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Synthesis of a tritium-labeled photo-affinity probe based on an atypical leukotriene biosynthesis inhibitor

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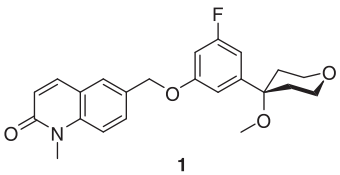
A radiolabeled photo-affinity probe containing a diazirine group was designed and synthesized based on an atypical leukotriene (LT) biosynthesis inhibitor to determine whether this type of inhibitor (such as 5) interacts with a novel protein, critical for LT biosynthesis. One of the key transformations was to introduce a tritium label in the thiazole ring. This was eventually achieved by treating the iodinated compound with tritium, Pt/C (sulfided) and triethylamine in anhydrous aprotic solvent, to give the tritiated compound with a specific activity of 303 GBq/mmol. The affinity probe will be used to identify the proteins and protein complexes with which this type of molecules interacts with in inflammatory cells.

Keywords: photo-affinity labeling; tritiation; leukotriene; 5-lipoxygenase

Introduction

Leukotrienes (LTs) are lipid mediators that are implicated in numerous diseases, including inflammation, atherosclerosis, and respiratory diseases such as asthma.[1–9] LTs are produced from arachidonic acid by the action of 5-lipoxygenase (5-LO) and the integral membrane protein 5-LO-activating protein (FLAP), which is an essential partner of 5-LO for LT production in cells. Previously, it has been shown that LT production can be controlled by either inhibiting 5-LO or FLAP, which could in turn lead to new therapies for treatment of diseases associated with LTs.[10–13]

Crawley *et al.* have developed 4-methoxytetrahydropyrans (**1**–**4**) as potent and selective inhibitors of 5-LO from which compounds **1** and **2** were chosen for clinical trials.[14,15] Independently, compound **5** that also incorporates 4-methoxytetrahydropyran moiety was designed and synthesized at Merck Frosst.[16] Compound **5** was found to be a moderately potent inhibitor of 5-LO and it does not have significant interactions with FLAP or LTC_4 synthase. However, compound **5** shows greater inhibitory potency in human whole blood cell assay than the level of 5-LO activity would predict. This suggests that **5** might have off-target activity within the same 5-LO pathway, perhaps with an as yet uncharacterized ancillary protein.[17] Hence, it is of interest to develop an affinity probe based on **5** to identify this putative new molecular target, which could serve as a new target for drug discovery and optimization.



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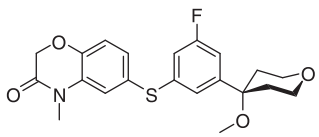

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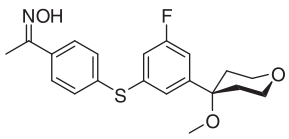

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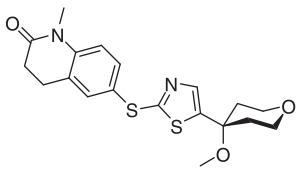

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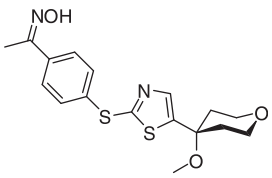

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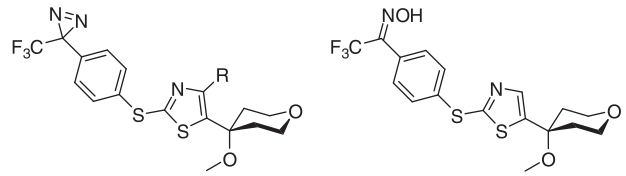
In order to explore the biomolecular target(s) of compound **5**, a radiolabeled photo-affinity probe would be useful. From structure activity relationship, we noted that the oxime moiety could be modified without having significant impact on the biological activity.[16] We envisioned that **6**, a closely analogous diarizine relative of **5**, should retain the activity and this could be used to identify these new targets. The radioactive photo-affinity ligand, such as compound **7**, could be incubated with inflammatory cells and photolysed to give covalent conjugation with target proteins. The tritium label could then be followed to characterize the complexes. Hence, we report herein the synthesis of the protio-compound **6** and the tritio-compound **7** in which the oxime group is replaced with a photo-labile diazirine group. In view of the fact that compounds **6** and **7** have a trifluoromethyl group, instead of a methyl group as in the parent oxime **5**, we also synthesized the trifluoromethyl derivative **8** to verify whether there is any impact in the biological activity when a methyl group is modified.



6: R = H
7: R = T

8

Experimental

General methods

^{1}H and ^{13}C NMR spectra were recorded at frequencies of 500 and 125 MHz, respectively. All assignments were confirmed with the aid of two-dimensional ^{1}H, ^{1}H (gCOSY) and ^{1}H, ^{13}C (gHMQC) experiments using standard Varian pulse programs. Processing of the spectra was performed with MestRec software. The high-resolution mass spectra were recorded in positive-ion mode with an ESI ion source on an Agilent Time-of-Flight LC/MS mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or dipped in $KMnO_4$ solution and heated. Column chromatography was performed with Silica gel 60 (230–400 mesh). All procedures with diazirine functionality were carried out in flask/column covered by aluminum foil to exclude light.

