Synthesis of Diazirin Based Photoreactive Saccharin Derivatives for the Photoaffinity Labeling of Gustatory Receptors.

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Abstract: Saccharine is well known as one of the most common artificial sweeteners and also has a bitter taste at high concentrations. At present, there has been no detailed functional analysis of these gustatory receptors. Therefore, we designed and synthesized photoreactive saccharine derivatives containing the trifluoromethyldiazirinyl moiety at the 5- or 6-position, for use as functional analysis tools for photoaffinity labeling.

Introduction

Humans distinguish gustatory sensations as five basic taste: bitterness, saltiness, sourness, sweetness, and savoriness. Sweetness is almost universally regarded as a pleasurable experience for human beings. Numerous chemical substances, both natural and artificial, have been reported as sweeteners. Saccharine is one of the most common artificial sweeteners in the world, owing to its several hundred times higher sweetness than that of sucrose, and has a bitter taste at high concentrations. Sweet and bitter taste receptors are both G protein-coupled receptors (GPCRs). For saccharin, the bioactivity underlying its sweetness involves binding with the sweet taste receptor, which has a heterodimeric structure with T1R2 and T1R3 subunits. Each subunit has a large amino-terminal domain (ATD) linked by a cysteine-rich domain (CRD) at the extracellular site to a seven transmembrane helical domain (TMD).[1] The human heterodimeric sweet taste receptor (hT1R2-hT1R3) responds to a wide variety of chemical substances, both natural and artificial.[2] Although these sweeteners have various chemical structures, all of the compounds bind to the same sweet taste receptor.[3,4] As the receptor can distinguish saccharin, the structural relationships between saccharin and the other sweeteners, as well as the structural features of saccharin derivatives that favor the activation of the sweet taste receptor, have been studied through conformational analysis using crystallography, NMR analysis, and molecular modeling. However, the receptor-bound conformations of the sweeteners remain unclear, owing to limited structural information on the ligands complexes with the receptor.

Photoaffinity labeling is a useful biochemical method to explore the structural and functional relationships between low molecular weight bioactive compounds and biomolecules.[5] This method is suitable for analyzing biological interactions as it is based on the affinity of bioactive compounds for biomolecules. Various photophores, such as phenyldiazirine, arylazide and benzophenone, are used. To the best of our knowledge, synthesis of saccharine derivatives for photoaffinity labeling has not been reported yet. Arylazide saccharin, which can utilize as photoaffinity labeling reagents, has been already reported very recently. But the arylazide moiety was utilized for click reaction substrates and did not apply for photoaffinity labeling.[6] Although comparative irradiation studies of these three photophores in living cells indicates that a carbene precursor, (3-trifluoromethyl)phenyldiazirine, is the most promising photophore,[7] the relatively complicated synthesis of the (3-trifluoromethyl)phenyldiazirinyl ring has resulted in fewer applications in biomolecular studies compared to other photophores. To resolve this problem, we have reported on the post-functional synthesis of a family of 3-trifluoromethyl-phenyldiazirines using many reaction conditions.[8] Suami et al reported on several structure-activity relationships for saccharine, and found that substitutions at the 5- and 6- positions in saccharine were tolerated with regards to its biological activities.[9] In addition, some sweet compounds also interact with other taste modalities. For example, saccharine triggers both the sweet and bitter taste modalities. The mechanisms underlying changes in taste modalities have not yet been elucidated.[10] In this report, we aim to describe the novel synthesis of photoreactive trifluoromethyldiazirinyl saccharine derivatives, which were utilized to elucidate the sweet and bitter mechanisms, with post-functional derivatizations[11] for (3-trifluoromethyl)phenyldiazirine derivatives.

