An Efficient Strategy for the Synthesis of 1-(Trifluoromethylsulfonamido)-propan-2-yl Esters and the Evaluation of Their Cytotoxic Activity

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An efficient method for the synthesis of 1-(trifluoromethylsulfonamido)propan-2-yl benzoates is described, the products of the reaction were characterized by heteronuclear single quantum coherence spectroscopy (HSQC), heteronuclear multiple bond correlation (HMBC) and NMR experiments. The overall process began with the activation of the oxazoline ring by triflic anhydride, followed by the opening of the five-membered ring in the 5-methyl-2-phenyl-4,5-dihydrooxazole system. The cytotoxic activity of the new trifluoromethyl sulfonamides was evaluated with six cancer cell lines and human gingival fibroblasts, posteriorly analyzing the influence on cytotoxicity exerted by the withdrawing and donor substrates at the para-position of the phenyl ring. Compounds 3b–e showed cytotoxic activity, with IC₅₀ values ranging from 17–17.44µM for the cell lines tested, finding the highest effect for compound 3e.

Key words ring opening reaction; sulfonamide; oxazoline; NMR; cytotoxic activity

Trifluoromethanesulfonamide (hereinafter “triflamide” for brevity) is a derivative of trifluoromethanesulfonic acid. Two main methods of synthesis of triflamides and other perfluoroalkanesulfonamides have been described: The reaction of the corresponding fluorooalkylsulfonyl fluorides RFSO₂F with ammonia or amines,1–6) and the reaction of trifluoromethanesulfonfonyl chloride CF₃SO₂Cl or triflic anhydride (CF₃SO₂)₂O normally, in the presence of triethylamine to bind the eliminated triflic acid.7–9) Different approaches based on the Mitsunobu reaction10) and the intermolecular bromoesterification of allylic sulfonamides have also been reported.11) However, the majority of these reactions have disadvantages such as: poor yield, multiple reaction steps, difficulty to workup, and the use of an excess of amine is required.12–14)

This class of compounds are also useful for industrial purposes, employed as nonflammable solvents for improving lithium-ion batteries.15,16)

The triflamide structure is a very important compound in medicinal chemistry, being present in several bioactive compounds.17,18) Trifluorosulfonamide is a bioisostere of a hydroxyl group as well as the hydroxymethyl and ureido groups.

Bioisosterism is a strategy of Medicinal Chemistry for the rational design of new drugs, extensively studied for modification of drug target, selectivity efficacy, potency, membrane permeability, biotransformation and toxicity profile.

The biological activity of triflamide derivatives is to a great extent due to their lipophilicity imparted by the presence of the CF₃SO₂NH moiety, and to their high acidity. Some triflamide derivatives have also been employed as anticonvulsant agents.19,20) Others have been used to treat Alzheimer’s disease by decreasing the level of Alzheimer’s amyloid-β peptides in the brain and cerebrospinal fluid.21) Moreover, the application of triflamides involve a broad range of biological activities such as antiinflammatory,22–24) anti-obesity,25) antihypertensive,26–27) antihyperglycemic,25) antibacterial,28,29) and antineoplastic,30) among others.30–32) Included in this kind of compound are perfluoroalkene sulfonamides and N-alkyl perfluoroalkanesulfonamides, which are reported to have antiinflammatory,33–35) and herbalicidal activity.36) It is noteworthy that the addition of fluorine atoms conferred them with high acidity and lipophilicity.4,5,37)

In the present study, we carried out the synthesis of a new series of 1-(trifluoromethylsulfonamido)propan-2-yl benzoate derivatives. The strategy employed the ring opening of the oxazoline precursor in the presence of trifluoromethanesulfonic anhydride to give compounds 3a–g in good overall yields.

The cytotoxicity of the new compounds was tested with six tumor cells: U251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), K-562 (human chronic myelogenous leukemia), HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), and SKLU-1 (human lung adenocarcinoma), and FGH (human gingival fibroblast).

Results and Discussion

Chemistry The synthesis of the new 1-(trifluoromethylsulfonamido)propan-2-yl benzoates 3a–g was carried out through the treatment of the corresponding 5-methyl-2-arylsulfonamido para-substituted 1a–g with an excess of triflic anhydride at −70°C (Chart 1).