5-(4-Methoxytetrahydropyran-4-yl)-2-(4-(2,2,2-trifluoro-1-(hydroxyimino)ethyl)phenylthio)thiazole (8)

To a stirred solution of **14** (505 mg, 2.08 mmol) in $CHCl_3$ (15 ml) at 0°C was added MCPBA (470 mg, 77%, 2.09 mmol) and the mixture was stirred for 90 min. $Ca(OH)_2$ (247 mg, 3.33 mmol) was added and the mixture was stirred at rt for 20 min, filtered, and concentrated to give essentially pure sulfoxide. The residue was dissolved in TFAA (8 ml) and refluxed for 30 min and concentrated. The mixture was diluted with MeOH–Et_3N (1:1, 20 ml) and evaporated to dryness. The residue was dissolved in $CHCl_3$ (150 ml) and washed with sat. NH_4Cl, dried over anhydrous Na_2SO_4, and concentrated to provide thiol **13**. In a separate flask, oxime **11** (500 mg, 1.59 mmol), Pd_2dba_3 (60 mg, 0.066 mmol), and dppf (148 mg, 0.267 mmol) was dissolved in NMP (8 ml) followed by the addition of Et_3N (0.45 ml, 3.23 mmol). The mixture was purged with N_2 for 15 min and then a solution of thiol **13** (~2.08 mmol) in NMP (3 ml) was added. The reaction mixture was heated at 60°C for 4 h and then partitioned between EtOAc (150 ml) and brine (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:3 → 1:1) to give **8** as a white solid (379 mg, 57%) as 1:9 inseparable mixture of isomers.

Major isomer: ^{1}H NMR (DMSO): δ 12.95 (1H, s, NO*H*), 7.78 (1H, s, H-4), 7.70 (2H, d, $J_{2'',3''}$ = 8.3 Hz, H-3″), 7.57 (2H, d, $J_{2'',3''}$ = 8.3 Hz, H-2″), 3.62–3.60 (4H, *m*, H-2′), 2.98 (3H, s, OC*H*$_3$), 1.99–1.90 (4H, *m*, H-3′); ^{13}C NMR (DMSO): δ 162.3 (C-2), 147.1 (C-5), 144.5 (1C, $J_{C,F}$ = 31.5 Hz, C-5″), 142.2 (C-4), 134.8 (C-4″), 132.7 (C-3″), 130.7 (C-2″), 127.8 (C-1″), 121.7 (1C, $J_{C,F}$ = 273.8 Hz, C-6″), 73.2 (C-4′), 63.4 (C-2′), 50.2 (O*C*H$_3$), 36.6 (C-3′).

HRMS calcd for $(C_{17}H_{17}F_3N_2O_3S_2+H)^+$ 419.0710, found 419.0705.

5-(4-Methoxytetrahydropyran-4-yl)-2-(4-(3-(trifluoromethyl)-diazirin-3-yl)phenylthio)thiazole (6)

Diazirine **12** (335 mg, 1.07 mmol), Pd_2dba_3 (45 mg, 0.049 mmol), and dppf (112 mg, 0.202 mmol) was dissolved in NMP (6 ml) followed by the addition of Et_3N (0.45 ml, 3.23 mmol) in dark. The mixture was purged with N_2 for 15 min and then a solution of freshly prepared thiol **13** (~1.52 mmol) in NMP (2 ml) was added. The reaction mixture was heated at 60°C for 5 h and then partitioned between EtOAc (150 ml) and brine (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:3 → 1:2) to give **6** as pale yellow syrup (128 mg, 29%). ^{1}H NMR (DMSO): δ 7.78 (1H, s, H-4), 7.70 (2H, d, $J_{2'',3''}$ = 8.6 Hz, H-3″), 7.38 (2H, d, $J_{2'',3''}$ = 8.8 Hz, H-2″), 3.63–3.60 (4H, *m*, H-2′), 2.97 (3H, s, OC*H*$_3$), 1.99–1.91 (4H, *m*, H-3′); ^{13}C NMR (DMSO): δ 162.0 (C-2), 147.3 (C-5), 142.2 (C-4), 135.1 (C-4″), 133.4 (C-3″), 129.1 (C-1″), 128.5 (C-2″), 122.4 (1C, $J_{C,F}$ = 274.9 Hz, C-6″), 73.2 (C-4′), 63.4 (C-2′), 50.2 (O*C*H$_3$), 36.6 (C-3′), 28.7 (1C, $J_{C,F}$ = 40.1 Hz, C-5″). HRMS calcd for $(C_{17}H_{16}F_3N_3O_2S_2+H)^+$ 416.0714, found 416.0719.

5-Bromo-2-(methylthio)thiazole (16)

To a stirred solution of 2-(methylthio)thiazole **9** (5.0 g, 38.2 mmol) in DMF (100 ml) was added NBS (8.15 g, 45.8 mmol). The mixture was stirred in dark for 16 h and then partitioned between EtOAc (300 ml) and H_2O (150 ml). The organic phase was washed with brine (150 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:8) to give **16** as pale yellow oil (6.3 g, 79%). ^{1}H NMR $CDCl_3$): δ 7.54 (1H, s, H-4), 2.75 (3H, s, S*C*H$_3$); ^{13}C NMR ($CDCl_3$): δ 167.7 (C-2), 144.0 (C-4), 106.7 (C-5), 16.7 (SCH$_3$). HRMS calcd for $(C_4H_4BrNS_2+H)^+$ 209.9046, found 209.9041.