Results and Discussion

Although several methods for the synthesis of saccharine have been reported,[12] we were required to choose the unaffected synthetic methods for the diazirine three-membered ring structure. The each synthetic steps for oxidative cyclization of *N*-*t*Bu-*o*-toluenesulfonamide derivatives to construct saccharin skeleton [13] could be applied without decomposition of trifluoromethyldiazirine moiety. 2,2,2-trifluoro-1-(*p*-tolyl)ethanone (**1a**) was treated with hydroxylamine hydrochloride in the presence of sodium hydroxide in ethanol at reflux temperature for 16 h, under previously reported conditions,[14] resulting in 87% yield. The isolated yield increased to 95% within 3 hours when using pyridine as a solvent at 70 °C.[15] The regioisomer (**1b**) was also converted to the corresponding oxime (**2b**) under

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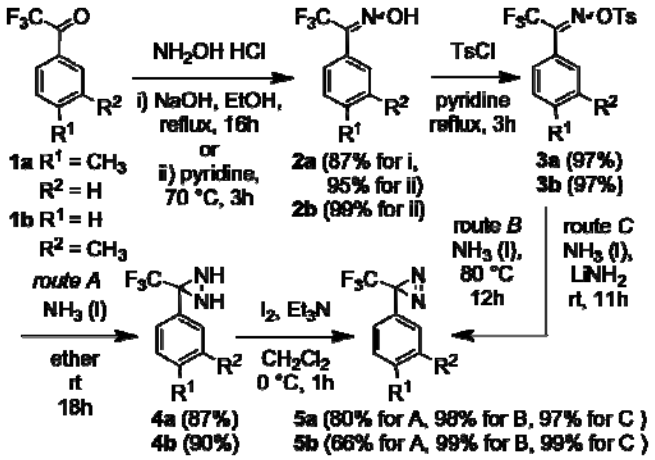
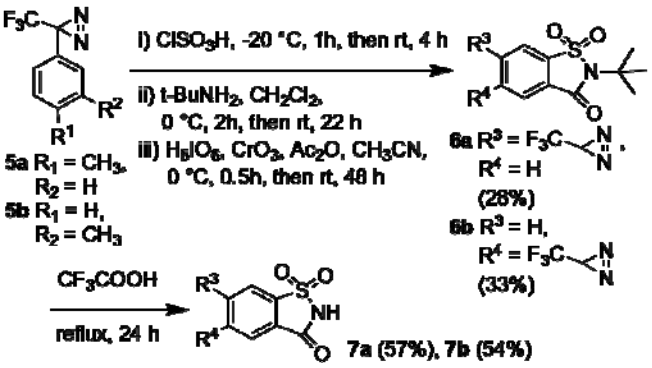
identical conditions with **1a** in 99% yield. The oximes were converted to tosyl oximes (**3a** and **3b**) with TsCl in pyridine with very good yield. We then subjected the tosyl oximes to classical stepwise conversions to diazirines (**5a**[14] and **5b**[16]) via diaziridines (**4a** and **4b**) with up to 70% yield for the two steps (Scheme 1, route A). We recently reported on effective one-pot conversions to diazirine derivatives from the corresponding tosyl oximes.[17] The tosyl oximes were subjected to two conditions. One was treatment of the tosyl oximes in liquid ammonia at 80 °C for 12 hours in a stainless pressure-resistant tube. A detailed product analysis revealed that diaziridine formation occurred within an hour, then slow conversion to diazirine with in situ generated $^{-}NH_2$ species at high temperature (Scheme 1, route B). The reaction rate for the latter step was improved by adding lithium amide to enhance the concentration of $^{-}NH_2$ at lower temperature (room temperature) than that in the conditions without lithium amide (Scheme 1 route C) than the condition without $LiNH_2$. The chemical yields of both one-step conversions to diazirine from tosyl oxime were almost quantitative.



Scheme 1. Improved synthesis of 3-(*m*- or *p*- tolyl)-3-(trifluoromethyl)-3*H*-diazirines