In order to generalize the scope of the method we employed the 5-methyl-2-(2-fluorophenyl)-4,5-dihydrooxazole 1b under the same reaction conditions. The 1-(trifluoromethylsulfonamido)propan-2-yl 2-fluorobenzoate 3h was obtained in 11% of yield. Additionally, a mixture of byproducts that could not be isolated and raw materials were identified during the purification process. The possible mechanism for the reaction was recently described by our group.38) The reaction was influenced by the substituent at the para-position (Table 1). The yields were higher with electron-withdrawing substituents (Cl,
NO₂, Br) than the electron-donor substituent (OMe).

In the 1H-NMR spectrum of N-alkyl trifluoromethyl sulfonamides, the signal of the amino group was observed at around 6 ppm for all derivatives. The 13C-NMR spectrum presented a quadruplet signal near 119 ppm corresponding to the triflyl moiety, and 2) the cleavage of the O–C bond with the loss of the alpha to carbonyl group with the loss of the benzoyljugated ester. On the other hand, two typical fragmentations were observed in the mass spectrum of series: 1) the cleavage of the triflamide system resulted in an easy, an efficient synthetic strategy against the aforementioned six human cancer cell lines (see end of Introduction). The IC₅₀ values are presented in Table 2. It is noteworthy that compounds 3a, f and g were not included in the table due to in preliminary assays were not active.

The results show that compounds 3b–e exhibited a cytotoxic effect on all the cancer cell lines, except for 3c in most cases. Furthermore, the compound with a CF₃ substituent yielded the highest cytotoxic effect for all cell lines tested.

The data for compounds 3b–e indicate that a change in the substituent at the para-position of the phenyl ring significantly affected cytotoxic activity. Moreover, closer inspection of the data demonstrates that with respect to the human glioblastoma cell line (U251), compounds with a Cl substituent were less active than those with Br or NO₂ groups on the aromatic ring.

Regarding the prostate cancer cell line (PC-3), the SAR of compounds 3b–e evidenced greater activity for compounds 3d and e, with a hard-withdrawing effect (R=NO₂, CF₃) on the aromatic ring. Compound 3e (R=Br), contrarily, was not active. In the case of human leukemia cell line (K562), the greatest toxicity (IC₅₀=13.48) was observed for 3e having the trifluoromethyl substituent. Once again, 3c was not active.

Compounds 3b and c exhibited significant cytotoxicity against the colon HCT-15 cell line, having a tendency similar to that found with U251. In both cases, the cytotoxic effect was dependent on the kind of withdrawing substituent on the aromatic ring. The best inhibitory effect was observed with 3b and e (IC₅₀=13.48–42.92). Compounds 3b, d and e also demonstrated potent cytotoxicity against the breast cancer cell line (MCF-7), with the best effect exhibited by compound 3e. Compound 3e was not active. Concerning the lung cell line (SKLU-1), compounds 3b and c exhibited significant cytotoxicity. On the other hand, the cytotoxic activity of the compounds 3b–e against human gingival fibroblasts was determined and only the compound 3e shown an inhibitory effect (41.82%). Similar to all previous tests, 3e (R=CF₃) showed the best result, and its high level of inhibition is attributed to the withdrawing effect of CF₃ in the para-position of the aromatic ring, which inhibits the biotransformation process of drugs in the hydroxylation stage. It is striking that compounds with electron donor substituents were not active in this assay. The values of inhibition of 3b–e indicate the importance of further anticancer testing with triflamides.

**Conclusion**

The synthesis route was based on the reaction of the oxazo-line ring with triflic anhydride, followed by the opening of the five-membered ring of the 5-methyl-2-phenyl-4,5-dihydrooxa-zole system resulted in an easy, an efficient synthetic strategy for the synthesis of 1-(trifluoromethylsulfonamido)propan-2-yl esters 3a–h. The yield of the reaction was significantly higher for para-substituents. The cytotoxicity assay showed that compounds 3b, d and e had activity on six human cancer cell

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**Table 1. Results of the Synthesis of 1-(Trifluoromethylsulfonamido)-propan-2-yl Benzoate Derivatives**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>4-H</td>
<td>86.1</td>
<td>62–64</td>
</tr>
<tr>
<td>3b</td>
<td>4-Cl</td>
<td>76.5</td>
<td>74–76</td>
</tr>
<tr>
<td>3c</td>
<td>4-Br</td>
<td>81</td>
<td>64–66</td>
</tr>
<tr>
<td>3d</td>
<td>4-NO₂</td>
<td>86</td>
<td>126–128</td>
</tr>
<tr>
<td>3e</td>
<td>4-CF₃</td>
<td>59</td>
<td>90–92</td>
</tr>
<tr>
<td>3f</td>
<td>4-CH₁</td>
<td>88</td>
<td>58–60</td>
</tr>
<tr>
<td>3g</td>
<td>4-OCH₃</td>
<td>56</td>
<td>59–60</td>
</tr>
<tr>
<td>3h</td>
<td>2-F</td>
<td>11</td>
<td>No solid</td>
</tr>
</tbody>
</table>

**Chart 1. Synthesis Route of 1-(Trifluoromethylsulfonamido)propan-2-yl Benzoates 3a–g**
lines, and that with most of these 3c was not active. Compound 3e turned out to be the most active but also show activity against the fibroblast. The nature of the respective substituents was analyzed in relation to the cytotoxic assay, finding an increased effect with the electron withdrawing groups and a lack of response with the electron donor groups.

