: 1.0 mmol of benzothiazole 1, 1.1 mmol of Tf₂O, 1.2 mmol of bis(TMS)ketene acetal 2a–d and 10 mL of CH₂Cl₂. b Isolated yield after SiO₂ column chromatography.

Thus, we have demonstrated the viability of bis(trimethylsilyl)ketene acetals to act as nucleophiles in their reaction with N-activated benzothiazoles. Thereafter, we focus in the issue of competition between the benzothiazole and pyridine fragments to be activated by triflic anhydride by using 2-(pyridin-3-yl)-1,3-benzothiazole 4 as substrate. This issue was initially addressed by reacting triflic anhydride with 4 followed by addition of bis(trimethylsilyl)ketene acetal 2a. The precursor compound 4 was efficiently prepared by SiO₂-catalyzed condensation of 2-aminothiophenol and 3-pyridinecarboxaldehyde under microwave irradiation for 20 min in an air atmosphere. Based on our previous findings,21-25 when 1.1 equiv of Tf₂O was added to a solution of 4 in dry CH₂Cl₂ at -78 °C and subsequently treated with 1.2 equiv of ketene acetal 2a for 2 hours at room temperature, the corresponding 1,4-dihydropyridine 5a was obtained as white solid in 90% yield. Thus, selective activation of 4 took place exclusively at the pyridine fragment. Attempts to introduce an extra triflyl group at the benzothiazole moiety by increasing the amount of Tf₂O failed, even when the reaction time with triflic anhydride was increased probably due to the steric hindrance of the bulky triflyl groups.

Afterward, under conditions described earlier, the reaction was tested with other nucleophiles bearing different cycloalkyl substituents (Table 2). The reaction is successful for the four ketene acetals used and gives very good yields of the resulting carboxylic acids, after recrystallization from hexane / dichloromethane. Compounds 5a-d were characterized by various spectroscopic techniques. According to their physical data, the observed products result from regioselective nucleophilic addition of the ketene acetal to C-4 of the pyridine fragment. In the ¹H NMR spectrum of 5a, the evidence for the addition of ketene acetal 2a to the pyridine component was supported by signals appearing as doublets at 6.94 (H-13) and 4.50 ppm (H-15), as well as a doublet of doublets at 5.63 ppm (H-14) corresponding to the three vicinal protons of the 1,4-DHP moiety. The ¹³C NMR spectra confirmed the incorporation of a carboxylic acid by the signal appearing at 176.2 ppm and the introduction of a triflyl group by showing a quartet signal at 119.4 ppm. In addition, the structural arrangement of 5b was unequivocally established by X-ray diffraction analysis (Figure 1).
Table 2. Synthesis of 1,4-dihydropyridines 5a-d using different bis(trimethylsilyl)ketene acetals

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketene acetal</th>
<th>Product</th>
<th>Yield(^b) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2a</td>
<td>5a</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>2b</td>
<td>5b</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>2c</td>
<td>5c</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>2d</td>
<td>5d</td>
<td>79</td>
</tr>
</tbody>
</table>

\(^a\) Reaction conditions: 1.0 mmol of 2-(pyridin-3-yl)-1,3-benzothiazole 4, 1.1 mmol of \(\text{ Tf}_2\text{O}\), 1.2 mmol of bis(trimethylsilyl)ketene acetal 2a–d and 25 mL of \(\text{CH}_2\text{Cl}_2\). \(^b\) Isolated yield after purification by recrystallization from hexane/dichloromethane.
Figure 1. ORTEP view of compound 5b. Thermal ellipsoids are at 30% probability level.

We next turned our attention to the synthesis of novel substituted δ-lactones. Since carboxylic acid bearing dihydropyridines are known to produce lactones by using typical hydroxy-, halo- or protolactonization conditions,27 the carboxylic acids 5 were transformed to the novel bicyclic δ-lactones 6 under established bromolactonization conditions which involve the addition of 1.1 equiv. of NBS to a dichloromethane solution of the carboxylic acids 5a-d and 20 mol % of TBAB to give, after 2 h at 0 °C and silica gel purification, white solids in moderate to good yields (Table 3).