3-(*p*- or *m*- tolyl)-3-(trifluoromethyl)-3*H*-diazirines (**5a** and **5b**) were subjected to aromatic chlorosulfonation at adjacent positions of the toluene methyl group with chlorosulfonic acid at -20 °C. Adding of the strong acid at a higher temperature promoted decomposition of the diazirinyl ring.[18] The sulfonyl chloride was converted to *N*-alkylated sulfonamide with *t*$BuNH_2$ at room temperature. The reaction in the triethylamine and *t*$BuNH_2$ system (both 2 equivalents; experimental conditions are reported in the reference[13]) afforded a more complex mixture than that in *t*$BuNH_2$ only (4 equivalents). Oxidative cyclization with H_5IO_6 and CrO_3 between the methyl group and *o*-oriented *N*-tert-butyl sulfonamide synthesized the saccharine skeleton (**6a** and **6b**) in a moderate yield. The purifications for each step generated lower isolated yields for the cyclized compounds **6a** and **6b** (less than 10%). We performed these three steps as one-pot reactions (~30%). The deprotection of the *t*Bu group with TFA under reflux conditions afforded diazirinyl saccharine derivatives (**7a** and **7b**, Scheme 2). The synthesized photoreactive saccharine derivatives were insoluble in water. We performed further purification of the synthesized saccharine derivatives, converting them to the corresponding sodium salts with aqueous sodium hydroxide, then subjecting them to reversed phase HPLC. Both sample were eluted at 22.5 and 21.0 min on an ODS column with 30% methanol for **7a** and **7b**, respectively, at 215 nm (Figure 1). These peaks were also detected at 350 nm measurements. The ^{19}F-NMR for the (trifluoromethyl)-3*H*-diazirines (-65 ppm) reveals three-membered ring, and is identical to that previous reported.[18] These results indicate that the diazirinyl groups are preserved in the final compounds.



Scheme 2. Post-functional synthesis of trifluoromethyldiazirinyl saccharin derivatives at 5- or 6-positions.

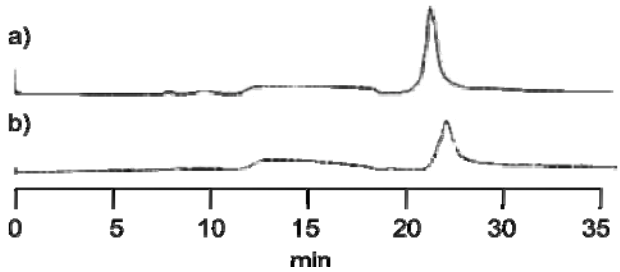
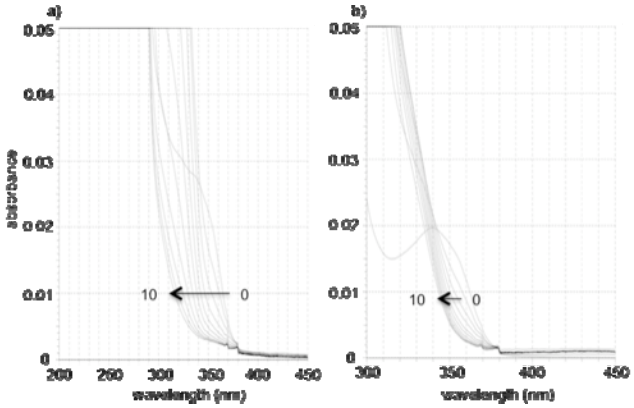


Figure 1. HPLC profiles of 5- or 6-trifluoromethyldiazirinyl sodium saccharin. The chromatograms for sodium salts of **7a** and **7b** are a) and b), respectively. HPLC conditions; Tosoh TSKgel ODS-80Ts (4.6 x 250 mm), 30% MeOH/ H_2O, flow rate 1 mL/ min, detection at 215 nm.

Irradiation studies of a 1 mM methanolic solution of **7a** and **7b** using 15 W black light have indicated that the diazirinyl moiety of both saccharine derivatives decomposed very rapidly. Reports have suggested that diazo derivatives, which are one of the main by-products of the irradiation of diazirines, cannot generate carbene under irradiation at 350 nm at a diazirine concentration of over 10 mM.[19] However, a diluted (< 1mM) solution can generate carbene from diazo compounds beyond 10 min of irradiation at 350 nm. The absorbance at around 350 nm, which is characteristic of diazirinyl three membered ring,[20] diminished with increasing irradiation time. The half-life of the diazirinyl

saccharine derivatives **7a** and **7b** were calculated at 77 and 107 sec, respectively. These half-lives are more rapid than those of the diazirinyl α-amino acid derivatives[21] that we have already reported on with the photoreactive D-isomer acting as the sweetener.[22] A required characteristic for photoaffinity labeling was sufficiently met, in that no influence of other biomolecules was promoted. The photoreactivities of these compounds were also consisted with previous ^{19}F-NMR studies, in which, ^{19}F-NMR chemical shifts changed from -65 ppm to -75 ppm after 10 min of irradiation. The results indicated that the diazirine moieties generated carbenes, which were then quenched by the solvent.