**Experimental**

All glassware was thoroughly oven-dried. Chemicals and solvents were purchased from commercial suppliers. Melting points (mp) were determined on a Melt Temp II apparatus and are reported without correction. The 1H- and 13C-NMR spectra were recorded on a Bruker Advance III, at 300 MHz (1H-NMR) and 75 MHz (13C-NMR), as well as on a Varian NMR system, at 500 MHz (1H-NMR) and 125 MHz (13C-NMR), in chloroform-d. Chemical shifts are given in parts per million with reference to internal tetramethylsilane (TMS). Electron ionization mass spectrometry (EI-MS) spectra were recorded on a JEOL JMS-AX505 spectrometer. IR spectra were obtained with a Bruker Tensor 27 spectrophotometer. The phenylidihydrooxazoles 3a–g were prepared according to previously reported methods using racemic 1-aminopropan-2-ol.41

**Cell Culture and Assay for Cytotoxic Activity**

Cell culture and assay for cytotoxic activity HCT-15, MCF-7, K-562, U-251, PC-3, and SKLU-1, were supplied by The National Cancer Institute (NCI), U.S.A. The cytotoxicity of tumors cells with the test compounds was determined using the protein-binding dye sulforhodamine B (SRB) in microculture plates and are reported without correction. The lack of response with the electron donor groups.

Table 2. IC50 (µM) Values of Cytotoxic Activity for Compounds 3b–e

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>U251</th>
<th>PC-3</th>
<th>K562</th>
<th>HCT-15</th>
<th>MCF-7</th>
<th>SKLU-1</th>
<th>FGH</th>
</tr>
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<tbody>
<tr>
<td>3b</td>
<td>Cl</td>
<td>45.22±1.9</td>
<td>57.17±5.4</td>
<td>59.60±6.0</td>
<td>45.67±0.57</td>
<td>26.08±0.1</td>
<td>34.43±1.3</td>
<td>NA</td>
</tr>
<tr>
<td>3c</td>
<td>Br</td>
<td>41.69±0.8</td>
<td>&gt;100 &gt;100 &gt;100</td>
<td>38.23±3.6</td>
<td>&gt;100 &gt;100</td>
<td>36.89±1.0</td>
<td>NA</td>
<td></td>
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<tr>
<td>3d</td>
<td>NO2</td>
<td>40.13±4.0</td>
<td>35.02±4.0</td>
<td>42.92±4.0</td>
<td>36.2±1.0</td>
<td>40.99±0.2</td>
<td>27.61±1.1</td>
<td>NA</td>
</tr>
<tr>
<td>3e</td>
<td>CF3</td>
<td>40.13±4.0</td>
<td>27.44±0.3</td>
<td>13.48±2.0</td>
<td>27.08±9.3</td>
<td>21.04±2.7</td>
<td>17.16±2.0</td>
<td>41.82</td>
</tr>
<tr>
<td>Cisplatin</td>
<td></td>
<td>3.3±0.6</td>
<td>8.3±0.7</td>
<td>5.3±1.2</td>
<td>10±1.0</td>
<td>9.4±1.1</td>
<td>4.3±0.5</td>
<td>—</td>
</tr>
</tbody>
</table>

*a) The cytotoxic activity for compound 3e is given in terms of inhibition percentage of cancer cells FGH (50 µM).**

**Procedure for the Synthesis of 5-Methyl-2-(4-(trifluoromethyl)phenyl)-4,5-dihydrooxazole (1e)**

The compound 1e was prepared according to the methodology described in the literature.42 The titrate compound was purified by chromatographic column using a mixture of hexane–ethyl acetate as eluent giving a yellow oil in good yield (396mg, 88.2%). 1H-NMR (500 MHz, CDCl3) δ: 1.44 (3H, d, J=6.0Hz, H-6), 3.64 (1H, dd, J=14.7Hz, J=7.5Hz, H-4), 4.18 (1H, dd, J=14.7Hz, J=9.6Hz, H-4), 4.89 (1H, m, H-5), 7.67 (2H, d, J=8.4Hz, H-9, H-11), 8.06 (2H, d, J=8.1Hz, H-8, H-12). 13C-NMR (75 MHz, CDCl3) δ: 21.1 (C-6), 61.7 (C-4), 76.7 (C-5), 120.2 (q, J=271.05Hz) CF3, 125.2 (q, J=375.7Hz) C-9, 121.8 (C-8, C-12), 132.5 (C-7), 132.9 (C-10), 162.7 (C-2).