Table 3. Synthesis of δ-bromolactones 6a-c

<table>
<thead>
<tr>
<th>Entry</th>
<th>Carboxylic acid</th>
<th>R1 + R2</th>
<th>Product</th>
<th>Yieldb %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5a</td>
<td>-CH3</td>
<td>6a</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>5b</td>
<td>-(CH2)3-</td>
<td>6b</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>5c</td>
<td>-(CH2)4-</td>
<td>6c</td>
<td>79</td>
</tr>
<tr>
<td>4</td>
<td>5d</td>
<td>-(CH2)5-</td>
<td>6d</td>
<td>65</td>
</tr>
</tbody>
</table>

a Reaction conditions: 0.5 mmol of 5a-d, 0.55 mmol of NBS, 0.6 mmol NaHCO3, 0.1 mmol of TBAB and 20 mL of CH2Cl2. b Isolated yield after SiO2 column chromatography.

The observed products result from a regioselective ring-closing step as was confirmed by spectroscopic data of compounds 6a-d. For example, high resolution mass spectrometry (ESI-TOF) confirmed the expected mass m/z = 510.96142 ([M + 1]+) for 6a in accordance with the molecular formula. Characteristic signals of protons H-13 and H-15 of compound 6a were observed in 1H NMR as doublets at 6.94 and 4.50 ppm (J = 6.1 Hz) respectively, while proton H-14 appears as a doublet of doublets signal at 5.63 ppm with the same...
coupling constant. Lactone 6a formation was verified in the $^{13}$C NMR spectrum by the appearance of the lactone carbonyl signal at 171.5 ppm and the alkyl oxygen-base carbon (site of lactone ring closure) at 82.9 ppm, as well as in the IR spectrum where characteristic lactone carbonyl group appears at 1761 cm$^{-1}$.

**Cytotoxic activity**

The cytotoxicity of the two different series of compounds 5a–d and 6a–d toward different cancer cell lines, including human glioblastoma (CNS U251), human prostatic adenocarcinoma (PC-3), human chronic myelogenous leukemia (K562), human colorectal adenocarcinoma (HCT-15), human mammary adenocarcinoma (MCF-7), and non-small cell lung cancer (SKLU) were determined by using the protein-binding dye sulforhodamine B (SRB) assay in microculture to determine cell growth.\(^{28}\)

**Table 4.** Inhibition of the growth (%) of human tumor cell lines for 5a–d and 6a–d at 50 μM in DMSO

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Entry</th>
<th>Compound</th>
<th>U251</th>
<th>PC-3</th>
<th>K562</th>
<th>HCT-15</th>
<th>MCF-7</th>
<th>SKLU-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5a</td>
<td>46.2</td>
<td>33.4</td>
<td>27.0</td>
<td>12.5</td>
<td>45.0</td>
<td>28.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5b</td>
<td>60.3</td>
<td>35.4</td>
<td>33.7</td>
<td>32.7</td>
<td>61.0</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5c</td>
<td>35.2</td>
<td>27.6</td>
<td>19.4</td>
<td>26.9</td>
<td>45.6</td>
<td>38.8</td>
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<tr>
<td>4</td>
<td>5d</td>
<td>22.9</td>
<td>23.3</td>
<td>6.3</td>
<td>10.7</td>
<td>21.5</td>
<td>29.6</td>
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</tr>
<tr>
<td>5</td>
<td>6a</td>
<td>33.4</td>
<td>64.7</td>
<td>64.7</td>
<td>68.2</td>
<td>75.6</td>
<td>67.7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6b</td>
<td>87.9</td>
<td>78.6</td>
<td>81.7</td>
<td>96.1</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6c</td>
<td>41.8</td>
<td>39.6</td>
<td>33.1</td>
<td>37.1</td>
<td>53.6</td>
<td>54.2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6d</td>
<td>33.1</td>
<td>33.1</td>
<td>39.9</td>
<td>21.8</td>
<td>35.3</td>
<td>31.8</td>
<td></td>
</tr>
</tbody>
</table>

The initial cytotoxicity screening data listed in Table 4 show moderate to good activities toward almost all tumor cell lines. From those data, we observe that in general lactones 6 were more active than their corresponding carboxylic acids 5. Regarding the carboxylic acids, the most promising results were obtained from compound 5b, which shows the highest cytotoxic activity against U251, MCF-7, and SKLU-1 tumor cell lines, whereas the least active compound was the compound 5d. Similarly, as with carboxylic acids, the lactone 6b with a cyclobutyl group exhibits the highest activity against the six tumor cell lines tested. It is worth noting that the activity of tested lactones decreases considerably for all tumor cell lines when the size of the cycloalkane ring is increased (Table 4, entries 6-8). Finally, we determined the IC$_{50}$ of the most active compounds over HCT-15, MCF-7 and SKLU-1 and compared with a well-known chemotherapeutic drug (cisplatin) and Gefinitib which, like some benzothiazole derivatives,\(^ {29}\) is a potent epidermal growth factor receptor (EGFR) inhibitor (Table 5).
Table 5. IC\textsubscript{50} (μM) of human tumor cell lines for compounds 5b, 6b, Cisplatin, and Gefitinib in DMSO