Scheme 3. Photolysis of diazirin-based saccharin derivatives **7a** (a) and **7b** (b) in methanol (1 mM) with black light (15W). UV spectra of the photolysis reaction were recorded every 1 min for 10 min.

The synthesized photoreactive saccharine derivatives were subjected to preliminary gustatory receptor assays at 10 mM. **7a** and **7b** have 70 and 90 % relative sweetness activity, respectively, against the same concentration of saccharin for the hT1R2-hT1R3 expressed HEK-293T cell.[23] The bitter receptor hTAS2R44 response to **7a** and **7b** at 10 mM activity was calculated as 65 % and 80 %, respectively. Other bitter receptors, such as hTAS2R43,[10] could not respond to 10 mM **7a** and **7b**. The photoreactive compounds will be subjected to further detailed biological analyses for their gustatory responses.

Conclusions

These results indicate that the preparation of the diazirinyl saccharine derivatives is effective, and that these photoreactive compounds have enough affinity for the sweet and bitter taste receptors to elucidate the binding sites of their ligands in these receptors. This will ultimately allow an understanding of the underlying molecular mechanisms of gustatory receptors.

Experimental Section

General Remarks: NMR spectra were measured by JEOL EX-270 or Bruker AMX500 spectrometers. All solvents were of reagent grade and distilled using the appropriate methods. ESI-TOF-MS data were obtained with a Waters UPLC ESI-TOF mass spectrometer.

2,2,2-trifluoro-1-(*p*-tolyl)ethanone oxime (2a): i) in EtOH: 2,2,2-trifluoro-1-(*p*-tolyl)ethanone **1a** (0.1041 g, 0.55 mmol) in EtOH (5 mL) was added to hydroxylamine hydrochloride (0.0459 g, 0.66 mmol) and NaOH (0.1114 g, 1.60 mmol) in EtOH (5 mL). The reaction mixture was refluxed for 16 h, and then concentrated. The residue was partitioned between ether and water. The organic layer was washed with 0.01 M HCl and water, dried over $MgSO_4$, filtrated and concentrated to afford colorless amorphous mass (0.0974 g, 87%). ii) in pyridine: 2,2,2-trifluoro-1-(*p*-tolyl)ethanone **1a** (1.1300 g, 6.01 mmol) was dissolved in pyridine (30 mL), then hydroxylamine hydrochloride (0.5005 g, 7.20 mmol) was added. The mixture was stirred at 70 °C for 1 h and was then subjected to rotary evaporation to remove pyridine. The residue was dissolved in ethyl acetate and washed with 1 M HCl, the organic layer was washed with H_2O and brine, dried over $MgSO_4$ and concentrated to afford colorless amorphous mass (1.1700 g, 96%). The product was mixture of *syn*- and *anti*- isomers: ^{1}H NMR (270 MHz, $CDCl_3$): δ = 9.01 (brs, 1H), 7.37–7.44 (m, 2H), 7.21–7.29 (m, 2H), 2.39 (s, 0.8H), 2.38 (s, 2.2H) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 148.2 (q, $^2J_{C,F}$ = 30.6 Hz), 141.2 and 141.0, 129.42 and 129.36, 128.7 and 128.3, 123.0, 118.5 (q, $^1J_{C,F}$ = 283.0 Hz), 21.3 and 21.2 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -62.4, -66.6 ppm. HRMS (ESI): calcd. for $C_9H_9F_3NO$ 204.0636; found 204.0632.