4-Bromo-5-(4-hydroxytetrahydropyran-4-yl)-2-(methylthio)thiazole (17)

A solution of compound **16** (3.0 g, 14.3 mmol) in THF (8 ml) was added to a stirred solution of freshly prepared LDA (n-BuLi (11.4 ml, 2.5 M in hexanes, 28.5 mmol) and diisopropylamine (4.0 ml, 28.5 mmol) in THF (20 ml)) at −78°C under N_2. After

stirring for 20 min at rt, the reaction mixture was cooled to −78°C, followed by addition of tetrahydropyran-4-one **10** (2.65 ml, 28.6 mmol) dropwise. The mixture was stirred at rt for 16 h and then partitioned between EtOAc (200 ml) and 1 M HCl (50 ml). The organic phase was washed with H_2O (50 ml), brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:2 → 1:1) to give **17** as brown syrup (1.92 g, 43%). ^{1}H NMR ($CDCl_3$): δ 3.87–3.84 (4H, *m*, H-2ax′, H-2eq′), 2.93 (1H, s, O*H*), 2.66 (3H, s, SCH_3), 2.43 (2H, ddd, $J_{2ax',3eq'}$ = 7.4 Hz, $J_{2ax',3ax'}$ = 10.0 Hz, $J_{3ax',3eq'}$ = 13.9 Hz, H-3ax′), 1.87–1.84 (2H, *m*, H-3eq′); ^{13}C NMR ($CDCl_3$): δ 164.9 (C-2), 139.7 (C-5), 118.4 (C-4), 69.6 (C-4′), 63.2 (C-2′), 37.0 (C-3′), 16.5 (S*C*H$_3$). HRMS calcd for $(C_9H_{12}BrNO_2S_2+H)^+$ 309.9571, found 309.9566.

4-Bromo-5-(4-methoxytetrahydropyran-4-yl)-2-(methylthio)thiazole (18)

NaH (100 mg, 60% in oil, 2.5 mmol) was added to a stirred solution of compound **17** (460 mg, 1.5 mmol) in DMF (10 ml) at 0°C under N_2. After stirring for 10 min, MeI (115 µl, 1.8 mmol) was added and the mixture was stirred at rt for 2 h. The reaction mixture was partitioned between EtOAc (100 ml) and H_2O (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:3) to give **18** as pale yellow solid (420 mg, 88%, mp 85–87°C). ^{1}H NMR $CDCl_3$: δ 3.81–3.79 (4H, *m*, H-2′), 3.17 (3H, s, OCH_3), 2.69 (3H, s, SCH_3), 2.22–2.12 (4H, m, H-3′); ^{13}C NMR ($CDCl_3$): δ 165.7 (C-2), 135.4 (C-5), 120.9 (C-4), 73.7 (C-4′), 63.3 (C-2′), 50.3 (O*C*H$_3$), 35.2 (C-3′), 16.3 (S*C*H$_3$). HRMS calcd for $(C_{10}H_{14}BrNO_2S_2+H)^+$ 323.9727, found 323.9724.

4-Bromo-5-(4-methoxytetrahydropyran-4-yl)-2-(4-(3-(trifluoromethyl)-diazirin-3-yl)phenylthio)thiazole (15)

To a stirred solution of **18** (450 mg, 1.39 mmol) in $CHCl_3$ (15 ml) at 0°C was added MCPBA (340 mg, 77%, 1.51 mmol) and the mixture was stirred for 90 min. $Ca(OH)_2$ (190 mg, 2.56 mmol) was added and the mixture was stirred at rt for 20 min, filtered, and concentrated to give essentially pure sulfoxide. The residue was dissolved in TFAA (8 ml) and refluxed for 45 min and concentrated. The mixture was diluted with MeOH–Et_3N (4:1, 10 ml) and evaporated to dryness under high vacuum to give crude thiol **19** as the triethylammonium salt. In a separate flask, diazirine **12** (450 mg, 1.45 mmol), Pd_2dba_3 (80 mg, 0.087 mmol), and dppf (196 mg, 0.354 mmol) was dissolved in NMP (8 ml) followed by the addition of Et_3N (0.60 ml, 4.31 mmol) in dark. The mixture was purged with N_2 for 15 min and then heated to 60°C. A solution of **19** (~1.39 mmol) in NMP (4 ml) was added over a 30-min period. The reaction mixture was stirred at 60°C for 16 h and then partitioned between EtOAc (150 ml) and brine (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:7 → 1:5) to give **15** as colorless syrup (115 mg, 22%). ^{1}H NMR (CD_3OD): δ 7.73 (2H, d, $J_{2'',3''}$ = 8.2 Hz, H-3′′), 7.36 (2H, d, $J_{2'',3''}$ = 8.6 Hz, H-2′′), 3.74–3.72 (4H, *m*, H-2′), 3.12 (3H, s, OCH_3), 2.19–2.03 (4H, *m*, H-3′); ^{13}C NMR (CD_3OD): δ 163.8 (C-2), 139.4 (C-5), 133.9 (C-3′′), 133.4 (C-4′′), 130.6 (C-1′′), 128.0 (C-2′′), 122.2 (1C, $J_{C,F}$ = 281.4 Hz, C-6′′), 121.6 (C-4), 73.9 (C-4′), 63.0 (C-2′), 49.7 (O*C*H$_3$), 34.9 (C-3′), 28.7 (1C, $J_{C,F}$ = 40.4 Hz, C-5′′). HRMS calcd for $(C_{17}H_{15}BrF_3N_3O_2S_2+H)^+$ 493.9819, found 493.9818.