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>HCT-15</th>
<th>MCF-7</th>
<th>SKLU-1</th>
<th>COS7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5b</td>
<td>86.8 ± 6.5</td>
<td>71.6 ± 4.3</td>
<td>74.7 ± 4.3</td>
<td>92.2 ± 1.3</td>
</tr>
<tr>
<td>2</td>
<td>6b</td>
<td>40.0 ± 4.7</td>
<td>27.4 ± 0.5</td>
<td>54.0 ± 3.1</td>
<td>47.7 ± 1.2</td>
</tr>
<tr>
<td>3</td>
<td>Cisplatin</td>
<td>21.4 ± 0.9</td>
<td>16.6 ± 0.4</td>
<td>2.2 ± 0.1</td>
<td>6.4 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>Gefitinib</td>
<td>10.4 ± 1.8</td>
<td>9.0 ± 0.8</td>
<td>9.7 ± 0.9</td>
<td>3.2 ± 0.4</td>
</tr>
</tbody>
</table>

Analyzing these results, we observe that the compounds 5b and 6b have higher IC\textsubscript{50} values in comparison with references Cisplatin and Gefitinib, which indicates their moderate activity against the evaluated tumor cell lines. Additionally, compounds were also tested against normal monkey kidney fibroblast (COS7 cell line). In general, IC\textsubscript{50} values determined for COS7 cells are higher than those found for cancer cell lines, which means that this type of compounds exhibit certain selectivity towards cancer cell lines.

Conclusions

We have demonstrated that bis(TMS)ketene acetals react efficiently with N-trifl fi quaternary salt of benzothiazole to affords 2-substituted 2,3-dihydrobenzothiazoles. In addition, a simple method for the regioselective synthesis of 4-substitut ed 1,4-dihydropyridines bearing a benzothiazole moiety was developed from 2-(pyridin-3-yl)-1,3-benzothiazole and different bis(trimethylsilyl)ketene acetals by using triflic anhydride as activating agent. In these reactions, N-activation of the pyridine moiety is preferred over benzothiazole fragment. Using a regioselective bromolactonization protocol, novel bicyclic δ-bromolactones were accessed. Finally, we have demonstrated that carboxylic acids and lactones shown moderate anticancer activity against human tumor cell lines where the derivatives bearing a cyclobutyl group present the highest activity.

Experimental Section

General. All the reactions were performed under an inert atmosphere of nitrogen or argon. All reagents were obtained from commercial suppliers and used without further purification. Merck silica gel (type 60, 0.063-0.200 mm) was used for column chromatography. All compounds were characterized by IR spectra, recorded on a Perkin-Elmer 283B or 1420 spectrophotometer, by means of ATR or KBr techniques, and all data are expressed as wavenumbers (cm\textsuperscript{-1}). Melting points were obtained on a Melt-Temp II apparatus. NMR spectra were recorded on a Bruker Avance III at 300 MHz (1H NMR) and 75 MHz (13C NMR) in chloroform-\textit{d}, acetone-\textit{d}\textsubscript{6} or DMSO-\textit{d}\textsubscript{6}. Chemical shifts are given in ppm with TMS as the reference. The MS-DART data were obtained on a Jeol AccuTOF; the values of the signals are expressed as mass/charge units (\textit{m/z}). Microwave irradiation experiments were performed using a Monowave 300 single-mode microwave reactor. The reusable 35 mL Pyrex vial is sealed with PEEK snap caps and standard PTFE coated silicone septa. Reaction cooling is performed using compressed air automatically after the heating period has elapsed. Suitable X-ray quality crystals of 5b were grown by slow evaporation of a \textit{n}-hexane-dichloromethane mixture at -5°C. The crystals were mounted on a glass fiber at room temperature, and then placed on a Bruker Smart Apex CCD.
diffractometer, equipped with Mo Kα radiation; decay was negligible in both cases. Systematic absences and intensity statistics were used in the space group determination. The structure was solved using direct methods. Anisotropic structure refinements were achieved using a full matrix, least-squares technique on all non-hydrogen atoms. All hydrogen atoms were placed in idealized positions, based on hybridization, with isotropic thermal parameters fixed at 1.2 times the value of the attached atom. Structure solutions and refinements were performed using SHELXTL V6.10.