2,2,2-trifluoro-1-(*m*-tolyl)ethanone oxime (2b): The same treatment of 2,2,2-trifluoro-1-(*m*-tolyl)ethanone **1b** (1.1302 g, 6.01 mmol) in pyridine as that just described gave **2b** (1.2100 g, 99%) as colorless amorphous mass. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.76 (brs, 0.6H), 8.59 (brs, 0.4H), 7.29–7.40 (m, 4H), 2.40 (s, 1.8H), 2.39 (s, 1.2H) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 148.4 (q, $^2J_{CF}$ = 30.7 Hz), 148.1 (q, $^2J_{C,F}$ = 32.4 Hz), 138.6, 131.6 and 131.4, 129.8 and 129.1, 129.0 and 128.6, 125.9 and 125.7, 125.5, 120.7 (q, $^1J_{C,F}$ = 274.8 Hz), 118.4 (q, $^1J_{C,F}$ = 282.9 Hz), 21.2 and 21.1 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -62.4, -66.8 ppm. HRMS (ESI): calcd. for $C_9H_9F_3NO$ 204.0636; found 204.0628.

2,2,2-trifluoro-1-(*p*-tolyl)ethanone O-tosyl oxime (3a): To a solution of oxime **2a** (0.9102 g, 4.48 mmol) in acetone (30 mL) at 0 °C, triethylamine (1.87 mL, 13.4 mmol) was added. Then, *p*-toluenesulfonyl chloride (0.9445 g, 4.95 mmol) was added to the reaction mixture that was stirred at room temperature for 1 h. After evaporation, the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:3) to afford colorless amorphous mass (1.4962 g, 93%, mixture of *syn*- and *anti*-isomers). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.90 (d, *J* = 8.4 Hz, 2H), 7.28–7.40 (m, 4H), 7.22 (d, *J* = 8.4 Hz, 2H), 2.48 (s, 0.6H), 2.46 (s, 2.4H), 2.40 (s, 0.6H), 2.39 (s, 2.4H) ppm. ^{13}C NMR (67 MHz, $CDCl_3$): δ = 154.1 (q, $^2J_{C,F}$ = 32.1 Hz), 146.2 and 146.1, 142.6 and 142.4, 131.5 and 131.3, 129.9, 129.5, 129.2 and 129.1, 128.8 and 128.4, 124.8, 117.5 (q, $^1J_{C,F}$ = 283.9 Hz), 21.5, 21.3, 21.2 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -61.5, -66.5 ppm. HRMS (ESI): calcd. for $C_{16}H_{15}F_3NO_3S$ 358.0725; found 358.0745.

2,2,2-trifluoro-1-(*m*-tolyl)ethanone O-tosyl oxime (3b): The same treatment of **2b** (1.0405 g, 5.12 mmol) as that just described gave **3b** as colorless amorphous mass (1.6830 g, 92%, mixture of *syn*- and *anti*-isomers). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.87–7.92 (m, 2H), 7.30–7.40 (m, 4H), 7.16–7.23 (m, 2H), 2.48 (s, 0.7H), 2.46 (s, 2.3H), 2.39 (s, 0.7H), 2.37 (s, 2.3H) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 154.3 (q, $^2J_{C,F}$ = 32.3 Hz), 146.3 and 146.1, 138.8 and 138.7, 132.6 and 132.5, 131.6 and 131.3, 129.9 and 129.4, 129.3 and 129.1, 128.7 and 128.6, 127.7, 126.0, 125.5, 117.4 (q, $^1J_{C,F}$ = 283.8 Hz), 21.5, 21.2 and 21.1 ppm. ^{19}F NMR

(470 MHz, $CDCl_3$): δ = -61.5, -66.9 ppm. HRMS (ESI): calcd. for $C_{16}H_{15}F_3NO_3S$ 358.0725; found 358.0710.

3-(*p*-tolyl)-3-(trifluoromethyl)diaziridine (4a): To liquid NH_3 (20 mL) at -78 °C in a sealed tube, tosyloxime **3a** (1.0760 g, 2.85 mmol) in ether (5 mL) was added. The reaction mixture was stirred at room temperature for 8 h. After evaporation of NH_3 gas, the reaction mixture was partitioned between ether and water. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was subjected to silica-column chromatography (AcOEt/hexane 1:5) to afford 3-(*p*-tolyl)-3-(trifluoromethyl)diaziridine **4a** as colorless amorphous mass (0.5021 g, 87%). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.50 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 2.76 (d, *J* = 8.0 Hz, 1H), 2.38 (s, 3H), 2.19 (d, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 140.3, 129.5, 128.9, 128.1, 123.7 (q, $^1J_{C,F}$ = 278.1 Hz), 57.8 (q, $^2J_{C,F}$ = 35.9 Hz), 21.1 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -75.7 ppm. HRMS (ESI): calcd. for $C_9H_{10}F_3N_2$ 203.0796, found 203.0785.