5-Bromo-2-(4-(3-(trifluoromethyl)-diazirin-3-yl)phenylthio)thiazole (21)

Diazirine **12** (600 mg, 1.92 mmol), Pd_2dba_3 (90 mg, 0.09 mmol), and dppf (230 mg, 0.41 mmol) was dissolved in NMP (12 ml) followed by the addition of Et_3N (0.70 ml, 5.0 mmol) in dark. The mixture was purged with N_2 for 15 min and then a solution of 2-mercaptothiazole **20** (320 mg, 2.73 mmol) in NMP (3 ml) was added. The reaction mixture was heated at 60°C for 24 h and then partitioned between EtOAc (150 ml) and brine (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. Polar impurities were removed by flash chromatography (EtOAc-hexanes, 1:9) and the residue was dissolved in DMF (50 ml). NBS (400 mg, 2.24 mmol) was added and the reaction mixture was stirred at rt in dark for 18 h. The mixture was diluted with EtOAc (150 ml) and washed with H_2O (50 ml), brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:10) to give compound **21** as pale yellow oil (378 mg, 51%).

^{1}H NMR ($CDCl_3$): δ 7.63 (1H, s, H-4), 7.57 (2H, d, $J_{2'',3''}$ = 8.4 Hz, H-3′′), 7.20 (2H, d, $J_{2'',3''}$ = 8.6 Hz, H-2′′); ^{13}C NMR ($CDCl_3$): δ 162.8 (C-2), 143.9 (C-4), 132.9 (C-4′), 131.7 (C-3′), 129.2 (C-1′), 126.6 (C-2′), 120.8 (1C, $J_{C,F}$ = 274.8 Hz, C-6′′), 109.2 (C-5), 27.2 (1C, $J_{C,F}$ = 40.7 Hz, C-5′′). HRMS calcd for $(C_{11}H_5BrF_3N_3S_2+H)^+$ 379.9138, found 379.9137.

Alternative method for the synthesis of 4-Bromo-5-(4-methoxytetrahydropyran-4-yl)-2-(4-(3-(trifluoromethyl)-diazirin-3-yl)phenylthio)thiazole (15)

A solution of compound **21** (635 mg, 1.67 mmol) in THF (4 ml) was added to a stirred solution of freshly prepared LDA (*n*-BuLi (1.6 ml, 2.5 M in hexanes, 4.0 mmol) and diisopropylamine (0.56 ml, 4.0 mmol) in THF (6 ml)) at −78°C under N_2. After stirring for 15 min at rt, the reaction mixture was cooled to −78°C, followed by addition of tetrahydropyran-4-one **10** (0.4 ml, 4.32 mmol) dropwise. The mixture was stirred at rt for 16 h and then partitioned between EtOAc (150 ml) and 1 M HCl (50 ml). The organic phase was washed with H_2O (50 ml), brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. Most impurities were removed by flash chromatography (EtOAc-hexanes, 1:3 → 1:2) and the residue was dissolved in DMF (15 ml). The mixture was cooled to 0°C and then NaH (49 mg, 60% in oil, 1.22 mmol). After stirring for 10 min, MeI (60 µl, 0.96 mmol) was added and the mixture was stirred at rt for 90 min. The reaction mixture was partitioned between EtOAc (50 ml) and H_2O (20 ml). The organic phase was washed with brine (20 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:4) to give **15** as colorless syrup (207 mg, 25%).

4-Iodo-5-(4-methoxytetrahydropyran-4-yl)-2-(methylthio)thiazole (22)

nBuLi (0.4 ml, 2.5 M in hexanes, 1 mmol) was added to a stirred solution of compound **18** (220 mg, 0.68 mmol) in Et_2O (10 ml) at −78°C under Ar. After stirring for 30 min, a solution of 1,2-diiodoethane (280 mg, 0.99 mmol) in Et_2O (3 ml) was added dropwise. The mixture was stirred at −78°C for 30 min and then

at rt for 2 h. The reaction was quenched with sat. NH_4Cl (5 ml) and partitioned between EtOAc (100 ml) and H_2O (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:3) to give **22** as a white solid (194 mg, 77%, mp 109–111°C). ^{1}H NMR $CDCl_3$: δ 3.81–3.79 (4H, *m*, H-2′), 3.14 (3H, s, OCH_3), 2.68 (3H, s, SCH_3), 2.24 (2H, ddd, $J_{2eq',3eq'} = 2.6$ Hz, $J_{2ax',3eq'} = 4.9$ Hz, $J_{3ax',3eq'} = 14.2$ Hz, H-3eq′), 2.08–2.04 (2H, m, H-3ax′); ^{13}C NMR ($CDCl_3$): δ 167.1 (C-2), 138.9 (C-5), 90.9 (C-4), 73.6 (C-4′), 63.3 (C-2′), 50.2 (OCH_3), 35.5 (C-3′), 16.5 (SCH_3). HRMS calcd for $(C_{10}H_{14}INO_2S_2+H)^+$ 371.9588, found 371.9584.