**Synthesis of bis(trimethylsilyl)ketene acetal.** The syntheses of ketene acetals 2a-d were carried out using the methodology previously described elsewhere.30

**Synthesis of 2,3-dihydrobenzothiazoles 3a-e, general procedure.** Benzothiazole 1 (1.0 mmol) in anhydrous dichloromethane (10 mL) were placed in a 100 mL round-bottom flask under nitrogen atmosphere. Trifluoromethanesulfonic anhydride (0.12 mL, 1.1 mmol) was added by syringe at -78 °C and the mixture was stirred at this temperature for 30 min. After this time, the corresponding bis(trimethylsilyl)ketene acetal 2a-d (1.2 mmol) was added. The mixture was warmed to room temperature and stirred for 4 h. The crude product was diluted with dichloromethane and washed with water and brine. The organic layer was dried over Na2SO4 and the solvent removed. Finally, the 2,3-dihydrobenzothiazoles were purified by silica gel column chromatography, eluting with a solvent mixture of hexane/ethyl acetate.

**2-Methyl-2-[3-[(trifluoromethyl)sulfonyl]-2,3-dihydro-1,3-benzothiazol-2-yl]propanoic acid (3a).** White crystals (305 mg, 86%), mp 147-148 °C. 1H NMR (300 MHz, Acetone-d6, ppm): δ 8.57 (s, 1H, OH), 7.55 (dd, J 7.8, 1.5 Hz, 1H, H-3), 7.45 (dd, J 7.6, 1.6 Hz, 1H, H-6), 7.32 (td, J 7.5, 1.5 Hz, 1H, H-2), 7.25 (td, J 7.7, 1.5 Hz, 1H, H-1), 6.06 (s, 1H, H-8), 1.39 (s, 3H, CH3), 1.06 (s, 3H, CH3). 13C NMR (75 MHz, Acetone-d6, ppm): δ 175.0 (C=O), 136.4 (C-4), 133.0 (C-5), 128.4 (C-2), 128.6 (C-1), 120.3 (q, CF3, J 327 Hz), 119.4 (C-3), 73.6 (C-8), 50.6 (C-10), 22.9 (CH3), 17.0 (CH3). IR (KBr, cm-1): vmax 1704 (C=O). MS (DART, m/z): 373 [M+18]+, 356 [M+H]+. HRMS (ESI-TOF): calcd for C13H13F3NO4S2 [M+H]+ 356.02381; found 356.02362.

**1-3-[(Trifluoromethyl)sulfonyl]-2,3-dihydro-1,3-benzothiazol-2-yl)cyclobutane-1-carboxylic acid (3b).** White crystals (227 mg, 62%), mp 140-142 °C. 1H NMR (300 MHz, Acetone-d6, ppm): δ 10.18 (s, 1H, OH), 7.62 (d, 1H, J 7.7 Hz, H-3), 7.43 (dd, J 7.3, 1.9 Hz, 1H, H-6), 7.24-733 (m, 2H, H-2 and H-1), 6.10 (s, 1H, H-8), 2.67-2.57 (m, 1H, CH2 cyclobutyl), 2.44-2.26 (m, 2H, CH2 cyclobutyl), 2.02-1.78 (m, 3H, CH2 cyclobutyl). 13C NMR (75 MHz, Acetone-d6, ppm): δ 174.2 (C=O), 135.9 (C-4), 132.8 (C-5), 128.4 (C-2), 126.1 (C-6), 122.8 (C-1), 120.3 (q, CF3, J=327 Hz), 119.2 (C-3), 70.8 (C-8), 54.5 (C-10), 27.4 (CH2 cyclobutyl), 24.3 (CH2 cyclobutyl), 15.3 (CH2 cyclobutyl). IR (KBr, cm-1): vmax 1705 (C=O). MS (DART, m/z): 385 [M+18]+, 367 [M]+. HRMS (ESI-TOF): calcd for C13H13F3NO4S2 [M+H]+ 367.0158; found 367.01518.