3-(*m*-tolyl)-3-(trifluoromethyl)diaziridine (4b): The same treatment of **3b** (1.0301 g, 2.88 mmol) as that just described gave **4b** as colorless amorphous mass (0.5240 g, 90%). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.40 - 7.42 (m, 2H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 1H), 2.77 (d, *J* = 8.0 Hz, 1H), 2.38 (s, 3H), 2.21 (d, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 138.7, 131.7, 131.0, 128.73, 128.69, 125.3, 123.6 (q, $^1J_{C,F}$ = 278.2 Hz), 58.0 (q, $^2J_{C,F}$ = 35.9 Hz), 21.2 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -76.0 ppm. HRMS (ESI): calcd. for $C_9H_{10}F_3N_2$ 203.0796, found 203.0796.

3-(*p*-tolyl)-3-(trifluoromethyl)-3*H*-diazirine (5a): Route A (stepwise conversions via diaziridine); The diaziridine **4a** (0.1434 g, 0.71 mmol) was dissolved in CH_2Cl_2 (5 mL) and triethylamine (0.29 mL), and cooled at 0 °C. Iodine (0.1985 g, 0.78 mmol) was added dropwisely. The reaction mixture was stirred for 1 h and was washed with 1 M NaOH, H_2O and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated. The residue was subjected to silica-column chromatography ($CHCl_3$/EtOAc, 19:1) to afford **5a** colorless oil (0.1140 g, 80%). Route B (one-pot synthesis); To liquid NH_3 (10 mL) at -78 °C in a sealed tube, tosyloxime **3a** (0.7105 g, 1.99 mmol) was added. The reaction was stirred at 80 °C for 11 h. The sealed tube was cooled at -78 °C and the reaction mixture was diluted with Et_2O (50 mL). The sealed tube was warmed at room temperature to remove the ammonia gradually. The organic layer was washed by H_2O, brine, dried over $MgSO_4$ and carefully evaporated (0 °C) to afford colorless oil (0.3920 g, 98%). Route C (one-pot with $LiNH_2$); To liquid NH_3 (5 mL) at -78 °C in a sealed tube, tosyloxime **3a** (0.7110 g, 1.99 mmol) and $LiNH_2$ (0.2295 g, 10.00 mmol) was added. The reaction was stirred at room temperature for 12 h. The sealed tube was cooled at -78 °C and the reaction mixture was diluted with Et_2O (50 mL). The sealed tube was warmed at room temperature to remove the ammonia gradually. The organic layer was washed by H_2O (three times), brine, dried over $MgSO_4$ and carefully evaporated (0 °C) to afford colorless oil **(0.**3880 g, 97%)**.** ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.20 (d, *J* = 8.2 Hz, 2H), 7.08 (d, *J* = 8.2 Hz, 2H), 2.36 (s, 3H) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 140.0, 129.6, 127.8, 126.5, 122.4 (q, $^1J_{C,F}$ = 274.5 Hz), 28.3 (q, $^2J_{C,F}$ = 40.4 Hz), 21.0 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -65.4 ppm. HRMS (ESI): calcd. for $C_9H_8F_3N_2$ 201.0640, found 201.0632.

3-(*m*-tolyl)-3-(trifluoromethyl)-3*H*-diazirine (5b): Route A (stepwise conversions via diaziridine); The same treatment of **4b** (0.1434 g, 0.71 mmol) as that just described gave **5b** (0.0941 g, 66%) as colorless oil with identical manner described above. Route B (one-pot synthesis); The same treatment of **3b** (0.4040 g, 2.00 mmol) as that just described gave **5b** (0.3960 g, 99%) as colorless oil. Route C (one-pot with $LiNH_2$); The same treatment of **3b** (0.4082 g, 2.02 mmol) as that just described gave **5b** (0.4000 g, 99%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.28 (t, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.97 (s, 1H), 2.35 (s, 3H) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 138.9, 130.5, 129.2, 128.8, 127.1, 123.7, 122.4 (q, $^1J_{C,F}$ = 274.5 Hz), 28.3 (q, $^2J_{C,F}$ = 40.4 Hz), 21.2 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -65.2 ppm. HRMS (ESI): calcd. for $C_9H_8F_3N_2$ 201.0640; found 201.0644.