**General Procedure for the Synthesis of 1-(Trifluoromethylsulfonyl)amido)propan-2-yl Benzoates (3a–g)**

To a solution of the corresponding 5-methyl-2-aryl-4,5-dihydrooxazole (1a–g; 3.1 mmol) in 40 mL of anhydrous dichloromethane, cooled to −78°C and under an inert atmosphere, trifluoromethane sulfonic anhydride (3.08 mmol, 2.0equiv.) was added. The reaction mixture was stirred for 1h at −78°C, allowed to reach room temperature (rt) and stirred for 1h, and then added to a flask containing H2O (30 mL) and extracted with CHCl3 (3×20 mL). The organic extracts were combined and dried (anhydrous Na2SO4) and the solvent was removed under reduced pressure to deliver the title compounds 3a–g, which were further purified by column chromatography on silica gel using a mixture of hexane and EtOAc as eluent.

1-(Trifluoromethylsulfonyl)amido)propan-2-yl Benzoate (3a)

Compound 3a was obtained from 5-methyl-2-phenyl-4,5-dihydrooxazole 1a and TfO by following the aforementioned procedure, giving a white solid in good yield (830 mg, 86.1%). The mp was 62−64°C.

1H-NMR (300 MHz, CDCl3) δ: 1.41 (3H, d, J=6.6Hz, H-11), 3.53 (2H, m, H-12), 5.26 (1H, m, H-10), 5.87 (1H, s, NH-13), 7.44 (2H, dd, J=7.8, J=6.5Hz), 8.01 (2H, d, J=7.2Hz, H-2, H-6).

13C-NMR
 Compound 3b was obtained from 5-methyl-2-(4-chlorophenyl)-4,5-dihydrooxazole 1 and Tf₂O by following the aforementioned procedure, giving a white solid in good yield (819 mg, 76.5%). The mp was 74–76°C.

1H-NMR (CDCl₃) δ: 1.41 (3H, d, J = 6.6 Hz, H-11), 3.53 (2H, m, H-12), 5.75 (1H, m, H-10), 5.85 (1H, s, NH-13), 7.40 (2H, d, J = 8.4 Hz, H-3, H-5), 7.94 (2H, d, J = 8.4 Hz, H-2, H-6). 13C-NMR (CDCl₃) δ: 17.3 (C-11), 48.5 (C-12), 60.1 (C-10), 119.5 (q, J = 318.52 Hz, CF₂-17), 127.8 (C-1), 128.3 (C-3, C-5), 131.1 (C-2, C-6), 140.1 (C-4), 165.6 (C-7). IR (CH₂Cl₂) cm⁻¹: 1152.4 (SO₂ asym), 1379.4 (C–O), 1688.34 (C=O), 1705.29 (C=O), 2852.64, 2921.06, 2954.38, 2992.64 (C–H sp³), 3107.14 (C–H sp²), 3286.06 (NH). EI-MS m/z (rel. int. %): 380.00 (100, [M⁺+1]⁺), 190.00 (75, M⁺−190) 173.00 (55, M⁺−207). HR-MS (FT-ICR) m/z [M⁺+1]⁺: 380.0395 (Calcd for C₁₂H₁₄F₂NO₅S: 380.0391).

1-(Trifluoromethylsulfonyl)imadazol-1-yl 4-Chlorobenzoate (3f)

Compound 3f was obtained from 5-methyl-2-(4-methylphenyl)-4,5-dihydrooxazole 1 and Tf₂O by following the aforementioned procedure, giving a white solid in good yield (887 mg, 88%). The mp was 58–60°C.