**1-3-[(Trifluoromethyl)sulfonyl]-2,3-dihydro-1,3-benzothiazol-2-yl)cyclopentane-1-carboxylic acid (3c).** White crystals (225 mg, 59%), mp 146-147 °C. 1H NMR (300 MHz, DMSO-d6, ppm): δ 13.08 (s, 1H, OH), 7.50 (dd, J 7.7, 1.5 Hz, 1H, H-3), 7.46 (dd, J 7.9, 1.3 Hz, 1H, H-6), 7.30 (td, J 7.6, 1.5 Hz, 1H, H-2), 7.23 (td, J 7.7, 1.5 Hz, 1H, H-1), 6.09 (s, 1H, H-8), 2.01-1.87 (m, 3H, CH2 cyclopentyl), 1.66-1.32 (m, 5H, CH2 cyclopentyl). 13C NMR (75 MHz, DMSO-d6, ppm): δ 175.9 (C=O), 135.8 (C-4), 133.3 (C-5), 129.1 (C-2), 126.6 (C-6), 123.4 (C-1), 120.2 (q, CF3, J=327 Hz), 119.7 (C-3), 72.7 (C-8), 61.9 (C-10), 33.5 (CH2 cyclopentyl), 30.0 (CH2 cyclopentyl), 25.3 (CH2 cyclopentyl), 25.1 (CH2 cyclopentyl). IR (KBr, cm-1): vmax 1706 (C=O). MS (DART, m/z): 399 [M+18]+, 382 [M]+. HRMS (ESI-TOF): calcd for C14H15F3NO4S2 [M+H]+ 382.03946; found 382.03880.

**1-3-[(Trifluoromethyl)sulfonyl]-2,3-dihydro-1,3-benzothiazol-2-yl)cyclohexane-1-carboxylic acid (3d).** White crystals (257 mg, 65%), mp 168-170 °C. 1H NMR (300 MHz, DMSO-d6, ppm): δ 12.94 (s, 1H, OH), 7.49 (dd, J 7.6, 1.5 Hz, 1H, H-3), 7.43 (dd, J 7.8, 1.4 Hz, 1H, H-6), 7.28 (td, J 7.6, 1.5 Hz, 1H, H-2), 7.22 (td, J 7.7, 1.6 Hz, 1H, H-1), 5.85 (s, 1H, H-8) 1.88 (dd, J 30.8, 11.9 Hz, 2H, CH2 cyclohexyl), 1.54 (d, J 10.4 Hz, 3H, CH2...
cyclohexyl), 1.33-1.01 (m, 5H, CH₂ cyclohexyl). ¹³C NMR (75 MHz, DMSO-d₆, ppm): δ 173.4 (C=O), 136.1 (C-4), 132.5 (C-5), 128.9 (C-2), 122.6 (C-6), 123.3 (C-1), 120.2 (q, CF₂, δ 372 Hz), 119.3 (C-3), 75.4 (C-8), 55.1.9 (C-10), 29.3 (CH₂ cyclohexyl), 28.8 (CH₂ cyclohexyl), 25.2 (CH₂ cyclohexyl), 22.9 (CH₂ cyclohexyl), 22.8 (CH₂ cyclohexyl). IR (KBr, cm⁻¹): νmax 1701 (C=O). MS (DART, m/z): 413 [M+18]^+, 396 [M+H]^+. HRMS (ESI-TOF): calcd for C₁₅H₁₇F₃NO₄S₂ [M+H]^+ 396.0511; found 396.0538.

**Synthesis of 2-(pyridin-3-yl)-1,3-benzothiazole (4).** To a 35 mL microwave vial equipped with a magnetic stirrer were added 2-aminothiophenol (1 g, 7.98 mmol), 3-pyridinecarboxaldehyde (854 mg, 7.98 mmol) and SiO₂ (24 mg, 0.4 mmol) in EtOH (15 mL). The reaction mixture was irradiated at 70 °C for 20 min. After cooling to room temperature, the reaction was filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography with a solvent mixture of hexane/ethyl acetate. Yellow powder (1.25 g, 74%), mp 135-136 °C. ¹H NMR (300 MHz, CDCl₃, ppm): δ 9.31 (s, 1H, H-11), 8.73 (dd, J 4.8, 1.6 Hz, 1H, H-13), 8.39 (dt, J 8.0, 2.0 Hz, 1H, H-3), 8.12 (d, J 8.1 Hz, 1H, H-15), 7.94 (d, J 1.0, J 7.6 Hz H-6), 7.57-7.52 (m, 1H, H-2), 7.47-7.4 (m, 2H, H-1, H-14). ¹³C NMR (75 MHz, CDCl₃, ppm): δ 164.57 (C-8), 153.95 (C-5), 151.62 (C-13), 148.61 (C-11), 134.96 (C-4), 134.52 (C-15), 129.68 (C-10), 126.64 (C-1), 125.72 (C-14), 123.79 (C-2), 123.49 (C-6), 121.75(C-3). The data obtained are in agreement with that previously reported.31