***N*-tert-butyl-6-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (6a):** Chlorosulfonic acid (0.38 mL, 5.7 mmol) was cooled at -20 °C. Compound **5a** (0.1150 g, 0.58 mmol) was added dropwise and the reaction mixture was stirred at same temperature for 1 h, warmed to room temperature, and stirred for 4 h, then poured into ether and ice water. The organic layer was washed with saturated $NaHCO_3$, dried over $MgSO_4$ and filtrated. The filtrate was concentrated to afford crude 2-methyl-5-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzene-1-sulfonyl chloride as pale yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.80 (d, *J* = 1.7 Hz, 1 H), 7.53 (dd, *J* = 1.1, 8.0 Hz, 2 H), 7.50 (d, *J* = 8.0 Hz, 2 H), 2.80 (s, 3 H) ppm. The crude residue in CH_2Cl_2 (1 mL) was added to *tert*-$BuNH_2$ (0.13 mL, 1.2 mmol) in CH_2Cl_2 (1 mL) at 0 °C. The reaction mixture was stirred at same temperature for 2 h, and then warmed to room temperature for 3 h. The reaction mixture was washed with 0.1N HCl and saturated $NaHCO_3$, dried over $MgSO_4$ and filtrated. The filtrate was concentrated to afford *N*-*tert*-butyl-2-methyl-5-(3-(trifluoromethyl)-3*H*-diazirin-3-yl) benzenesulfonamide as pale yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.84 (d, *J* = 2.3 Hz, 1 H), 7.35 (d, *J* = 8.0 Hz, 1 H), 7.29 (dd, *J* = 2.3, 8.0 Hz, 1 H), 4.45 (s, 1 H), 2.67 (s, 3 H), 1.23 (s, 9 H) ppm. CrO_3 (6.0 mg, 0.06 mmol) and acetic anhydride (0.43 mL, 4.5 mmol) were added to ortho-periodic acid (1.0576 g, 4.64 mmol) in CH_3CN (10 mL). The crude material in minimum volume in CH_3CN was added at 0 °C. The reaction mixture was stirred at room temperature for 2 d and concentrated. The residue was redissolved in EtOAc and washed with saturated $NaHCO_3$, saturated $Na_2S_2O_3$ and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated. The crude oil was subjected to silica gel column chromatography (hexane/CH_2Cl_2, 3:1) to afford **6a** as pale yellow amorphous mass (0.0560 g, 28%). λ_{max} (ε) (CH_3OH) 290 (850), 340 (300). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 8.0 Hz, 1 H), 7.64 (s, 1 H), 7.59 (d, *J* = 8.0 Hz, 1 H), 1.77 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$) δ 158.8, 138.7, 136.2, 131.9, 128.2, 125.2, 121.4 (q, $^1J_{C,F}$ = 275.5 Hz), 118.4, 61.8, 28.4 (q, $^2J_{C,F}$ = 41.6 Hz), 27.8 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -64.7 ppm. HRMS (ESI): calcd. for $C_{13}H_{13}F_3N_3O_2S$ 348.0630; found 348.0646.