1H-NMR (CDCl₃) δ: 1.43 (3H, d, J = 6.6 Hz, H-11), 3.55 (2H, m, H-12), 5.29 (1H, m, H-10), 5.87 (1H, s, NH), 7.69 (2H, d, J = 8.11 Hz, H-3, H-5), 8.12 (2H, d, J = 8.11 Hz, H-2, H-6). 13C-NMR (CDCl₃) δ: 17.2 (C-11), 48.4 (C-12), 70.5 (C-10), 119.5 (J = 318.01 Hz, CF₂-17), 125.5 (q, J = 3.68 Hz, C-3, C-5), 130.1 (C-2, C-6), 132.6 (C-1), 135.1 (C-4), 165.2 (C-7). IR (KBr) cm⁻¹: 1155.86 (SO₂ sym), 1189.4 (CF₂), 1275.93 (C–O), 1376.51 (SO₂ asym), 1585.92 (C=C stretch), 1705.29 (C=O), 2852.64, 2921.06, 2954.38, 2992.64 (C–H sp³), 3107.14 (C–H sp²), 3286.06 (NH). EI-MS m/z (rel. int. %): 380.00 (100, [M⁺+1]⁺), 190.00 (75, M⁺−190) 173.00 (55, M⁺−207). HR-MS (FT-ICR) m/z [M⁺+1]⁺: 380.0395 (Calcd for C₁₂H₁₄F₂NO₅S: 380.0391).

1-(Trifluoromethylsulfonyl)imadazol-1-yl 4-Methoxybenzoate (3g)

Compound 3g was obtained from 5-methyl-2-(4-methoxyphenyl)-4,5-dihydrooxazole 1 and Tf₂O by following the aforementioned procedure, giving a white solid in good yield (500 mg, 47.3%). The mp was 58–60°C.

1H-NMR (CDCl₃) δ: 1.38 (3H, d, J = 6.6 Hz, H-11), 3.52 (2H, m, H-12), 3.84 (1H, s, OMe-18), 5.22 (1H, m, H-10), 5.88 (2H, dt, J = 8.4, 2.1 Hz, H-3, H-5), 7.58 (1H, dd, J = 7.5, 7.5 Hz, H-4), 8.01 (2H, d, J = 7.2 Hz, H-3, H-5), 7.95 (2H, dt, J = 9.0, 2.1 Hz, H-2, H-6). 13C-NMR (CDCl₃) δ: 17.4 (C-11), 48.6 (C-12), 55.4 (OMe-18), 69.5 (C-10), 113.7 (C-3, C-5) 119.6 (q, J = 318.98 Hz, CF₂-17), 121.6 (C-1), 131.8 (C-2, C-6), 163.7 (C-4), 166.3 (C-7). IR (KBr) cm⁻¹: 1169.10 (SO₂ sym), 1186.31 (CF₂), 1256.99 (C–O), 1373.90 (SO₂ asym), 1605 (C=C stretch), 1688.34 (C=O), 2844.10, 2940.88 (C–H sp³), 2984.54 (C–H sp²), 3225.77 (NH). EI-MS m/z (rel. int. %): 342.00 (100, [M⁺+1]⁺), 135.00 (45, M⁺−190). HR-MS (FT-ICR) m/z [M⁺+1]⁺: 342.0623 (Calcd for C₁₁H₁₂F₂NO₄S: 342.0623).

1-(Trifluoromethylsulfonyl)imadazol-1-yl 2-Fluorobenzoate (3h)

Compound 3h was obtained from 5-methyl-2-(2-fluorophenyl)-4,5-dihydrooxazole 1 and Tf₂O by following the
The aforementioned procedure, giving a white paste in low yield (102 mg, 11%).

1H-NMR (CDCl3): δ 1.42 (3H, d, J=6.6 Hz, H-11), 3.53 (2H, m, H-12), 5.26 (1H, m, H-10), 5.62 (1H, s, NH), 7.13 (1H, ddd, J=7.5, 7.4, 1.0 Hz, H-3), 7.22 (1H, td, J=7.8, 1.2 Hz, H-5), 7.54 (1H, m, H-4), 7.92 (1H, td, J=7.8, 1.2 Hz, H-6). 13C-NMR (CDCl3): δ 17.2 (C-11), 48.4 (C-12), 70.5 (C-10), 117.0 (d, J_C,F=22.5 Hz, C-3), 118.0 (d, J_C,F=22.5 Hz, C-1), 119.6 (J_C,F=318.1 Hz, CF3), 124.2 (d, J_C,F=3.8 Hz, C-5), 132.2 (d, J_C,F=9.25 Hz, C-6), 135.1 (d, J_C,F=9.25 Hz, C-4), 161.9 (J_C,F=257.87 Hz, C-2), 164.1 (J_C,F=3.8 Hz, C-7).

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Conflict of Interest The authors declare no conflict of interest.

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