**Synthesis of 1,4-dihydropyridines 5a-d, general procedure.** 2-(Pyridin-3-yl)-1,3-benzothiazole 4 (212 mg, 1.0 mmol) and 35 mL of anhydrous dichloromethane were placed in a 100 mL round-bottom flask under a nitrogen atmosphere. Trifluoromethanesulfonic anhydride (0.17 mL, 1.1 mmol) was added by syringe at -78 °C and the mixture was stirred at this temperature for 30 min. After this time, the corresponding bis(trimethylsilyl)ketene acetal 2a-d (1.2 mmol) was added. The mixture was warmed to room temperature and stirred for 8 h. The crude product was diluted with 20 mL of dichloromethane and washed with water and brine. The organic layer was dried over Na₂SO₄ and the solvent removed. Finally, the crude product was purified by recrystallization from hexane/dichloromethane to afford the desired product.

2-{3-[1,3-Benzothiazol-2-yl]-1-[(trifluoromethyl)sulfonyl]-1,4-dihydropyridin-4-yl}-2-methylpropanoic acid (5a). White powder (389 mg, 90%), mp 198-199 °C. ¹H NMR (300 MHz, Acetone-d₆, ppm): δ 10.94 (s, 1H, OH), 8.04 (d, J 7.7 Hz, 1H, H-3), 7.97 (d, J 7.7 Hz, 1H, H-6), 7.58-7.42 (m, 3H, H-1, H-2, H-11), 6.94 (d, J 7.7 Hz, 1H, H-13), 5.63 (dd, J 7.9, 5.6 Hz, 1H, H-14), 4.50 (d, J 5.6 Hz, 1H, H-15), 1.19 (s, 3H, CH₃), 1.08 (s, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃, ppm): δ 176.2 (C=O), 166.1 (C-8), 153.2 (C-5), 134.3 (C-4), 126.8 (C-11), 126.5 (C-1), 125.8 (C-2), 123.2 (C-13), 123.6 (C-6), 121.8 (C-3), 119. 4 (q, CF₃, J 320 Hz) 118.8 (C-10), 112.1 (C-14), 47.8 (C-16), 41.3 (C-15), 23.7 (CH₃), 19.5 (CH₃). IR (ATR, cm⁻¹): νmax 1679 (C=O). MS (DART, m/z): 433 [M+H]^+. HRMS (ESI-TOF): calcd for C₁₇H₁₆F₃NO₄S₂ [M+H]^+ 433.05036; found 433.05072.

1-{3-[1,3-Benzothiazol-2-yl]-1-[(trifluoromethyl)sulfonyl]-1,4-dihydropyridin-4-yl)cyclobutane-1-carboxylic acid (5b). White powder (391 mg, 88%), mp 184-185 °C. ¹H NMR (300 MHz, DMSO-d₆, ppm): δ 12.63 (s, 1H, -OH), 8.11 (d, J 7.7 Hz, 1H, H-3), 8.01 (d, J 7.3 Hz, 1H, H-6), 7.59-7.42 (m, 3H, H-1, H-2, H-11 ), 6.90 (d, J 7.9 Hz, 1H, H-13),), 5.51 (dd, J 8.0, 5.2 Hz, 1H, H-14), 4.28 (d, J 5.2 Hz, 1H, H-15), 2.11-1.93 (m, 4H, CH₂ cyclobutyl), 1.83-1.52 (m, 2H, CH₂ cyclobutyl). ¹³C NMR (75 MHz, DMSO-d₆, ppm): δ 176.7 (C=O), 166.3 (C-8), 153.1 (C-5), 134.2 (C-4), 127.1 (C-11), 126.2 (C-1), 125.8 (C-2), 123.2 (C-13), 122.7 (C-6 and C-3), 119. 4 (q, CF₃, J 325 Hz) 118.8 (C-10), 112.1 (C-14), 53.7 (C-16), 39.7 (C-15), 28.7 (CH₂ cyclobutyl), 27.9 (CH₂ cyclobuty 16.3 (CH₂ cyclobutyl). IR (ATR, cm⁻¹): νmax 1689 (C=O). MS (DART, m/z): 445.0 [M+H]^+. HRMS (ESI-TOF): calcd for C₁₈H₁₆F₃NO₄S₂ [M+H]^+ 445.05036; found 445.05024.