4-Iodo-5-(4-methoxytetrahydropyran-4-yl)-2-(4-(3-(trifluoromethyl)-diazirin-3-yl)phenylthio)thiazole (24)

To a stirred solution of **22** (302 mg, 0.81 mmol) in $CHCl_3$ (10 ml) at 0°C was added MCPBA (195 mg, 77%, 0.88 mmol) and the mixture was stirred for 90 min. $Ca(OH)_2$ (142 mg, 1.92 mmol) was added and the mixture was stirred at rt for 20 min, filtered, and concentrated to give essentially pure sulfoxide. The residue was dissolved in TFAA (8 ml) and refluxed for 45 min and concentrated. The mixture was diluted with MeOH-Et_3N (4:1, 10 ml) and evaporated to dryness under high vacuum to give crude thiol **23 as the** triethylammonium salt. In a separate flask, diazirine **12** (350 mg, 0.98 mmol), Pd_2dba_3 (80 mg, 87 μmol), and dppf (196 mg, 0.35 mmol) was dissolved in NMP (8 ml) followed by the addition of Et_3N (0.40 ml, 2.8 mmol) in dark. The mixture was purged with N_2 for 15 min and then heated to 60°C. A solution of **23** (~0.81 mmol) in NMP (4 ml) was added over a 20-min period. The reaction mixture was stirred at 60°C for 16 h and then partitioned between EtOAc (150 ml) and brine (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc-hexanes, 1:7 → 1:5) to give **24** as pale yellow syrup (118 mg, 27%). ^{1}H NMR (CD_3OD): δ 7.72 (2H, d, $J_{2'',3''} = 8.3$ Hz, H-3′′), 7.35 (2H, d, $J_{2'',3''} = 8.7$ Hz, H-2′′), 3.76–3.73 (4H, *m*, H-2′), 3.10 (3H, s, OCH_3), 2.26–2.21 (2H, *m*, H-3eq′), 2.03–1.97 (2H, *m*, H-3ax′); ^{13}C NMR (CD_3OD): δ 164.9 (C-2), 143.0 (C-5), 133.8 (C-4′′), 133.7 (C-3′′), 130.5 (C-1′′), 127.9 (C-2′′), 122.2 (1C, $J_{C,F} = 281.5$ Hz, C-6′′), 91.9 (C-4), 73.7 (C-4′), 63.0 (C-2′), 49.5 (OCH_3), 35.2 (C-3′), 28.2 (1C, $J_{C,F} = 40.3$ Hz, C-5′′). HRMS calcd for $(C_{17}H_{15}F_3IN_3O_2S_2+H)^+$ 541.9680, found 541.9683.

Hydrogenation of compound 24

Pt/C sulfided (10 mg, 5% on C) was added to a stirred solution of compound **24** (5 mg, 9 μmol) in EtOH (2 ml). The suspension was briefly purged with hydrogen gas and then a balloon filled with hydrogen gas was attached. The suspension was stirred at room temperature for 18 h. The solid material was filtered and washed with EtOH (1 ml). The filtrate was concentrated and the residue was purified by flash chromatography (EtOAc-hexanes, 1:2) to give **6** as colorless syrup (3 mg, 78%).

Deuteriation of compound 24

Pt/C sulfided (9 mg, 5% on C) was added to a stirred solution of compound **24** (3 mg, 5.5 μmol) in EtOAc (1.5 ml) and Et_3N (5 μl, 35 μmol). The suspension was briefly purged with deuterium gas and then a balloon filled with deuterium gas was attached. The suspension was stirred at room temperature for 48 h. The solid material was filtered and the filtrate was concentrated. ^{1}H NMR and MS of the crude material show that there was 50% deuterium incorporation.

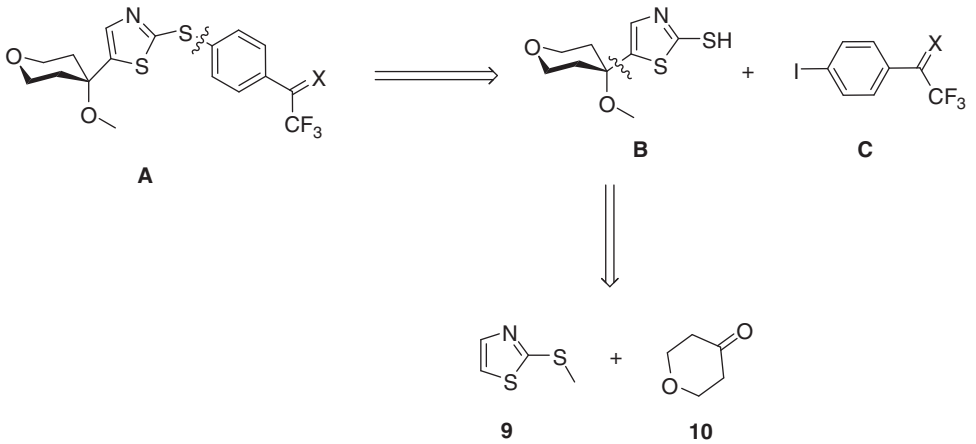
Tritiation of compound 24

Following the deuteriation procedure with tritium gas (at ca. 200 mm Hg and extending the reaction period to 4 days) gave the tritiated compound **7**. The crude product was purified by reverse-phase HPLC (Luna Polar RP, CH_3CN/H_2O 30:70 isocratic) to give probe **7** (0.222 GBq) with specific activity of 303 GBq/mmol representing a 28% radiochemical yield. Identity of **7** was confirmed by LC/MS and HPLC co-elution with unlabeled reference.

Results and discussion

Retrosynthetic analysis indicated that thioether **A** could be synthesized by coupling thiol **B** with aryl iodide **C** (Scheme 1). The key intermediate **B** could, in turn, be prepared from commercially available 2-(methylthio)thiazole (**9**) and tetrahydropyran-4-one (**10**). This route was chosen in order to provide flexibility in synthesizing compounds that have different functional groups attached to the phenyl ring.