***N*-tert-butyl-5-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (6b):** The same treatment of **5b** (0.1355 g, 0.68 mmol) as that just described for **5a** gave 2-methyl-4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzene-1-sulfonyl chloride. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.10 (d, *J* = 8.6 Hz, 1 H), 7.24 (d, *J* = 8.6 Hz, 1 H), 7.16 (s, 1 H), 2.80 (s, 3 H) ppm. The same treatment of the residue as that just described for above gave *N*-tert-butyl-2-methyl-4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl) benzenesulfonamide. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.05 (d, *J* = 8.6 Hz, 1 H), 7.12 (d, *J* = 8.6 Hz, 1 H), 7.04 (s, 1 H), 4.44 (s, 1 H), 2.66 (s, 3 H), 1.22 (s, 9 H). The same treatment of the residue with H_5IO_6, CrO_3 and acetic anhydride as that just described for **6b** (0.0781 g, 33%). λ_{max} (ε) (CH_3OH) 280 (900), 340 (300). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.89 (d, *J* = 8.0 Hz, 1 H), 7.82 (s, 1 H), 7.63 (d, *J* = 8.0 Hz, 1 H), 1.77 (s, 9 H) ppm. ^{13}C NMR (270 MHz, $CDCl_3$): δ = 158.7, 138.5, 135.8, 132.2, 128.3, 122.7, 121.4 (q, $^1J_{C,F}$ = 281.1 Hz), 120.9, 61.8, 28.3(q, $^2J_{C,F}$ = 38.8 Hz), 27.7 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -64.7 ppm. HRMS (ESI): calcd. for $C_{13}H_{13}F_3N_3O_3S$: 348.0630; found 348.0626.

6-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (7a): Compound **6a** (13.5 mg, 39 μmol) was dissolved in TFA (2 mL). The reaction mixture was refluxed for 24 h, and then concentrated. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$,

1N HCl, then brine. The organic layer was dried over $MgSO_4$ and filtrated. The filtrate was concentrated, then the residue was recrystallized from EtOAc and hexane at -20 °C to afford **7a** (6.5 mg, 57%) as colorless amorphous mass. λ_{max} (ε) (CH_3OH) 290 (920), 337 (303). ^{1}H NMR (270 MHz, CD_3OD): δ = 8.11 (1H, d, *J* = 8.0 Hz), 7.90 (1H, s), 7.84 (1H, d, *J* = 8.0 Hz) ppm. ^{1}H NMR (270 MHz, CD_3OD): δ = 161.3, 142.3, 137.3, 133.8, 130.6, 126.8, 123.1 (q, $^1J_{C,F}$ = 275.1 Hz), 120.4 29.7 (q, $^2J_{C,F}$ = 41.2 Hz) ppm. ^{19}F NMR (470 MHz, CD_3OD): δ = -66.7 ppm. HRMS (ESI): calcd. for $C_9H_5F_3N_3O_3S$: 292.0004; found 292.0020.

5-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)-1,2-benzisothiazole-3-one-1,1-dioxide (7b): Compound **6b** (13.2 mg, 38 μmol) in TFA (2 mL) was treated same manner described for **6a**. The residue was recrystallized from EtOAc and hexane at -20 °C to afford **7b** as afford colorless amorphous mass (6.0 mg, 54%). λ_{max} (ε) (CH_3OH) 280 (1005), 340 (280). ^{1}H NMR (270 MHz, CD_3OD): δ = 8.11 (d, *J* = 8.0 Hz, 1 H), 7.86 (s, 1 H), 7.83 (d, *J* = 8.0 Hz, 1 H) ppm. ^{13}C NMR (68 MHz, CD_3OD): δ = 161.2, 142.3, 136.5, 134.3, 130.5, 123.9, 123.1, 123.0 (q, $^1J_{C,F}$ = 275.1 Hz), 29.5 (q, $^2J_{C,F}$ = 41.2 Hz) ppm. ^{19}F NMR (470 MHz, CD_3OD): δ = -62.7 ppm. HRMS (ESI): calcd. for $C_9H_5F_3N_3O_3S$: 292.0004; found 292.0014.

HPLC purification for 7a and 7b. The suspension of 3a and 3b in aqueous solution were made to alkaline with 1M NaOH. The sodium salts were subjected to HPLC (Tosoh TSKgel ODS-80Ts (4.6 x 250 mm)、30% MeOH, 1 mL / min, detection at 215 nm or 350nm).

Photolysis of methanolic solution of 7a and 7b. Methanolic solutions of **7a** and **7b** (1 mM) were irradiated with black light (15W) at distance 3 cm. The spectrum of each minute was measured. Decrease of absorbance at around 350 nm was plotted to calculate half-life.

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