1-{1,3-Benzothiazol-2-yl]-1-[(trifluoromethyl)sulfonyl]-1,4-dihydropyridin-4-yl)cyclopentane-1-carboxylic acid (5c). White powder (426 mg, 93%), mp 193-194°C. ¹H NMR (300 MHz, DMSO-d₆, ppm): δ 12.48 (s, 1H, -OH).
OH) 8.07 (d, J 8.1 Hz, 1H, H-3), 7.98 (d, J 7.8 Hz, 1H, H-6), 7.54-7.41 (m, 3H, H-1, H-2), 7.34 (s, 1H, H-11), 6.68 (d, J= 7.9 Hz, 1H, H-13), 5.54 (dd, J 7.9, 5.4 Hz, 1H, H-14), 4.32 (d, J 5.3 Hz, 1H, H-15), 1.93-1.83 (m, 2H, CH₂ cyclopentyl), 1.45-1.35 (m, 6H, CH₂ cyclopentyl). ¹³C NMR (75 MHz, DMSO-d₆, ppm): δ 176.7 (C=O), 166.6 (C-8), 153.1 (C-5), 134.2 (C-4), 127.1 (C-11), 126.5 (C-1), 126.2 (C-2), 123.2 (C-13), 122.8 (C-6), 122.6 (C-3), 119.5 (C-10), 119.3 (q, CF₃, J 325 Hz), 113.3 (C-14), 60.8 (C-16), 40.3 (C-15), 33.8 (CH₂ cyclopentyl), 31.8 (CH₂ cyclopentyl), 24.1 (2 CH₂ cyclopentyl). IR (ATR, cm⁻¹): νmax 1683 (C=O). MS (DART, m/z): 459.0 [M+H]⁺. HRMS (ESI-TOF): calcld for C₁₅H₁₈F₃N₂O₄S₂ [M+H]⁺ 459.0661; found 459.06658.

3-{1,3-Benzothiazol-2-yl}-1-{[trifluoromethyl]sulfonyl}-1,4-dihydropyridin-4-yl)cyclohexane-1-carboxylic acid (5d). White powder (373 mg, 79%), mp 216-217 °C. ¹H NMR (300 MHz, DMSO-d₆, ppm): δ 12.47 (s, 1H,OH), 8.06 (d, J 7.7 Hz, 1H, H-6), 7.97 (d, J 7.4 Hz, 1H, H-3), 7.56-7.35 (m, 3H, H-1, H-2, H-11), 6.95 (d, J 7.7 Hz, 1H, H-13), 5.50 (dd, J 7.8, 5.8 Hz, 1H, H-14), 4.12 (d, J 5.8 Hz, 1H, H-15), 1.51-1.38 (m, 2H, CH₂ cyclohexyl), 1.26-0.90 (m, 8H, CH₂ cyclohexyl). ¹³C NMR (75 MHz, DMSO-d₆, ppm): δ 175.4 (C=O), 166.6 (C-8), 153.1 (C-5), 134.3 (C-4), 126.9 (C-11), 126.7 (C-1), 126.1 (C-2), 123.5 (C-13), 123.2 (C-6), 122.6 (C-3), 119.3 (q, CF₃, J 325 Hz), 118.8 (C-10), 112.2 (C-14), 53.4 (C-16), 42.7 (C-15), 31.4 (CH₂ cyclohexyl), 29.5 (CH₂ cyclohexyl) 23.5 (CH₂ cyclohexyl), 23.3 (CH₃ cyclohexyl). IR (ATR, cm⁻¹): νmax 1675 (C=O). MS (DART, m/z): 473.0 [M+H]⁺. HRMS (ESI-TOF, m/z): calcld for C₂₀H₂₁F₃N₂O₅S₂ [M+H]⁺ 473.08166; found 473.08166.

General procedure for the synthesis of 6-bromolactones 6a-d. A 100 mL round-bottom flask was charged with the corresponding carboxylic acid 5a-d (0.5 mmol), sodium bicarbonate (50 mg, 0.6 mmol), N-bromosuccinimide (98 mg, 0.55 mmol), and tetrabutylammonium bromide (32 mg, 0.1 mmol) in dry dichloromethane (20 mL). The mixture was stirred at 0 °C and kept away from light. The reaction progress was monitored by TLC and once it reached completion, the crude mixture was washed with water (3 × 20 mL). The organic phase was dried over sodium sulfate and evaporated under reduced pressure. The obtained lactones were purified by silica gel column chromatography, eluted with a mixture of hexane/ethyl acetate.