The oxime (**11**) and the diazirine (**12**) were synthesized from 1,4-diiodobenzene using the procedure of Topin *et al.* (Scheme 2).[18] The key intermediate **13**, corresponding to **B**, was prepared from



Scheme 1. Retrosynthetic analysis of compound **A**.

commercially available 2-(methylthio)thiazole (**9**) (Scheme 3).[19] Palladium catalyzed cross-coupling of thiol **13** with either oxime **11** or diazirine **12** gave the target compounds **8** and **6**, respectively (Scheme 4).

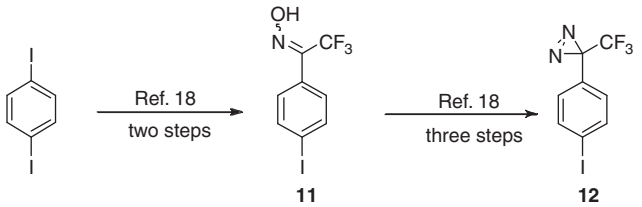
To save the expensive isotopically enriched reagent and to reduce the number of steps involving radioactive intermediates, we envisioned that the tritium could be introduced as the final synthetic step by tritium reduction of brominated compound **15**. Several attempts were made to directly introduce a bromine atom at C-4 of the thiazole moiety of compound **6**. However, in each case, there was considerable decomposition under the standard condition for lithiation/bromination or with NBS and the desired product could not be isolated. Hence, we introduced bromine at an earlier step, as shown in Scheme 5. 2-(Methylthio)thiazole (**9**) was treated with NBS to give compound **16** with bromine at C-5. When compound **16** was subjected to LDA, it underwent the 'halogen dance' (halogen migration) reaction where the bromide migrates to C-4.[20] Quenching the reaction with water gave compound with a bromide at C-4 position. The reason for halogen dance reaction can be explained in terms of the acidity of position 4 and 5 of thiazole, which correlates with the stability of the lithiated species formed. The 5-position is more acidic than the 4-position, hence, the ultimate lithiation is more favored at position 5.[20] Treating the lithiated intermediate with tetrahydropyran-4-one (**10**) gave alcohol **17**, which was subsequently methylated to give thioether **18**. The methyl group on the sulfide was cleaved using a mild, one-pot, three-step procedure via Pummerer rearrangement of the corresponding sulfoxide to give thiol **19** as the triethylammonium salt.[21] Palladium-catalyzed cross-coupling of **19** with diazirine **12** gave the desired compound **15**.

Alternatively, compound **15** was synthesized from commercially available 2-mercaptothiazole (**20**), as shown in Scheme 6. Diazirine **12** was first coupled with 2-mercaptothiazole (**20**) and on treatment with NBS gave compound **21**. The brominated compound **21** underwent halogen dance reaction when subjected to LDA and quenching the lithiated intermediate with tetrahydropyran-4-one (**10**) gave an alcohol, which was subsequently methylated to give compound **15**.

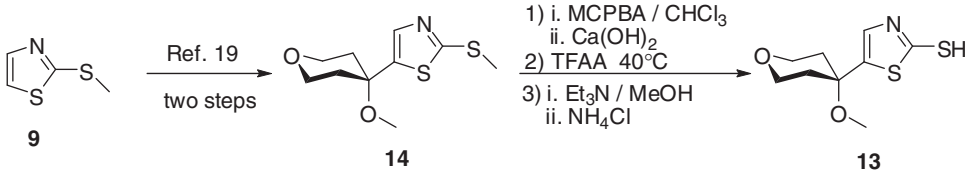
Attempts were made to dehalohydrogenate compound **15** using H_2. Various conditions and catalysts were tried; however, we could not isolate the desired product in which the bromide is exchanged with hydrogen. With Pd as catalyst, only decomposed starting material was isolated, whereas with Pt, there was no reaction as indicated by TLC. Presumably, the rate at which Pt reacts is too slow, whereas Pd is reacting faster with thioether than with bromide, which in turn, cleaves the thioether and poisons the catalyst. Hence, we envisioned that an iodinated compound might be more appropriate, as it will undergo hydrogenation reaction faster than bromide and perhaps the iodide would react faster than thioether with Pd as catalyst. In addition, the iodo-hydrogen exchange might be fast enough to be carried out with Pt as catalyst.

Numerous attempts were made to iodinate compound **15** directly by metallation; however, no desired product was obtained. The lithium–iodine exchange was successful with intermediate **18** and treating the resulting lithiated intermediate with 1,2-iodoethane gave compound **22** (Scheme 7). The methyl group on the sulfide was cleaved to give intermediate **23,** which was subsequently coupled with diazirine **12** to furnish iodinated compound **24**.