6-{1,3-Benzothiazol-2-yl}-9-bromo-4,4-dimethyl-8-{[trifluoromethyl]sulfonyl]}-2-oxa-8-azabicyclo[3.3.1]non-6-en-3-one (6a). White powder (224 mg, 88%), mp 218-219 °C. ¹H NMR (300 MHz, CDCl₃, ppm): δ 8.0 (d, J 8.0 Hz, 1H, H-6), 7.88 (d, J 7.9 Hz, 1H, H-3), 7.57-7.37 (m, 3H, H-1, H-2, H-11), 6.18 (s, 1H, H-13), 5.14 (t, J 3.1 Hz, 1H, H-14), 3.98 (t, J 2.4 Hz, 1H, H-15), 1.63 (s, 3H, -CH₃), 1.31 (s, 3H, -CH₃). ¹³C NMR (75 MHz, CDCl₃, ppm): δ 171.5 (C=O), 164.9 (C-8), 153.2 (C-5), 133.9 (C-4), 126.6 (C-11), 126.1 (C-1), 123.3 (C-2), 123.1 (C-6), 121.6 (C-3) 119.2 (q, CF₃, J 321 Hz), 117.28 (C-10), 82.94 (C-13), 45.65 (C-14), 43.87 (C-16), 35.76 (C-15), 28.08 (CH₃), 25.9 (CH₃). IR (ATR, cm⁻¹): νmax 1761 (C=O). MS (DART, m/z): 513 (Br, 81) [M+H⁺]⁺, 511 (Br, 79) [M⁺]⁺. HRMS (ESI-TOF): calcld for C₁₄H₁₂BrF₃N₂O₂S₂ [M⁺]⁺ 510.96087; found 510.96142.

8-{1,3-Benzothiazol-2-yl}-9-bromo-6-{[trifluoromethyl]sulfonyl]}-4-oxa-6-azaspiro[bicyclo[3.3.1]nonane-2,1'-cyclobutan]-7-en-3-one (6b). White powder (235 mg, 90%), mp 168-169 °C. ¹H NMR (300 MHz, DMSO-d₆, ppm): δ 8.11 (d, J 7.1 Hz, 1H, H-6), 8.04 (d, J 7.9 Hz, 1H, H-6), 7.61-7.40 (m, 3H, H-1, H-2, H-11 ), 6.43 (br, 1H, H-13), 5.31 (t, J 3.1 Hz, 1H, H-14), 4.24 (t, J 2.4 Hz 1H, H-15), 2.77-2.67 (m, 1H, CH₂ cyclobutyl), 2.29-2.18 (m, 4H, CH₂ cyclobutyl), 1.98-1.93 (m, 3H, CH₂ cyclobutyl). ¹³C NMR (75 MHz, DMSO-d₆, ppm): δ 171.2 (C=O), 165.3 (C-8), 153.0 (C-5), 134.2 (C-4), 127.4 (C-11), 126.6 (C-1), 123.3 (C-2) 123.1 (C-6), 122.8 (C-3), 119.4 (q, CF₃, J 324 Hz), 117.6 (C-10), 83.2 (C-13), 49.9 (C-14), 43.0 (C-16), 36.8 (C-15), 34.14 (CH₂ cyclobutyl), 27.3 (CH₂ cyclobutyl) 15.7 (CH₂ cyclobutyl). IR (ATR, cm⁻¹): ν 1756 (C=O). MS (DART, m/z): 525 (Br, 81), 523 (Br, 79) [M⁺]⁺. HRMS (ESI-TOF, m/z): calcld for C₁₈H₁₅BrF₃N₂O₂S₂ [M⁺]⁺ 522.98093; found 522.98093.
The supernatant was discarded, and the plates were washed three times with water and air. Cells suspension was seeded in 96-well microtiter plates and incubated to allow for cell attachment. After 24 h, 100 ml of each test compounds and positive substances (Cisplatin or Gefitinib) were added to each well. 48 h later, adherent cell cultures were fixed by adding 50 ml of cold 50% (wt/vol) trichloroacetic acid (TCA) for 60 min at 4 °C. The supernatant was discarded, and the plates were washed three times with water and air. Cultures fixed with TCA were stained for 30 min with 100 ml of 0.4% SRB solution. Bound dye was extracted with 10 mM unbuffered Tris base and the optical densities were read on an Ultra Microplate Reader (Elx 808, BIO-TEK Instruments, Inc.), with a test wavelength of 515 nm.

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**Supplementary Material**

Copies of $^{1}H$ and $^{13}C$ NMR spectra of the new compounds. CCDC 1898179 for 5b contain the supplementary crystallographic data for this paper.
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