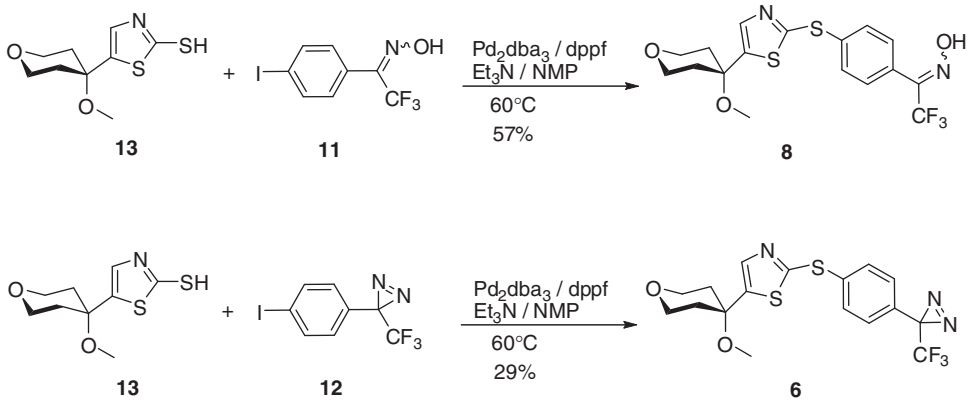
The hydrogenation of compound **24** proceeded smoothly with H_2 and 5% Pt/C (sulfided) in ethanol to give compound **6** in



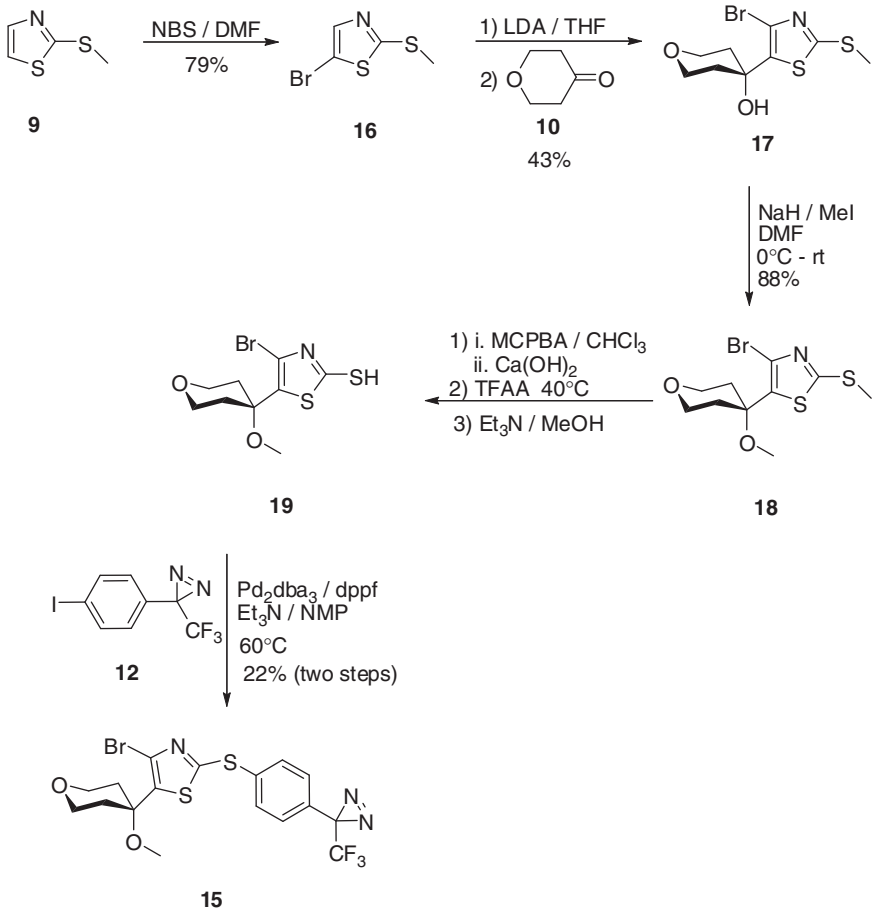
Scheme 2. Synthesis of oxime **11** and diazirine **12**.



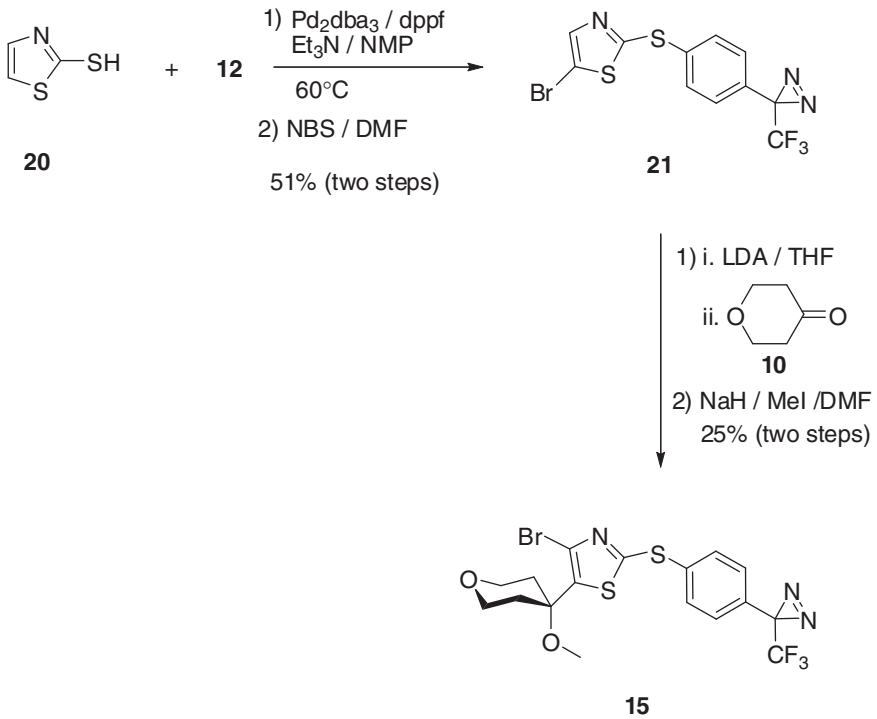
Scheme 3. Synthesis of thiol **13**.



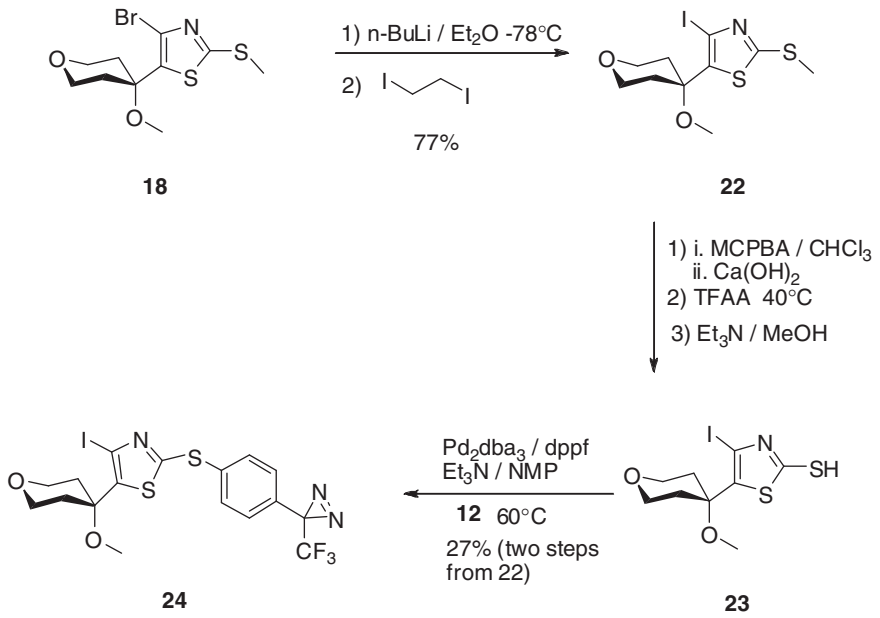
Scheme 4. Palladium catalyzed cross-coupling of thiol **13** with oxime **11** and diazirine **12**.



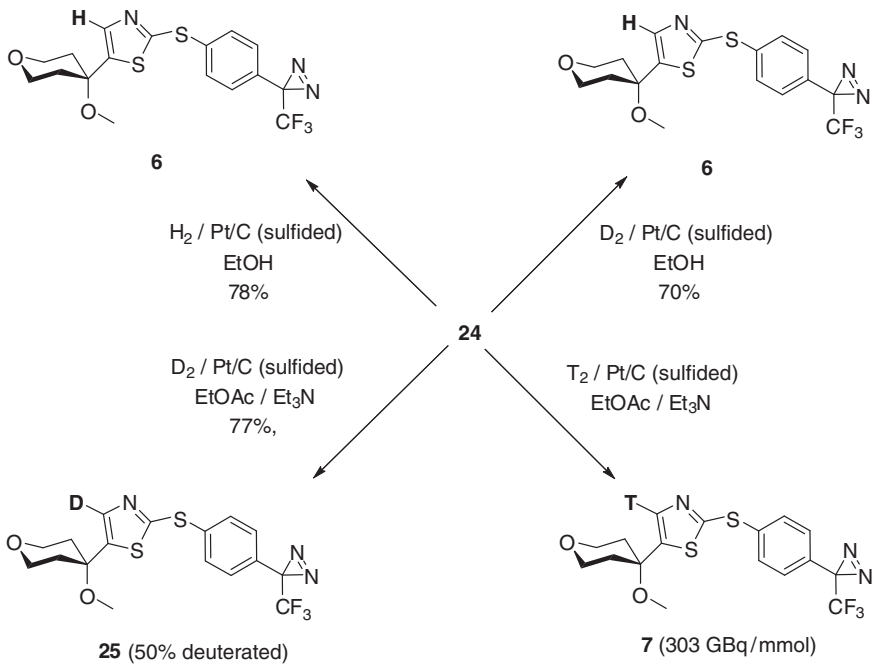
Scheme 5. Synthesis of compound **15**.



Scheme 6. Alternative method for the synthesis of compound **15**.



Scheme 7. Synthesis of iodinated compound **24**.



Scheme 8. Hydrogenation/deuteriation/tritiation of compound **24**.

78% yield (Scheme 8). The hydrogenation reaction also worked with Pd as catalyst, however, there was formation of side products as indicated by TLC. Unfortunately, when the reaction was repeated using deuterium gas, there was no incorporation of deuterium as indicated by MS and ^{1}H NMR. The proton introduced is most likely coming from the protic solvent. When the same reaction was carried out in aprotic solvent, 50% deuterated product (**25**) was isolated. The Pt/C catalyst and/or the deuterium gas might contain trace amount of H_2O, which could account for the 50% of the undesired protonated product **6** although no improvement in incorporation was obtained even after rigorously drying of the solvents and reagents. Similar reaction with tritium gas afforded the desired tritiated compound **7,** which was confirmed by LC/MS and HPLC co-elution with cold standard **6**. The specific activity of compound **7** was determined to be 303 GBq/mmol (28% radiochemical yield).

In summary, we have prepared oxime (**8**), photo-affinity ligand (**6**) and tritiated photo-affinity probe (**7**) with a trifluoromethyldiazirine unit. These compounds are currently being tested against 5-LO and in human whole blood cell assay. Compound **7** will be used to photo-affinity label protein and protein complexes with which this type of compound interact with in inflammatory cells.

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